

Agroecological drivers connecting soil management practices, soil health, plant health and nutritional and sanitary qualities of grain yield: the case of soft winter wheat to illustrate the "One Health" approach

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Doctorat de l'Université de Toulouse

préparé à Toulouse INP

Déterminants agroécologiques liant pratiques de gestion des sols, santé des sols, santé des plantes et qualités nutritionnelle et sanitaire des produits : le cas du blé tendre d'hiver pour illustrer l'approche "One Health"

Thèse présentée et soutenue, le 20 décembre 2024 par **Clara LEFEVRE**

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Spécialité Ecologie fonctionnelle

Unité de recherche CRBE - Centre de Recherche sur la Biodiversité et l'Environnement

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Membres invités

Mme Léa Lugassy, Pour une Agriculture Du Vivant

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« Les SCV [Systèmes sous Couverture Végétale] ne pourront encore progresser au bénéfice de la production agricole et de l'environnement que si la recherche a l'humilité de retourner dans la nature, en force, pour voir, apprécier, comprendre comment s'exerce le génie de la vie *in situ*, et comment cette nature si riche et si admirable dans sa complexité (*systémique, holistique*) peut servir toujours mieux l'agriculture durable, pour passer graduellement et de manière viable des agrosystèmes actuels aux écosystèmes cultivés. »

"DMC [Direct Mulch Seeding] will only be able to make further progress for the benefit of agricultural production and the environment if research has the humility to return to nature, in full, to see, appreciate and understand how the genius of life is exercised *in situ*, and how this nature, so rich and so admirable in its complexity (*systemic, holistic*), can serve sustainable agriculture better and better, in order to move gradually and sustainably from current agrosystems to cultivated ecosystems."

Lucien Séguy & Serge Bouzinac, 20081

¹Séguy, L. & Bouzinac, S. 2008. La symphonie inachevée du semis direct dans le Brésil Central : le système dominant dit de 'semi-direct'. https://agritrop.cirad.fr/546845/1/ID546845.pdf

<u>Résumé</u>

La transition agroécologique représente un enjeu majeur pour une agriculture performante, plus respectueuse des sols, de l'environnement et de la santé. L'urgence et l'ampleur de la tâche transcrivent la nécessité de tous les acteurs de la filière alimentaire, du champ, à l'assiette, à s'associer et travailler ensemble. Ces liens entre acteurs et disciplines se retrouvent dans le concept d'« Une seule santé » (« *One Health* »), basé sur le principe qu'il existe une connexion entre la santé des humains, des animaux, des plantes et de l'environnement. Bien que le rôle clé des pratiques agricoles dans le concept « *One Health* » soit plutôt bien renseigné d'un point de vue théorique, jusqu'à maintenant peu d'applications existent pour démontrer ces liens de cause à effet sur la base de données de terrain, du fait de la complexité de la mise en place de telles études.

Ce travail de thèse visait à démontrer que la théorie « *One Health* » appliquée à la production de blé mais en incluant les pratiques culturales qui en sont au point de départ, n'est pas qu'un concept théorique, mais que ces liens entre les pratiques, la santé des sols, celle des plantes et les qualités nutritionnelle et sanitaire des produits récoltés, sont bien vérifiables sur la base de données de terrain.

Pour ce faire, nous avons mis en place en 2021 un réseau d'étude à la ferme (OFE dans la suite du document : *On-Farm Experiment*), situé pour l'essentiel dans le quart nord-ouest de la France et composé de 86 parcelles agricoles cultivées en blé tendre d'hiver. La moitié de ces parcelles étaient cultivées selon les principes de l'Agriculture de Conservation des Sols (ACS), et l'autre moitié de manière « conventionnelle » (CONV). Les parcelles d'un agriculteur en ACS et d'un agriculteur en CONV ont été sélectionnées de manière à ce qu'elles puissent se comparer en binôme. Les parcelles de chaque binôme ACS/CONV possédaient donc les mêmes caractéristiques pédoclimatiques et étaient cultivées avec la même variété (ou mélange variétal) de blé tendre pour éviter toute multiplication de facteurs confondants. Sur les 43 paires de parcelles suivies, 22 ont été suivies sur l'année de campagne 2022-2023. Le blé tendre a été choisi comme culture d'étude car il représente aujourd'hui la céréale la plus cultivée et consommée en France et en Europe. Après avoir mis en place ce réseau de parcelles, nous avons suivi chacune d'entre elles sur une campagne de production.

En complément d'un diagnostic agroécologique (à l'aide de l'Indice de Régénération), nous avons procédé à une analyse fine et systémique des pratiques culturales appliquées à chaque parcelle étudiée, en prenant en compte les cinq campagnes précédant celle du blé étudié, ainsi que cette dernière. Un panel d'indicateurs de santé du sol, de santé de la plante, de qualité nutritionnelle et sanitaire de la récolte et de performances socio-économiques et environnementaux, a ensuite été testé sur chaque parcelle. Des investigations supplémentaires ont aussi été menées pour étudier des aspects spécifiques de composition des communautés microbiennes sur le continuum sol-plante-grain, en collaboration avec le Muséum d'Histoire Naturelle de Paris. Afin de clore la boucle de la « fourche à la fourchette », nos recherches sont allées jusqu'à la fabrication de pain, afin de potentiellement mettre en évidence les effets de ces pratiques agricoles contrastées sur le produit de consommation final.

Notre étude a permis de mettre en évidence des niveaux de performance agroécologique plus ou moins élevés au sein même des groupes ACS et CONV, menant à des effets variables sur la santé des sols. De manière générale, nous démontrons pour ce réseau les effets positifs des systèmes ACS comparés aux systèmes CONV sur les principales fonctions qui contribuent à la santé des sols, c'est-à-

dire le cycle des nutriments, la transformation du carbone, la stabilité structurale des agrégats et la régulation biologique. Les effets sur la qualité du grain ont été plus délicats à mettre en évidence, du fait d'un important effet de l'année d'étude. Toutefois, nous avons pu observer les effets positifs de l'ACS sur les qualités nutritionnelles du grain (concentration plus élevée en P, K et ergothionéine). Enfin, nous montrons, dans nos conditions d'étude, que l'ACS se pratique sans perte de rendement tout en diminuant la consommation de gasoil et la charge de travail pour l'agriculteur. Nous montrons aussi qu'il est possible chez les ACS les plus performants, de limiter la dépendance aux engrais minéraux et aux pesticides de synthèse par rapport à leurs homologues CONV, ce qui est encourageant pour le développement de cette pratique d'un point de vue environnemental et de santé publique. Tous ces résultats suggèrent des effets positifs de la pratique de l'ACS sur l'environnement et sur la santé des consommateurs (humains ou animaux).

Cette étude est le résultat d'un travail collaboratif à plusieurs échelles : avec les agriculteurs tout d'abord, qui ont pris part à la création du réseau d'étude, puis avec les multiples partenaires techniques, scientifiques et industriels, qui ont permis le déroulement de cette étude tout en horizontalité. Ce travail a finalement permis d'investiguer des aspects méthodologiques et analytiques de la santé des sols, des plantes et de la qualité nutritionnelle du blé en conditions réelles.

<u>Mots-clefs</u>: Agriculture de conservation, Une seule santé, Gestion durable des sols, Agroécologie, France

Abstract

The agroecological transition represents a major challenge for an efficient agriculture, more respectful of soils, the environment and health. The urgency and magnitude of the task reflect the need for all stakeholders in the food industry, from the field to the plate, to join forces and work together. These links between stakeholders and disciplines are reflected in the concept of "One Health", based on the principle that there is a connection between the health of humans, animals, plants and the environment. Although the key role of agricultural practices in the "One Health" concept is rather well documented from a theoretical point of view, until now few applications exist to demonstrate these cause-and-effect links based on field data, due to the complexity of setting up such studies. This thesis aimed at demonstrating that the "One Health" theory applied to wheat production but including the cultural practices that are at the starting point, is not just a theoretical concept. Indeed, these links between practices, soil health, plant health and the nutritional and health qualities of the harvested products, are verifiable on the basis of field data. To do this, we set up in 2021 an on-farm study network (OFE in the rest of the document: On-Farm Experiment), located mainly in the north-western quarter of France and composed of 86 agricultural plots cultivated with soft winter wheat. Half of these plots were cultivated according to the principles of Conservation Agriculture (CA), and the other half in a "conventional" manner (CONV). The plots of a farmer in CA and a farmer in CONV were selected so that they could be compared in pairs. The plots of each CA/CONV pair therefore had the same pedoclimatic characteristics and were cultivated with the same variety (or varietal mixture) of wheat to avoid the multiplication of confounding factors. Of the 43 pairs of plots monitored, 22 were monitored during the 2021-2022 campaign year, and 21 during the 2022-2023 campaign year. Wheat was chosen as the study crop because it is currently the most cultivated and consumed cereal in France and Europe. After setting up this network of plots, we monitored each of them over a production campaign. In addition to an agroecological diagnosis (using the Regeneration Index), we carried out a detailed and systemic analysis of the cultivation practices applied to each plot studied, taking into account the five campaigns preceding that of the wheat studied, as well as the latter. A panel of indicators of soil health, plant health, nutritional and sanitary quality of the crop and socio-economic and environmental performances were then tested on each plot. Additional investigations were also conducted to study specific aspects of microbial community composition on the soil-plant-grain continuum, in collaboration with the Natural History Museum of Paris. In order to close the loop from "farm to fork", our research went as far as bread making, in order to potentially highlight the effects of these contrasting agricultural practices on the final consumer product. Our study highlighted more or less high levels of agroecological performance within the CA and CONV groups, leading to variable effects on soil health. In general, we demonstrate for this network the positive effects of CA systems compared to CONV systems on the main functions that contribute to soil health, i.e. nutrient cycling, carbon transformation, structural stability of aggregates and biological regulation. The effects on grain quality were more difficult to highlight, due to a significant effect of the year of study. However, we were able to observe the positive effects of CA on the nutritional qualities of the grain (higher concentration of P, K and ergothioneine). Finally, we show, under our study conditions, that the practice of CA is carried out without loss of yield while reducing diesel consumption and the workload for the farmer. We also show that it is possible for the most efficient CA systems to limit dependence on mineral fertilizers and synthetic pesticides compared to their CONV counterparts, which is encouraging for the development of this practice from an environmental and public health point of view. All these results suggest positive effects of the practice of CA on the environment and on the health of consumers (humans or animals).

This study is the result of collaborative work on several scales: first with farmers, who took part in the creation of the study network, then with the multiple technical, scientific and industrial partners, who allowed this study to be carried out horizontally. This work ultimately made it possible to investigate methodological and analytical aspects of soil health, plants and the nutritional quality of wheat in real conditions.

Keywords : Conservation agriculture, One Health, Sustainable soil management, Agroecology, France

SCIENTIFIC PUBLICATIONS, COMMUNICATIONS AND LECTURES

Publications

Lefèvre, C., Husson, O., Lugassy, L. & Sarthou, J.-P. submitted. On-farm systemic approaches to unravel the role of soil and crop management in One Health. A review. *Agronomy for Sustainable Development*.

Lefèvre, C., Husson, O., Dumora, B, Grudé, O., Lugassy, L. & Sarthou, J-P. submitted. Data from extensive monitoring of agricultural practices, soil health, and wheat grain production in 44 farms in Northwestern France from 2021 to 2023. *Data in Brief*.

Lugassy, L., Boivin, P., Duru, M., Lefèvre, C., Peeters, A., Sarthou, J.-P., Selosse, M.-A. & Husson, O. submitted. The Regeneration Index, an agronomic tool to scale up agroecology. *Sustainable Futures*.

Dataset and metadata

Lefèvre, C., Husson, O., Lugassy, L., Dumora, B. & Sarthou, J-P. 2024. Data of on-farm assessment of cropping practices in a "One Health" perspective, Winter wheat, France. https://doi.org/10.18167/DVN1/SI026U, CIRAD Dataverse, V1.

Communications

Lefèvre, C. 2022. Application of Biofunctool[®] on 44 agricultural fields of Northwestern France. Comparison of winter wheat cropping systems in Conservation Agriculture and "Conventional" (In French). Presentation at Biofunctool[®] day, 24th August 2022. Online.

Lefèvre, C. 2022. Agroecological drivers connecting soil management practices, soil health, plant health and nutritional and sanitary quality of grain yield: the case of winter wheat to illustrate the "One Health" approach. Presentation to the LEFE laboratory, 21 October 2022, Toulouse, France.

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Posters

Lefèvre, C., Sarthou, J-P. 2021. Agroecological drivers connecting soil management practices, soil health, plant health and nutritional and sanitary quality of grain yield: the case of winter wheat to illustrate the "One Health" approach. Poster at the SEVAB Doctoral School Day, December 2021, Toulouse, France.

Lefèvre, C., Husson, O., Lugassy, L. & Sarthou, J.-P. 2024. Assessing the effects of sustainable soil management on One Health. Poster at the 18th Congress of the European Society for Agronomy, August 2024, Rennes, France.

Lectures

Directed work on the Regeneration Index (4 x 2 hours), ENSAT, March 2022, March 2023, December 2023 & March 2024.

Meetings and feedback to partners

<u>2021</u>

13 December	Study launch	Farmers, Extension	Vienne Agricultural
		services from Vienne,	Chamber, Mignaloux-
		Deux-Sèvres and	Beauvoir (86)
		Nouvelle-Aquitaine	
		region	

<u>2022</u>

4 January	Study launch	Financial partners	Online meeting
15 June	Mi-year feedback	Financial partners	Online meeting
27 October	Semester 2 feedback	Financial partners	Online meeting
21 December	Bread-making and 2022	Farmers, extension	Girardeau Mill, Boussay
	sampling campaign	services	(44)
	feedback		

<u>2023</u>

20 January	Feedback on study year	Farmers, extension	Vienne Agricultural
	1	services, financial	Chamber, Mignaloux-
		partners	Beauvoir (86)
9 March	Trimester 1 feedback	Financial partners	Online meeting
8 September	Feedback plant health	Farmers, extension	Deux-Sèvres Agricultural
	study + field campaign	services	Chamber, Bressuire (79)

<u>2024</u>

29 March	Trimester 1 feedback	Financial partners	Hybrid: Online and ENSAT,
			Toulouse (31)
21 May	Bread-making 2023	Farmers, extension	Girardeau Mill, Boussay
	feedback and restitution	services	(44)
	to farmers		
11 July	Trimester 2 feedback	Financial partners	Online meeting

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LIST OF ABBREVIATIONS

All abbreviations linked to studied indicators in this manuscript are available in **Annexe 1** of the manuscript.

AMPA : Aminomethylphosphonic Acid ANMF: Association Nationale de la Meunerie Française (National Association of French Millers) **ASV: Amplicon Sequence Variants** BPMF: Wheat suitable for French milling (« Blé Pour la Meunerie Française ») **CA:** Conservation Agriculture **CONV: Conventional Agriculture** DM: Dry Matter GHG : Greenhouse Gas MCPA: 2-methyl-4-chlorophenoxyacetic acid (post-emergence herbicide) NA: Non available NPO : Non-profit Organisation **OC: Organic Carbon OM: Organic Matter OTU: Operational Taxonomic Unit** PADV: Pour une Agriculture du Vivant, non-profit organisation PCA : Principal Component Analysis PCR: Polymerase Chain Reaction PLS/PM: Partial Least Square Path Modelling POXC: Permanganate Oxidisable Carbon **RI:** Regeneration Index **TFI: Treatment Frequency Index**

Laboratory methods

HPLC: High Performance Liquid Chromatography is a technique used to separate, identify, and quantify specific components in mixtures (Source: Wikipedia).

ICP-MS: Inductively coupled plasma mass spectrometry is a type of mass spectrometry that uses an inductively coupled plasma to ionize the sample. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected (Source: Wikipedia).

LC-MS/MS: Liquid chromatography (LC) tandem mass spectrometry (MS) is a technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS).

Chemical elements

B: Boron	Mn: Manganese	P ₂ O ₅ : Phosphorous pentoxide
Cu: Copper	Mo: Molybdenum	S: Sulphur
Fe: Iron	Na: Sodium	SO ₃ : Sulphur trioxide (Sulphite)
K ₂ O: Potash	Na ₂ O: Sodium Oxyde	SO ₄ ²⁻ : Sulphate
Mg: Magnesium	N: Nitrogen	Zn: Zinc

GENERAL INTRODUCTION

In a context of crisis (health, social and environmental) where traditional agricultural practices are increasingly being criticised, accelerating the agroecological transition is a significant challenge and a source of hope for sustainable agriculture that is less destructive of the planet's soils. There is a wealth of research on the subject of agroecological transition, and the importance of soil management no longer needs to be demonstrated (Adhikari and Hartemink, 2016; Keesstra *et al.*, 2016). Specifically, amongst other already well researched cropping systems (*e.g.* organic agriculture), Conservation Agriculture (CA) was evidenced as a promising alternative to more sustainable though productive agriculture (Derpsch *et al.*, 2024).

The urgency of making an agroecological transition and the scale of the task ahead mean that all the players in the food supply chain need to work together based on a shared understanding and vision. In France, *Pour une Agriculture du Vivant* (PADV) non-profit organisation (NPO) is supporting the development of agroecological sectors, bringing together the various stakeholders around a clear, pragmatic vision of sustainable agriculture: a path to progress aimed at building farming systems able to ensure soil fertility, plant nutrition and crop protection.

These links between players and themes are reflected in the concept of "One Health", which is based on the principle that there is a connection between the health of humans, animals and the environment. In other words, the quality of the environment in which we live, from the health of the soil to the food we eat, has a direct impact on our health (van Bruggen *et al.*, 2019; Keith, Schmidt and McMahon, 2016; Poch *et al.*, 2020).

Although the theoretical framework of the "One Health" concept is well known and widely used by institutions, until now, few scientific studies on agroecology and sustainable soil management have ventured to implement it in real conditions, due to its complexity of implementation in the field (Lebov *et al.*, 2017). Most research therefore proposes visions centred on some of the components of the "One Health" concept, for example by studying the impact of agroecological practices alone on soil health and the provision of certain ecosystem services (*e.g.* Perego *et al.* (2019), Pheap *et al.* (2019)), or the effects of agroecology on plant productivity and the nutritional and health aspects of production (*e.g.* Calzarano *et al.* (2018)).

The objectives of the thesis were therefore multiple and detailed as follows:

- 1. Conduct a comprehensive and systemic field study to compare Conservation and Conventional Agriculture following a "One Health" approach;
- 2. Obtain field data to support the scaling-up of large-scale agroecological farming practices;
- 3. Create a multi-stakeholder platform bringing together farmers, research and industry;

Around one main question: What are the effects of cropping practices on soil health, plant health and product nutritional and sanitary qualities, when growing soft winter wheat in a temperate climate?

Our central hypothesis is that far from being just a concept, the One Health theory is verifiable throughout the production and processing chain of soft winter wheat², from "field to fork".

² "Winter wheat" in the rest of the manuscript

This study was set up in Northwestern metropolitan France on winter wheat. Winter wheat is the major staple food in most temperate countries, including Europe and France. Specifically, France produces one quarter of winter wheat in the European Union (Agreste, 2021). Winter wheat represents an essential source of carbohydrates, proteins, fibres, lipids, vitamins and minerals. After processing, it is used for flour, cereals, biscuits and bread or added to other food and feed products, making it essential for the health of people and livestock (Shewry and Hey, 2015).

This study was driven by the NPO "Pour une Agriculture du Vivant", thanks to the financial support of five main partners, which are *Brioche Pasquier* Group, *Nutrition & Santé* Group, *Gaïago, Valorex* and the *Pour un Autre Monde* foundation (Figure 1). The research supervision was operated by *CNRS* and *CIRAD* research centres, and was hosted in *AgroToulouse (ENSAT)*, Engineering School of Agronomy in the Southwest of France, in the Research Centre for Biodiversity and Environment (CRBE) laboratory unit. Four research partnerships were set up during the study : with Aurea Agrosciences laboratory, which handled most soil analyses, the French Museum of Natural History, with whom we worked on microbiological aspects of the soil-plant-production continuum (**Case study n°2**), the "Moulin Girardeau" mill, who proposed their support in making Bread-making tests (**Case study n°3**), and lastly with the Penn State College of Medicine (University of Pennsylvania) who worked on a specific amino acid analysis in wheat grain (Ergothionein, **Chapter 4**). We also deeply acknowledge the technical support received from the local agricultural chambers of the Vienne and Deux-Sèvres administrative departments and of the Nouvelle-Aquitaine region (Figure 1).



Figure 1. Articulation of the research project.

This manuscript is organised into four chapters, written in scientific article formats and three case studies. **Chapter 1** provides a literature review on the importance of systemic studies in the studies of crop production systems, and the need for science to open to these new forms of studies. **Chapter 2** acts as a general material and methods, going through all the study design and indicators. It introduces the dataset and analyses methods mobilised during this PhD work. **Chapter 3** introduces the On-Farm Experimentation (OFE) setup for this study. It describes and analyses the different farmers' practices, which is key to allowing for any conclusion on their effects on the rest of the crop

production system. **Chapter 4** provides insights on the effects of the different identified cropping practices on the soil and production compartments, associated with performance externalities (environmental, economic and social). The end of this document is made of **three case studies**, aiming to show concrete applications of the potential of OFE networks to tackle specific research questions. The first case study (**Case study n°1**) aims to unravel the role and implication of plant health in the soil-production nexus. This case study is presented as a summary of an MSc thesis realised within the OFE network. The second case study (**Case study n°2**) tackles the specific relations between cropping practices and microbiology at the interface between soil, roots and production. This case study is the result of a joint project named "Agribiodiv", for "agricultural biodiversity", led in collaboration with the French National Museum of Natural History. The last case study (**Case study n°3**) represents the "final" step of production since it aimed to identify possible influences of cropping systems on the final edible product, *i.e.* bread. These three case studies were all set on sub-samples of fields and not on the complete setup as their aim was mainly exploratory (Figure 2).



Figure 2. Graphical overview of the structure of this PhD thesis.

CHAPTER 1: ON-FARM SYSTEMIC APPROACHES TO UNRAVEL THE ROLE OF SOIL AND CROP MANAGEMENT IN ONE HEALTH

Keywords: transdisciplinarity, systemic science, food production

This chapter was submitted as a review article to the Agronomy for Sustainable Development journal on August 27th, 2024.

This chapter aims to propose a state-of-the-art overview of the current knowledge on the effects of cropping systems on One Health, as well as demonstrate the importance of on-farm approaches to evidence these links in real world applications.

1. Introduction

In addition to eating enough food, eating nutritious and safe food supports human and animal health. Producing food in quantity has been successfully achieved since the late twentieth century through the Green Revolution and the development of machinery and chemistry in agricultural systems.

However, this came at a tremendous cost for the people and the environment: the current global food system, including food production and global diets, has been at a crossroads for years (Campbell *et al.*, 2017; Foley *et al.*, 2011; Welch and Graham, 1999), destroying more value than it created. Nowadays, it largely threatens the environment (1/3 of today's Greenhouse gas (GHG) emissions are due to the global food system (Crippa *et al.*, 2021)) and people's health at a colossal associated hidden cost exceeding ten trillion USD per year (FAO, 2023; Ruggeri Laderchi *et al.*, 2024). Intensive cropping systems contribute to the current mass extinction of biodiversity through the overuse and misuse of pesticides, ploughing, monocultures, and cutting down of native forests and hedges for global agricultural area expansion (Dudley and Alexander, 2017). In addition to causing environmental harm, agricultural intensification contributes to agricultural soil degradation at alarming rates (Montanarella *et al.*, 2016), hindering their ability to produce food under intensive management (Karlen and Rice, 2015; Kopittke *et al.*, 2019; Oliver and Gregory, 2015).

Although the linkages between soil degradation and quantitative food production have been evidenced, the ones between soil condition and nutritional quality of food are harder to demonstrate (Marles, 2017; Silver *et al.*, 2021). Indeed, in complex systems such as the food production system, traditional approaches have failed to evidence possible links between soils and food quality, possibly because they tend to study the food production system compartments in isolation from each other ("siloed approach" (den Boer *et al.*, 2021)). This prevents the identification of connections and interactions between various parts of the system and how a compartment influences the others (Sterman, 2012). Traditional research on cropping systems has historically focused on technological solutions applied to soil and plants (*e.g.* mineral fertilisation, fortification or genetic manipulations) (McKevith, 2004; Welch, 2002), leading to a relatively good knowledge of technical solutions that now need to be integrated into wider studies to translate research into action (Lal *et al.*, 2021). Linear traditional research and innovation efforts have therefore contributed to improving specific parts of the food system, such as agricultural production and food safety, but have largely failed to offer solutions to persistent problems that affect food systems due to their lack of engagement with trade-offs, unforeseen and undesired side-effects, and systemic feedback loop (den Boer *et al.*, 2021).

Today's food production urgent challenge is to sustain food security and safety while having limited negative impacts on the environment (including soil) and ensure human and animal health. The resulting challenge for researchers is to adopt a One Health perspective to demonstrate the links between crop management, soil health, plant growth and health, food security and safety, and the environment (Muramoto *et al.*, 2022). Carrying out robust scientific studies considering these interconnections between compartments (systemic approach) while covering each compartment in the best-integrated way (holistic approach) will be key to providing useful information to decision-makers and promoting the transition towards a more sustainable food system (Lebov *et al.*, 2017; Mettenleiter *et al.*, 2023).

One Health research has traditionally been considered from a veterinarian and epidemiologic perspective (Lebov *et al.*, 2017; Mackenzie, McKinnon and Jeggo, 2014), and the implication of soil and

plants in it has been recently claimed by researchers and practitioners (Boa, Danielsen and Haesen, 2015; Fletcher, Franz and Leclerc, 2009; Keith, Schmidt and McMahon, 2016). Hence very little research has been so far based on hard data in order to demonstrate the role, in a One Health approach, of the whole cropping system (that must be seen as the cropping practices-soil-plant-product chain) (Montgomery *et al.*, 2022; Montgomery and Biklé, 2021; Rekik and van Es, 2022).

Major difficulties in carrying out such research on complex systems rely on the need to address the many and diverse:

- Assessment methodologies, especially the absence of a global framework and thresholds to assess sustainable soil management practices, soil health, and their effects on plants and productions,
- **Pedoclimatic and socio-economic contexts** in which food is produced, leading to diverse rules and regulations, diverse production objectives (*e.g.* closing the yield gap, increasing crop quality), and different soil responses to agricultural management,
- **Stakeholders and disciplines** involved along the food chain, from production to consumption, and the associated antagonisms and trade-offs that hamper an efficient transition to a sustainable food system.

In this review, we propose a conceptual framework for understanding cropping systems and provide step-by-step guidance for assessing, in a systemic and holistic approach, the effects of cropping system management on One Health. We also give insights to overcome the current limits to systemic studies applied to cropping systems.

2. Understanding the cropping system and the need for systemic approaches

Any cropping system can be divided into four compartments: cropping practices, soil, plants, and harvested products. These four compartments are interconnected and interdependent. This system operates in a large diversity of climates and soil types around the globe (Figure 3).

Cropping practices strongly influence the capacity of soils to ensure their functions and to efficiently provide their ecosystem services (soil health concept) (Cadel, Cousin and Therond, 2023; Christel, Maron and Ranjard, 2021; Derpsch *et al.*, 2024; Khangura *et al.*, 2023; Ma *et al.*, 2023; Yang, Siddique and Liu, 2020). As a result, healthy soils are more likely to allow plants to grow thanks to an increased ability to catch nutrients and water through their roots and to defend themselves against pests and diseases (plant health concept) (van der Heijden, Bardgett and van Straalen, 2008; Muramoto *et al.*, 2022; Sahu *et al.*, 2019). Healthy plants are, in return, more likely to ensure a good production (Romero *et al.*, 2024; Savary *et al.*, 2017). Plants are crucial in the system since they are the only beings able to convert solar energy into food and oxygen for aerobic organisms such as humans and animals, therefore providing all the energy in the system (Fletcher, Franz and Leclerc, 2009). Retroactions exist between cropping practices, soil health, and plant health. Indeed, soil health issues such as fertility loss or the disruption of soil food webs lead to increased needs for synthetic fertiliers or pesticides. This often results in the loss of soil natural biological regulation mechanisms and in unbalanced and unhealthy plant nutrition. This subsequently leads to the loss of natural biological regulation of crop pest and disease, resulting in a vicious circle of increased use of agrochemicals (Derpsch *et al.*, 2024).

This whole system is thought to provide externalities that impact human, animal, and environmental health (One Health) (Muramoto *et al.*, 2022). In a nutshell, we theoretically know that a healthy life is

not possible without healthy soils (Banerjee and van der Heijden, 2023; Brevik *et al.*, 2020; Lehmann *et al.*, 2020; Poch *et al.*, 2020; Steffan *et al.*, 2018), and farmers' management choices are crucial if the most positive externalities are to be met (Ma *et al.*, 2023; Muramoto *et al.*, 2022).

It is necessary to understand what these four previously defined compartments are composed of to get an accurate holistic vision of the system. Indeed, each of these can be subdivided into interacting sub-compartments that need to be correctly assessed in any analysis of the cropping practices-soil-plants-product continuum (Figure 3).

First, cropping practices are the combination of different strategies adopted by farmers, which can be summarised in six sub-compartments that are (1) Soil tillage, (2) Pesticide use, (3) Crop nutrition including fertilisation and water use, (4) Crop variety, (5) Soil cover, and (6) Crop diversification. These six items are interconnected since a farmer's management choice on one of the sub-compartments may impact the others (Khangura et al., 2023; Rillig and Lehmann, 2019). For example, stopping pesticide use may influence the intensity of soil tillage and/or the diversity of crop rotation in order to manage weed pressure and ensure crop production. Additionally, as a standalone practice, no-tillage does not automatically lead to a fully functioning sustainable cropping system, suggesting that a set of complementary practices is needed to enable synergistic benefits and a fully functioning cropping system and the entire agroecosystem to deliver a wide range of ecosystem services (Derpsch et al., 2014, 2024). Therefore, the combination of all the management choices allows the assessment of the practices positive or negative externalities. The incomplete consideration and management of all these sub-compartments may lead to confusing conclusions about what the management really is. It is, for example, what may happen with labels or conceptual formulations used to describe cropping systems (Therond et al., 2017) (e.g. climate-smart agriculture (Lipper et al., 2014), carbon farming (Lal, 2023), sustainable or ecological intensification (Wezel et al., 2015), regenerative agriculture (Tittonell et al., 2022), agroecology (Bezner Kerr et al., 2021; D'Annolfo et al., 2017; Frison and Clément, 2020; Poux and Aubert, 2018)). A holistic description of the practice compartment is critical to every study that would aim to analyse the effects of cropping practices on any other item of the food system compartments without taking the risk of shortfall conclusion.

Similarly, the soil compartment may be divided into three sub-compartments: (1) physical properties, including soil texture; (2) chemical properties; and (3) biological properties. These three sub-compartments are intrinsically connected and are at the foundation of soil health. Further, each sub-compartment can be subdivided: for example, biological soil properties cover all soil organisms from mega to micro-fauna. Conclusions on soil biological activity based on only one type of organism are insufficient since each type of organism has different roles in soil and the soil biological status results from complex interactions between soil organisms (Khangura *et al.*, 2023). Soil health assessments need to accurately cover not only each soil compartment and sub-compartment but also the functional result of the interactions between all of them to provide reliable conclusions on the soil health status (Bünemann *et al.*, 2018; Kibblewhite, Ritz and Swift, 2008; Stewart *et al.*, 2018).

In the context of One Health, plant health refers to the overall vigour of plants (Boa, Danielsen and Haesen, 2015). Plant or crop health has historically been defined in relation to possible contamination with pests, diseases, or chemicals, but researchers now estimate that plant health is the combination of an absence of plant contamination and the plant capacity to catch nutrients and water through its roots to grow correctly.

Finally, crop product quality depends on three different factors of quality: (1) nutritional quality (through macro- and micro-nutrient profiles), (2) sanitary quality (*e.g.* presence of pesticide residues, heavy metals, mycotoxins), (3) technological quality. The overall production quality requires that all three parameters are taken in account. Complemented by yield data, this allows the linking of aspects of food production to food security and safety (Gomiero, 2018; Lairon, 2010).

The system complexity and the numerous interactions occurring within it, make traditional reductionist approaches fail when trying to demonstrate the effects of one item from that system on another one (*e.g.* no-till effects on soil microbiology). The absence of integration and the lack of consideration of these complex interactions often lead to shortfalls and confusions in the conclusions presented (Turner, 2021).

Systemic approaches are key to better understand the system behaviour (Veerman *et al.*, 2020) and the effects of cropping practices on the soil-plant-production chain, making the One Health approach suitable for agronomy. However, operationalizing One Health approaches remains a challenge (Pepin *et al.*, 2024).



Figure 3. The crop production system, interlinkages and relations between compartments, and link to One Health. Dashed arrows represent the interactions within sub-compartments of the crop production system. Bold arrows represent the interlinkages and retroactions between the different compartments of the system. Only a systemic assessment of the cropping system will allow to provide relevant and robust conclusions on its effects on One Health, including human and animal health.

3. Assessing the effects of cropping system management on One Health

We assume that all studies follow a logical path in four main steps: (1) Study design, (2) Data collection, (3) Data analysis and results interpretations, and (4) Valorisation and diffusion of results. In a One Health perspective, we propose a methodological shift right from the beginning of any study and all along the research development and valorisation of results to allow more accurate conclusions to be drawn on the effects of the food system on One Health (Figure 4).



Figure 4. From traditional to a systemic approach of the cropping system, following a One Health perspective. This novel approach requires a paradigm shift from researchers to increase collaborations between disciplines and stakeholders to share tools and approaches for maximised benefits to the food production system transformation.

3.1. Step 1: Study design

Study design is a crucial step and needs to be thought carefully to ensure the systemic aspect of the study, paying attention to the following points:

- Scale and duration of the study
- Choosing the most adapted indicators
- Setting on-farm research with farmers
- Multi-stakeholders' involvement

3.1.1. Scale and duration of study

A systemic approach must consider the time specificities of each system compartment. In the case of the effects of cropping management on soil health, it must be reminded that soil health at a given time is the result of years of soil management practices, which often have long-term impacts on the structure and function of that soil (the legacy effect) (Turner, 2021). Therefore, long-term results on soil health often differ from short-term outcomes due to time delays embedded in the system feedback structure (Turner, 2021). In the first years of the transition to sustainable crop management, the damage to the soil from many years of conventional tillage and cropping begins to be repaired, and the soil functions and soil-mediated ecosystem services are re-established (Derpsch *et al.*, 2024). It is estimated that it can take ten years or more to reach a new agroecological "equilibrium" (Derpsch *et al.*, 2024). This difference in time frame needs to be considered in the soil-plant-production chain
assessment since systemic studies intend to study the combined and cumulated effects and interlinkages between compartments. Therefore, it appears that studying the effects of cropping management on other items of the production chain, relying only on data from one or two-year past management with no supplementary information on the past practices, is not sufficient to correctly inform cropping management practices (Büchi *et al.*, 2019). Long-term studies are therefore to be privileged in a One Health perspective of a cropping system assessment.

3.1.2. Selecting the most adapted indicators

Data management has been recognised as a redundant brake to One Health studies operationalisation, and not only in the agronomic field (Destoumieux-Garzón *et al.*, 2018). Indeed, in the frame of One Health studies, multiple indicators need to be selected to holistically characterise each system compartment studied. In addition, there cannot be a "one-size fits all" rule for indicators because of specific local conditions where they are intended to be applied, so indicators need to be chosen specifically (Lazicki and Geisseler, 2021; Stott, 2019). For instance, in a soil health assessment, the OM:clay ratio used to characterise the vulnerability of the soil stability is an example of a soil health indicator that may not be applicable in all soil types (Dupla *et al.*, 2022; Johannes *et al.*, 2017; Poeplau and Don, 2023; Prout *et al.*, 2021; Rabot *et al.*, 2024).

The choice of indicators also needs to be made regarding the analysis and repetitions needed to cover the large diversity of pedoclimates and diverse management combinations, even at smaller study scales (Champeil, Fourbet and Doré, 2004; Deluz *et al.*, 2020; Stott, 2019). Indicators must be adapted to the sampling scheme, methods, and be relevant regarding the timing of sampling (*e.g.* seasonal, annual). The whole needs to be anticipated and chosen carefully, since these may infer on the final cost and overall relevance of the studies (Stott, 2019).

Due to the complexity of the soil system (Turner, 2021), many researchers have worked on the definition of guiding principles to choose the best indicators in systemic assessments of soil health, although these are extended to a general set of indicators in the other system compartments. A sound indicator should be (1) Sensitive to management practices, (2) Easy to use and measure, (3) Costeffective, (4) Accurate and repeatable, and (5) Easily interpretable and useful (Stott, 2019; Toor *et al.*, 2021).

3.1.3. On-farm research with farmers

On-farm and participatory research have been identified as a necessary solution toward accomplishing the UN Sustainable Development Goals (SDGs) by 2030 (Lal *et al.*, 2021). Indeed, until now, most agronomic research has been realised in experimental fields, allowing good control of different abiotic or biotic parameters to facilitate the work in line with the requirements of each research domain. Onfarm research is a more adapted solution than experimental field studies to cover the diversity of systems and approaches used by farmers, since it allows to monitor innovative practices that are adapted to time and space and allow to cover a wide diversity of agricultural practices (Veerman *et al.*, 2020). This leads to a better representability of the diverse systems potentials (Lacoste *et al.*, 2022), farmers' insights being generally more relevant to local conditions (MacMillan and Benton, 2014). Onfarm research also allows farmers to become knowledge producers rather than solely considered as users, therefore making them more likely to adopt innovative cropping practices arising from research (Šūmane *et al.*, 2018; Waters-Bayer *et al.*, 2015). Since on-farm research is associated with increased pedo-climate variability and thus more confounding factors, it is crucial to work as rigorously as possible. This can be done for instance through the inclusion of more samples replicates (Deluz *et al.*, 2020) and a precise description all system components.

3.1.4. Multi-stakeholders' involvement

In addition to including land managers, multi-stakeholder approaches involving partnerships between researchers, private actors, and policymakers have been identified as a key lever to promote the adoption of innovation in agriculture and the agri-food sector (den Boer *et al.*, 2021; Lal *et al.*, 2021; McPhee *et al.*, 2021). Such approaches are crucial in systemic studies that aim to adopt a One Health perspective (Lebov *et al.*, 2017) since :

- The necessary shift in agricultural production system concerns the entire food production chain, from producers to consumers (locola *et al.*, 2023),
- A higher level of inter-disciplinarity is needed to run systemic approaches compared with traditional studies, hence requires the involvement of a larger body of knowledge,
- The higher on-the-ground experience of community members (Lacoste *et al.*, 2022; Lebov *et al.*, 2017) is likely to enhance the research team's ability to collect new data and understand its context through collaboration with them (Lebov *et al.*, 2017; Šūmane *et al.*, 2018).

Making scientists from different disciplines communicate with each other is a challenge which has already been identified as a main operational brake to One Health approaches studies (Destoumieux-Garzón *et al.*, 2018). Early involvement from each domain will encourage broader thinking in the planning process and will facilitate the aggregation of resources available in each one, such as funding, staff, and data.

Until now in traditional approaches, agronomists have rather focused on the practices compartment, soil scientists on the soil compartment, plant specialists on plant pathologies or crop nutrition, and food scientists on the production part (quality of products). Like the subdivision of each compartment into sub-compartments, more specialised scientists may focus on these specific sub-compartments, such as soil physicians, soil chemists, or soil biologists. These specialists often have very detailed and specific knowledge of each compartment or sub-compartment, but they may lack knowledge of how compartments interact. In a One Health perspective, the systemic approach needed requests that scientists adopt a large vision of the cropping system. Developing such an understanding of the system can take time, thereby making projects longer. Such weaknesses can be overcome with interdisciplinary teams working collaboratively, especially when at least one member, fluent in system science (Burger, 2024), can help translate information between team members (Turner, 2021). Fostered cooperation between scientists will be key to One Health approaches being successfully studied and then applied (Destoumieux-Garzón *et al.*, 2018).

3.2. Step 2: Data collection

Once the best fitting indicators are selected, the research team needs to identify the best collection and assessment methods, which can be either direct (on field) or indirect (obtained remotely). Data collection requires expert knowledge of the local agricultural practices, soil types, or production types (Bégué *et al.*, 2018), therefore highlighting the absolute necessity of researchers going to the field (Rafiq *et al.*, 2024).

Researchers are working towards defining more cost-efficient indicators to assess cropping practices (Bégué *et al.*, 2018), soil health (Haney *et al.*, 2018; Hughes *et al.*, 2023), or plant health (Abd-Elsalam

et al., 2024; Hobart, Giebel and Schirrmann, 2023) through direct or indirect measurements. Developing efficient tools to facilitate participatory data collection directed to research or non-business activities would help the data collection part.

Direct assessments often lead to more significant costs, increasing as the study area is large and numerous repetitions are needed. For instance, surveying and farmers' interviews remain the most common approaches to gathering information on cropping practices in on-farm studies (Akakpo *et al.*, 2021; Büchi *et al.*, 2019; Chabert and Sarthou, 2020; Dupla *et al.*, 2022; Nkurunziza *et al.*, 2017; Renaud-Gentié, Burgos and Benoît, 2014). These are time-consuming since in-person meetings with farmers might take time, although they have been recognised as an efficient method to collect reliable on-farm management data (Thompson *et al.*, 2019). Indirect measurements, through remote sensing pictures, facilitate data collection and alleviate costs while gathering a large amount of data in a short time (Bégué *et al.*, 2018; Diaz-Gonzalez *et al.*, 2022). These can be used, *e.g.* to estimate soil-tilled areas, plant growth –Normalised Difference Vegetation Index, NDVI-, soil organic carbon contents, or other soil parameters, amongst others. However, they do not seem to be a good solution for all scales of studies since the resolution of such data may be insufficient or of too low resolution, specifically at field or farm scale (Diaz-Gonzalez *et al.*, 2022). Indeed, the wide variety and variability of agricultural practices or soil typologies cannot be adequately captured and described at the plot scale over large areas due to the lack of suitable satellite data (Bégué *et al.*, 2018).

Once a suite of indicators has been selected, there is a critical need for the standardisation of sampling and handling procedures in the field, as well as harmonised field and laboratory methods and protocols (Stott, 2019).

The integrated nature of One Health approaches leads to mixed data collection methodologies resulting in complex data structures with numerous and interconnected variables (Lebov *et al.*, 2017). Researchers therefore need to choose appropriate analytical methods that enable an adequate interpretation of these study data (Lebov *et al.*, 2017).

3.3. Step 3: Data analyses and results interpretation

Due to the quantity of data collected in systemic studies, some authors propose to aggregate data around scores, which may be translated into cropping practices scores (Craheix *et al.*, 2016), soil health (or quality) scores (or indexes) (Rinot *et al.*, 2019; Thoumazeau *et al.*, 2019b), or nutrition scorings (or nutrient profiling) (Bionutrient Institute, 2020a; Nicklas, Drewnowski and O'Neil, 2014). Scores are appreciated by the non-expert population since they allow for results harmonisation and provision of general conclusions and recommendations. However, some authors argue that they may hide the complexity around data interconnections and interdependencies (Janzen, Janzen and Gregorich, 2021; Powlson, 2020). Also, specifically in soil health assessments, many scoring methodologies exist with diverging results leading to different and inconsistent conclusions on soil health status based on the same initial data (Chang *et al.*, 2022), showing the limits of diverging methodologies around scoring calculations.

A main limitation to scoring calculations stands in the need to set thresholds for all the indicators studied according to their trajectory: more is better, less is better, or optimum (Chang *et al.*, 2022). The choice of thresholds may raise issues, since most threshold values are site-dependent and may vary according to pedo-climates or crop varieties. The complexity to establish precise thresholds that fit all possible pedo-climatic situations, leads to an absence of harmonised framework although

international efforts and discussions are ongoing toward their establishment to specifically assess soil health (European Environment Agency, 2023).

In response to the difficulty of setting thresholds, visual analysis such as colour coding or graphical representation of standalone or grouped indicators may be more helpful than building and interpreting complex indices (Toor *et al.*, 2021). Applying statistical methods such as profiling or clustering (possibly associated with other more complex analyses) allows to avoid the threshold issues while providing detailed information on the variable behaviour and the direct and indirect links between variables. However, these results are more difficult to scale up since they cannot provide absolute references. Other types of multivariate analysis, such as structural equation models (SEM), look well suited to simultaneously examine multiple outcomes (Wade *et al.*, 2022). Eventually, emerging data analysis tools such as machine learning techniques (*e.g.* multiple linear models, random forest, artificial neural networks), deep learning, or game theory are promising to quantify the effect of soil health on food production (Lehmann *et al.*, 2020; Wang *et al.*, 2024).

Eventually, valorisation and diffusion of results on the four compartments treated together are likely to facilitate acculturation to the need and benefits of a One Health systemic approach (step 4).

4. Conclusions and perspectives

The insights provided in this article target the cropping system but could be extrapolated to other production systems requiring to be studied using systemic approaches. We expect that setting these bases for such innovative studies will support researchers in implementing more systemic studies. These will have the potential to better understand the effects of sustainable cropping system management on the soil-plant-production nexus and foster the adoption of innovative and more sustainable cropping practices. With all the current advances in research, where installations and analytic capabilities have reached an unprecedented level of precision and accuracy, tools are no longer a limiting factor for implementing systemic studies with a One Health perspective. Unravelling the links between compartments of cropping systems will make it possible to identify influencing factors that could not be seen in traditional approaches, providing additional elements to foster the food system shift to more virtuous systems. Hence, it will make it possible to go deeper in understanding the cropping system and make accurate and relevant conclusions on the effects of crop production on One Health. It may reveal the potential of understudied or even unknown but more sustainable on-farm cropping systems and foster their adoption to scale up the sustainable transition of our food system (Garcia, Osburn and Jay-Russell, 2020).

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Conflicts of interest/Competing interests

The authors declare no competing interests.

Authors' contributions

C.L conceptualisation, investigation and writing of the original draft. O.H, J-P.S supervision, review and contribution to final draft. L.L. contribution to final draft. L.L. and J-P.S. funding acquisition.

This state-of the art section reminded us that the current global food system, while successful in producing food in quantity, poses significant threats to environmental and human health. We highlighted the need for <u>systemic approaches</u> to properly assess the effects of crop management on the crop production system, defined as the soil-plant-production chain, incorporating a One Health perspective to ensure human, animal and environment health. We reviewed the currently understood effects of cropping practices on the crop production system and the interconnections between its components and One Health, and showed that comprehensive assessments of these interconnections based on hard data were scarce. One likely reason may be that scientists often fail to overcome the limits to systemic studies. This section set the basis for a framework to support the application of proper systemic studies of the effects of cropping practices involving long-term studies, adapted indicators, and multi-stakeholders' involvement, in real conditions.

On this basis, the following chapters propose an application of the presented framework in real farm conditions.

CHAPTER 2: GENERAL MATERIALS AND METHODS

Keywords: Conservation agriculture, One Health, Food production, On-farm experimentation, Food quality

These general materials and methods were written in the form of an article submitted in <u>Data in Brief</u> journal. The article is entitled "Data from extensive monitoring of agricultural practices, soil health, and wheat grain production in 44 farms in Northwestern France from 2021 to 2023". The structure of this chapter follows the template requested by the Data in Brief journal. The full dataset can be accessed at <u>https://doi.org/10.18167/DVN1/SI026U</u>.

All indicators abbreviations included in the dataset are available in **Annexe 1** to facilitate the reading of the manuscript. Farmers' survey included in the dataset is available in **Annexe 2** of the manuscript.

1. Background

This data results from a two-year on-farm monitoring of 44 conservation agriculture (CA) and conventional (CONV) farms in Northwestern France (2021-2023). Our objective was to operationalise a One Health approach adapted to cropping practices, following the framework for One Health research adapted to cropping practices. To our knowledge, it is the first on-farm study proposing hard data on different compartments of the food production system, from practices to production. The data was collected on a farm and field scale. Data collection and analysis were organised through multi-stakeholder collaborations.

2. Data description

This article describes an extensive dataset of cropping practices, soil, plant and grain data collected on 44 farms between 2021 and 2023. On each farm, two fields were monitored, *i.e.* one in the growing campaign 2021-2022 and one in the growing campaign 2022-2023 (except for 6 farmers who provided the same field for two consecutive years and two other farmers who left the study in the second year). Growing campaigns 2021-2022 and 2022-2023 are written as "Year 1" and "Year 2," respectively, in the rest of the text to facilitate reading. In total, 86 winter wheat fields were monitored for one campaign over two consecutive years in Northwestern France (Figure 5). The 86 monitored fields included 43 fields conducted in CA and 43 fields conducted in CONV.

The dataset is made of one .xlsx file (*data_field.xlsx*) containing all the measured and computed variables separated into the different related compartments of the food system (column "Compartment") (*i.e.* Practice, soil, plant, grain, bread, performance) and associated sub-compartments (Column "Sub_Compartment") (*e.g.* grain: nutritional, technological, sanitary). The dataset has already been formatted and adapted for use on statistical software such as RStudio® (R Core Team, 2021). This dataset shows one variable per plot, referring, depending on the assessment method, to a unique measurement on a composite sample or to an averaged value of multiple replicates for on-field measured data. Two datasets are available for on-field measured data. The first (*dataABC.xlsx*) shows results for in-field measurements at each inner replicate. The second (*planthealth.xlsx*) presents the results of plant pest and disease assessments run in Year 2 on volunteer farmers' fields. Indicators presented in these two .xlsx files are averaged and named identically in the *data.field.xlsx* file and will be described only once in the rest of this article.

The three datasets are complemented by one metadata file (*metadata.xlsx*) providing supplementary information for each variable, such as a short description in English (*Description_EN*) and in French (*Description_FR*), the variable unit (*Unit*), the laboratory or institution in charge of the indicator measurement or computation (*Laboratory_Insitution*), the method of calculation or measurement (*Method_Protocol*), the indicator orientation (*i.e.* more the better, optimum, less the better) (*Orientation*) and the date of sampling or acquisition (*Date_of_sampling*). Each studied variable is classified according to its compartment (*Compartment*), *i.e.* "General", "Local_Condition", "Soil", "Plant", "Grain", "Bread", "Performance" and sub-compartment (*Sub-compartment*), *e.g.* for the compartment "Soil": "Biological", "Chemical", or "Physical". The description of indicators in the rest of this article follows this Compartment/Sub-compartment hierarchy. All the indicators and their description are available in **Annexe 1** of this manuscript.

The dataset also contains one .docx document (*Template_Farmers_survey_2023.docx*, also available in **Annexe 2** at the end of the manuscript) corresponding to the survey provided to farmers in Years 1

and 2 (in Year 1, the survey was filled with farmers through in-person interviews) to record their fiveyear historical cropping practices on their monitored field. The information deriving from this survey is noted as "Farmers" in the metadata file for the column *Laboratory_Institution* (also see **Annexe 1**).



Figure 5. Location of study plots in the 2021-2022 campaign (Year 1, yellow) and 2022-2023 campaign (Year 2, pink). Farmers taking part in the study were the same between study Years 1 and 2 (except for two farmers who withdrew from the study in Year 2). The study was carried out on winter wheat crops. Therefore, most farmers proposed two different plots between Years 1 and 2, since most of them did not grow wheat on the same plot in two consecutive years. However, six farmers who proposed the same plots in the two successive years, therefore grew wheat for two successive years. Grey lines correspond to the limits of the French administrative departments. To facilitate the analyses, study plots were clustered in four zones according to their geographic and pedoclimatic positions: Zone 1 = plots from Charente and south Vienne, Zone 2 = North Charente-Maritime, and south of Deux-Sèvres, Zone 3 = Indre-et-Loire and north and middle Vienne, Zone 4 = West of Deux-Sèvres and Maine-et-Loire

3. Experimental design, materials and methods

3.1. Experimental design

As each plot was of a different size (from 1 to 30 hectares) (*FieldSize*), zones of homogeneous size were defined on each plot to standardise studied zones throughout the experimental set up.

Each field was monitored for one campaign, as indicated in Figure 6. Data linked to the cropping system was collected each year during winter and cropping management data collection lasted during the whole campaign period, especially since some data concerned the harvested products (*e.g.* yield) and, therefore, was not yet available in winter. Soil data were obtained through three sessions of sampling (Figure 6, Table 1). A rectangular area of about one hectare was defined on each study plot, on which five points A, B, C, D, and E were laid out in a "W" pattern on the principal study zone (*Zone* = P) (Figure 7). In Year 2, volunteer farmers were proposed to leave a non-treated strip (control zone, *Zone* = C) of about 1 ha where they would not apply any fungicide or insecticide. Four inner replicates were set on that zone positioned in transect, *i.e.* T1, T2, T3 and T4. The C zone was used to monitor plant pests and diseases in non-treated conditions and allowed to compare plant health between non-treated and treated conditions in one field and between two non-treated zones of the same pair of farmers with contrasted cropping systems (CA and CONV). Each study zone (P, C) was positioned on

the most homogeneous possible areas of the plots, and plot edges were avoided to prevent possible edge effects (Figure 7). A buffer zone of about 3 m was considered between the C and the P zones.



Figure 6. Organisation of field monitoring for each monitored year.

"Survey" corresponds to the cropping practices data collection phase, "1" corresponds to the first sampling campaign in spring, "2" to the second sampling campaign in spring, and "3" to the third sampling campaign just before the harvest. "Aur" corresponds to the soil sampling by the Aurea laboratory, and "Grain" corresponds to the grain collection after harvests in summer.



Figure 7. On-field experimental design in 2022 and 2023 for soil, plant and wheat sampling.

SD_1, SD_2 and SD_3 refer to the three sampling dates, as described in Figure 6 and Table 1. No samplings were taken in the "buffer zones" to avoid any edge effects that could influence the results.

3.2. Description of indicators

The description of the dataset variables is organised in sections. Each section corresponds to the *Compartment* column, and each sub-section corresponds to the *Sub-compartment* column of the main dataset.

3.3. General

3.3.1. Identification

Plots were arranged in pairs of neighbouring plots (*Pair*), a pair was defined as two plots of neighbouring farmers with contrasted cropping systems (*Type*), *i.e.* conventional agriculture (*Type* = CONV) and conservation agriculture (*Type* = CA) for at least five years. The pairs of farmers were selected according to specific criteria on pedo-climatic conditions and management practices defined at the beginning of the study (more information on the detailed methodology is provided in **Chapter 3**). Since the monitoring was carried out over two years, *Year* indicated the corresponding study campaign for each studied plot. *Zone* differentiated the principal study zone (Zone=P), where farmers' practices were monitored without any request to adapt treatments or operations, and the control zone (Zone=C), where in Year 2, twenty-four farmers accepted to leave a non-treated strip allowing to monitor plant health under non-treated conditions (see **Case study n°1**).

3.3.2. Dates

As explained previously, as part of the plot monitoring, several sampling campaigns were carried out, and specific data was collected at each sampling campaign, as described in Table 1. Wheat seeding date (*date_seeding*) and harvesting date (*date_harvest*) were also recorded for the two years of study. Since it is difficult to make calculations with date formats on software such as RStudio[®], these dates were converted into a number of days to enable their inclusion into calculations and models. *d.samp1* represented the number of days between seeding in 2021 or 2022 and the first spring sampling in 2022 or 2023, *d.samp2* was the number of days between seeding in 2021 or 2022 and the second spring sampling in 2022 or 2023 and *d.samp3* was the number of days between seeding in 2021 or 2022 and the summer sampling in 2022 or 2023. *d.samp_aur* was the number of days between 1/01/2021 or 01/01/2022 and the seeding date in 2021 or 2022, while *d.harv* was the number of days between 1/01/2022 or 2023 and the harvest date in 2021 or 2022. The difference *d.harv-d.seed* led to *d.growth*, which was the number of days of wheat growth in 2022 or 2023.

3.3.3. Farm and field

Farms were classified according to their type (*Farmtype*) since some farms involved livestock or other animals' breeding (*Farmtype* = 1), and others did not involve livestock or other animals' breeding but only crops (*Farmtype* = 0). Field size (*FieldSize*) was also recorded, although the monitoring was conducted on a standardised square of one hectare in each field.

Table 1. Explanation of the different sampling campaigns and corresponding measured variables.

Indicator	Dates		Type of	
	Year 1	Year 2	sampling	corresponding collected data
Sampling 1 (SD_1)	5/04/22 to 15/04/22	17/04/23 to 28/04/23	Three inner replicates (A, B, C)	Soil data at 0-10 cm depth: Setting the lamina_baits in 2022 (<i>Lamina</i>) and litter bags in 2023 (<i>Litter_bags</i>), Visual Evaluation of Soil Structure (<i>VESS</i>), Soil water infiltration (<i>Beerkan</i>), soil aggregate stability (<i>Agg</i>), soil moisture (<i>Moist_s1</i>), temperature (<i>Temp_s1</i>), and water conductivity (<i>ECp_s1</i>). Only in 2023: bulk conductivity (<i>ECb_s1</i>) and permittivity (<i>Perm_s1</i>).
Sampling 2 (SD_2)	25/04/22 to 3/05/22	7/05/23 to 17/05/23	Three inner replicates (A, B, C)	Soil data at 0-10 cm depth: sampling for paramagnetism (<i>Paramag_LF</i> and <i>Paramag_Xld</i>) and enzymatic activities (<i>NAG</i> , <i>Beta_Glu</i> and <i>Phosphatase</i>) measurements, soil respiration with Biofunctool® method (<i>SituResp24</i> and <i>SituResp48</i>) (Thoumazeau <i>et al.</i> , 2017), soil moisture (<i>Moist_s2</i>), temperature (<i>Temp_s2</i>), water conductivity (<i>ECp_s2</i>). Only in 2023: soil and plant data: bulk conductivity (<i>ECb_s2</i>), permittivity (<i>Perm_s2</i>) and foliar pest and disease.
Aurea (SD_Aurea)	19/05/22 to 31/05/22	25/05/23 to 9/06/23	One composite sample across the "W"	All soil data analysed by Aurea Agrosciences laboratory at 0-20 cm depth.
Sampling 3 (SD_3)	16/06/22 to 7/07/22	23/06/23 to 7/07/23	Three inner replicates (A, B, C)	Soil data at 0-10 cm depth: removing lamina baits (in 2022) (<i>Lamina</i>) and litter bags (2023) (<i>Litter_bags</i>). Grain technological parameters: Wheat sampling for measurement of all grain technological parameters, plant growth, diseases visible on ears (<i>Fusarium</i> and eyespot) and experimental yield (<i>ExpYield_n</i>).
Grain	1 to 15/08/22	1 to 15/08/23	One composite sample from P zone	Grain collection for measurement of parameters analysed by Phytocontrol laboratory, Valorex, James Hutton Institute, University of Pennsylvania and Moulins Girardeau.

3.4. Local conditions

3.4.1. Location

Longitude (*GPS_X*) and latitude (*GPS_Y*) corresponded to the GPS coordinates expressed in WGS84 at point A of the P zone (Figure 7). The variable was summarised in *Location* which specified the field administrative department (*i.e.* 16: Charente, 17: Charente-Maritime, 37: Indre-et-Loire, 49: Maine-et-Loire, 79: Deux-Sèvres and 86: Vienne) and their geographic position in the administrative department (south, north, east, west). These geographic zones were subsequently clustered into four zones corresponding to similar pedo-climatic basins in the analyses, as follows: (1) Charente and south of Vienne departments, (2) north of Charente-Maritime and south of Deux-Sèvres, (3) Indre-et-Loire and north/middle Vienne, and (4) West of Deux-Sèvres and east/west of Maine-et-Loire (Figure 5).

3.4.2. Soil texture

Soil texture was measured at the laboratory (https://aurea.eu/) according to the protocol NF X31-107. Texture was measured with decarbonation (*Clay, Silt, Sand*) in 2022 and 2023. Likewise, CaCO₃ concentration (*CaCO3*) was analysed following the protocol NF ISO 10 693. Dry matter (*DM*) was measured following the ISO 11465:1993 protocol. Texture with no decarbonation was analysed in 2023 only (*Clay_no_decarb, Silt_no_decarb, Sand_no_decarb*), as well as coarse elements (*Coarse_elements*). Soil texture with decarbonation was also expressed in the USDA referential (*Texture_USDA*). An attempt to measure soil bulk density was made but failed, since most soils were either too hard because of spring drought in 2022, or with too many coarse elements to allow for a correct sampling.

3.4.3. Semi-natural habitats

We considered three types of semi-natural habitats, *i.e.* hedges, forests, and water streams. *Hedge* corresponds to the presence (*Hedge* = 1) or absence (*Hedge* = 0) of a hedge at a 200-meter distance from the P zone. *SNH* is a score from 0 to 3 defined as follows:

- 3 points if forest (as identified by the Corine Land Cover 2021) and/or watercourse less than 200 m from the P zone,
- 2 points if forest AND watercourse are more than 200 m from the study area but less than 1 km from the P zone,
- 1 point if forest OR watercourse is more than 200 m from the study area but less than 1 km from the P zone,
- 0 if neither forest nor watercourse is within 1 km of the P zone.

Distance calculations were made using QGIS (QGIS Development Team, 2009) and the layers © IGN BD Ortho® 50cm 2021 edition. Forests were derived from the CORINE Land Cover 2021 version with 20 cm resolution and watercourses with the BCAE georeferenced watercourses 2021, which are watercourses concerned by the European regulation over the good agricultural and environmental conditions ("Règles des Bonnes conditions agricoles and environmentales" – BCAE).

3.5. Cropping practices

Information on management practices in each of the 86 selected fields was collected whether at the five-year historical management period and monitored campaign (called "rotation" in the rest of the document") or at the year scale, corresponding to the monitored year, *i.e.* Year 1 and Year 2. Data collection in year 1 was carried out through in-person interviews. In year 2 we provided farmers with a paperwork to fill, containing the same content asked in year 1 (survey file attached to the dataset and available in **Annexe 2**). Complementary information was obtained through personal communications with farmers using emails, phone or text messages. Cropping practices were classified into six sub-compartments and two indexes as follows:

3.5.1. Tillage

The tillage classification for the seeding at the year scale in year 1 or 2 (*Tillage_n*) corresponds to a score from 0 to 3 with:

- 3 if direct seeding,
- 2 if light tillage, *i.e.* up to three machinery interventions and none of them exceeded 10 centimetres depth,
- 1 if heavy tillage, *i.e.* more than three machinery interventions needed for seeding and/or one the tillage operations was more than 10 centimetres depth,
- 0 if ploughing

This tillage score from 0 to 3 was also calculated for the five previous years of field management history and the studied year, leading to a score going up to 18 at the rotation scale (*Tillage_intensity_rot*), a score of 18 meaning that all crops were implanted through direct seeding, and 0 meaning that all crops were implanted after ploughing. The variable *LastPlough* corresponds to the number of years without ploughing on the monitored field. The reference year is 2021 for the plots monitored years 1 and 2022 for plots monitored in year 2. *System_age* derives from *LastPlough* as follows:

- "Very old" if the last ploughing was done more than 20 years before the monitored year,
- "Old" if the last ploughing was done 10 to 20 years before the monitored year,
- "Recent" if the last ploughing was done between 4 and 10 years before the monitored year,
- "Very recent" if the last ploughing was done in the four years preceding the monitored year.

3.5.2. Pesticide use

For each of the two monitored campaigns, we recorded the number of applied fungicides, herbicides, insecticides and molluscicides (*nbFungi_n*, *nbHerbi_n*, *nbIns_n*, *nbMoll_n*) as well as the number of applications of a specific fungicide family called succinate dehydrogenase inhibitors (*nbSDHI_n*). The frequency treatment index for herbicides (*TFI_h_n*) and insecticides, herbicides, and molluscicides taken together (*TFI_eh_n*) was calculated based on the information provided by the French Ministry of Agriculture (<u>https://alim.agriculture.gouv.fr/ift/</u>). Pesticide use average consumptions were not calculated at the rotation scale.

3.5.3. Crop nutrition

Mineral N, K_2O , SO_3 and P_2O_5 fertilisation were recorded at the year and rotation scale (respectively *minN_n* and *minN_rot*, K_2O_n and K_2O_rot , SO_3_n and SO_3_rot , $P_2O_5_n$ and $P_2O_5_rot$). At the rotation scale, we calculated the average yearly mineral fertilisation. Average yearly organic fertilisation was recorded at the year scale for N (*ON_rot*) and C (*C_entries_rot*). These organic fertilisation inputs were

estimated using the SIMEOS-AMG model (<u>https://simeos-amg.org/</u>) and corresponded to the quantity of C and N returned to soil through organic matter additions, crop roots, and crops returned to soils (cover crops or crop residues). The cumulated number of legumes cropped at the rotation scale was also recorded (*nbLeg_rot*).

3.5.4. Crop variety

Three indicators of different complexity were used to describe wheat varieties used by farmers. First, *Var_mix* equalled to 0 if a unique variety was grown on the field and 1 if a mix of varieties was grown. Second, *nbVar* gives the precise number, when available, of varieties contained in the mixes when *Var_mix* equalled to 1. Third, *Wheatvar* provides farmers' used names of varieties when available. Some farmers produced their own wheat mixes from year to year and were unable to provide specific information on the available varieties in their mix.

3.5.5. Crop diversification

The preceding crop to monitored wheat was recorded (*Prec_crop_n*). In the rest of the analyses, previous crops were clustered into different groups, i.e.: (1) Spring Cereal including buckwheat, grain maize, grain sorghum, seed maize and silage maize, (2) Legume including alfalfa, lentil, meslin dominated by legumes and pea, (3) Winter Cereal including winter barley and winter wheat (4) Oilseed including oilseed flax, rapeseed, rapeseed + legumes in co-culture and sunflower. The number of crops grown at the rotation scale including cover crops and lays, was calculated (*CropDiv_rot*). We also counted the number of years before the previous wheat crop (*Time_return_wheat*). When the period was bigger than five years, it was noted as "6" in the database.

3.5.6. Soil cover

We recorded the number of intercrops seeded at the rotation scale (*nbCC_rot*). Values ranged from 0 (no intercrop seeded) to 5 (cover seeded at each intercrop period). The presence of volunteer oilseed rape in the intercropping period, in the case of no-tillage, was counted as a plant cover. In addition, we calculated the number of opportunities to implant a cover crop in the crop succession at the rotation scale (*OppCC_rot*). To be considered as an opportunity; there must be a period of eight weeks or more between the harvesting of one crop and the seeding of the next one. We considered no opportunity when the soil was permanently covered by a perennial crop (e.g. alfalfa), by a meadow, or by a catch crop. The ratio OppCC_rot: nbCC_rot provides information on the cover efficiency (*EffCC_rot*). The ratio ranges from 0 (of all the opportunities to plant a cover crop, none were seized) to 1 (of all the opportunities to plant a cover crop, all were seized). Finally, *RestitRes_n* corresponds to 1 if the residues of the previous crop have been returned to soil and 0 if the residues of the previous crop have been exported. The equivalent of *RestitRes_n* was computed at the rotation scale (RestitRes_rot) indicating the cumulated number of residue returns of the principal crops (cover crops were excluded) over the five-year historical management and the monitored years. RestitRes_rot therefore corresponds to a score ranging from 0 (residues were never returned at the rotation scale) to 6 (residues were systematically returned at the rotation scale).

3.5.7. Indexes

Two indexes were calculated at farm (*RI_farm*) and field (*RI_field*) scales. At the farm scale, an agronomic diagnosis called the "Regeneration Index" was run in each of the 44 selected farms from December 2021 to February 2022. RI farm scores were calculated for each farm based on data from the growing campaign 2019-2020, at the farm scale. *RI_farm* was measured through a one-to-one

interview with each farmer using the tool available on <u>https://agroecologie.org/indice-de-regeneration</u> in winter 2021 (Lugassy *et al.,* submitted). *RI_field* is an adaptation of the *RI_farm* score adapted to the year-year historical management at the field scale (also see **Annexe 3**).

3.6. Soil health

To ensure to avoid any experimenter bias, all the soil health in-field monitoring diseases and pests monitoring were performed by the same observer.

3.6.1. Physical properties

The Visual Evaluation of Soil Structure (VESS), Aggregate stability in water (Agg) and water infiltration (Beerkan) tests available in the Biofunctool[®] kit, as described in Thoumazeau *et al.* (2019a, 2019b), were measured in-field on three replicates at A, B, and C positions. We adapted the Beerkan protocol as follows: the measurement time was limited to a maximum of 30 min instead of 40 min as indicated in the original protocol. In other words, if the ten water bottles were not all poured after 30 min of measurement, the test was interrupted. An adaptation of "VESS" calculation score was also performed to shift the orientation of results from an optimum to a "more the better" response curve. Also, we set a different optimum value of VESS scores compared with the one proposed in (Thoumazeau *et al.*, 2019b) (*scoreVESS*). Indeed, in our case, soil horizons were rated from 0.5 (very friable) to 5 (very compacted), instead of 1 to 5 in the original protocol (Guimarães, Ball and Tormena, 2011). We set the optimum value as the interval (1.2-1.9]. New adapted VESS scores ranged from 0 to 4 as follows (*scoreVESS*):

- scoreVESS = 4 if the initial rating was in the interval (1.2,1.9],
- scoreVESS = 3 if the initial rating was in the interval [0.5,1.2] or (1.9,2.6],
- scoreVESS =2 if the initial rating was in the interval (2.6,3.3],
- scoreVESS =1 if the initial rating was in the interval (3.3,4],
- scoreVESS =0 if the initial rating was in the interval (4,4.7]

Soil moisture (*Moist_s1* and *Moist_s2*) and temperature (*Temp_s1* and *Temp_s2*) were measured twice in spring, during the two sampling sessions using a WET Sensor kit (Photo 1). Specifically for these indicators, three sub-replicates were measured at each A, B, and C point at 0-10 cm depth and averaged to obtain one value per position.

A crusting index (*CIndex*) was calculated according to Rémy and Marin-Laflèche (1974) based on the following formula :

$$CIndex = \frac{1.5 \text{ x fine silt } + 0.75 \text{ x coarse silt}}{\% \text{clay } + 10 \text{ x \% OM}} - C$$

if pH < 7, C= 0; if pH > 7, C= -0.2 (pH - 7)

Since the crusting index was calculated based on texture data measured by Aurea, only one value per plot based on composite soil samples was available.



Photo 1. WET sensor on a CA plot, after VESS measurement. WET sensors measurements were done on the non-disturbed part of the VESS measurement square. Note the visible darker soil colour in the superficial horizon. © Clara Lefèvre, April 2023.

3.6.2. Chemical properties

Soil total and bioavailable elements were analysed at the laboratory (<u>https://aurea.eu/</u>) on a composite soil sample taken at 0-20 cm depth: Nitrogen (N, *s.TN*), Magnesium (Mg, *s.TMg* and *s.AMg*), Potassium (K₂O, only in 2023: *s.TK2O* and *s.AK2O*), Sodium (Na₂O, *s.TNa2O* and *s.Ana2O*), Zinc (Zn, *s.TZn* and *s.AZn*), Manganese (Mn, *s.TMn* and *s.AMn*), Iron (Fe, *s.TFe* and *s.AFe*), Boron (B, *s.TB* and *s.AB*), Sulphur (S, *s.TS* and *s.ASO4*), Molybdenum (Mo, *s.TMo* and *s.AMo*) and Copper (Cu, *s.TCu* and *s.ACu*). Associated extraction protocols are given in Table 2. Ratios of bioavailable: total elements were calculated (*s.ratioMg*, *s.ratioK2O* -2023 only-, *s.ratioNa*, *s.ratioZn*, *s.ratioMn*, *s.ratioFe*, *s.ratioB*, *s.ratioS*, *s.ratioOu*, allowing to understand the proportion of bioavailable element as part of the total.

Organic Carbon (OC) and Organic matter (OM) concentrations were determined by dry combustion after NF ISO 10 694. C/N was then calculated as the ratio between *OC:s.TN* (*C_N*). Soil water pH (*pH*) was measured according to NF ISO 1039. Metson Cation exchange capacity (*CEC*) was measured after NF X 31-130. It involved an exchange by percolation of cations (or bases) fixed to the soil with neutral ammonium acetate at pH 7. The solution was rinsed with alcohol, and then fixed ammonium (NH₄⁺) was measured on soil. The quantity of ammonium absorbed or fixed was then determined.

Biologically mineralisable nitrogen (BMN) was measured based on the incubation of a raw soil sample sieved to 2 mm under controlled conditions. Samples were completely immersed in water (anaerobic environment) and incubated at 40 °C for seven days. Since these anaerobic conditions block

nitrification (conversion of NH₄ to NO₃), only the conversion of organic nitrogen to ammoniacal nitrogen was monitored. The difference between ammonia levels at the start and end of incubation was used to calculate the BMN. Since there is no measurement standard, the laboratory based its method on the protocols developed by Waring and Bremner (1964) and subsequently adopted by Stanford and Smith (1976). BMN was expressed as a percentage of total N (*BMN_Ntot*) and in mg/kg of dry soil (*BMN_tot*).

The concentration of KMnO₄ carbon (also known as Permanganate Oxidisable Carbon - POXC) was analysed based on Weil *et al.* (2003) and Culman *et al.* (2012). After sampling, soils were dried at 38 °C and sieved to 2 mm and coldly oxidised by a potassium permanganate solution, causing the reagent to discolour. The decolourisation was measured using spectrophotocolourimetry. The result was expressed as a percentage of total OC (*s.POXC_OC*) or as mg/kg of dry matter at 38 °C (*s.TPOXC*).

Granulometric fractionation of OC (C_0_{50} , C_{200}_{2000} , C_{50}_{200} , C_{50}_{2000}), N ($N_0_{50}_{50}$, N_{200}_{2000} , N_{50}_{200} , N_{50}_{2000}) and C/N ($C_N_0_{50}$, $C_N_{200}_{2000}$, $C_N_{50}_{200}$, $C_{N}_{50}_{200}$, $C_{N}_{50}_{2000}$) in the 0-50, 50-200, 200-2000 and 50-2000 µm fractions were measured based on standard NF X31-516. Samples were dried at 38 °C and sieved to 2 mm underwater to separate the 0-50 µm, 50-200 µm and 200-2000 µm fractions. After drying and weighing, OC was measured in the 50-200 µm and 200-2000 µm fractions (by sulphochromic oxidation). These proportions of OC in the fractions were expressed as a percentage of total OC, the proportion of N, as a percentage of total N, and the C/N corresponded to the C/N of each of the soil fractions. For the 0-50 µm fraction, the results were obtained by difference with the total fraction.

Pore water conductivity (*ECp_s1* and *ECp_s2*), soil bulk electrical conductivity (*ECb_s1* and *ECb_s2*) and soil permittivity (*Perm_s1* and *Perm_s2*) at 0-10 cm depth were measured in-field on three sub-replicates in the two spring sampling sessions year 2 using the WET Sensor kit (Figure 7). Pore water conductivity refers to the electrical conductivity of the water within soil pores, while soil bulk electrical conductivity measures the overall ability of soil to conduct electricity, including pore water and soil solid particles. Permittivity reflects the quantity of electrical energy that can be stored in soils (Corwin and Lesch, 2005; Robinson *et al.*, 2003)

Measurements of paramagnetism in low frequency (*Paramag_LF*) and high frequency (not shown in the dataset) were realised on year 1 samples taken at 0-10 cm depth, at CIRAD research Centre (Montpellier) on dry soil sieved at 2 mm. Measurement was run using a Barrington MS3 device associated with an MS2B sensor, measuring at two frequencies (465 Hz and 4.65 kHz) in 10 ml containers with very low magnetic susceptibility (<10⁻⁸). Measurement was brought down to the mass of the sample (Mass Magnetic Susceptibility, m³/kg). *Paramag_Xld* derives from these two measurements according to the following formula:

XId (%) = (LF-HF)/LF.

Although included in the database, these data were measured with an exploratory goal and analyses of paramagnetism data are not included in the rest of the manuscript.

Table 2. Extraction protocols for available soil nutrients.Based on the information provided by Aurea Laboratory.

Available element	Protocol			
	Standard NF X31-122: 25 g fine earth was mixed with a 50 mL calcium chloride			
B, Mo	solution and boiled for 5 min. The mixture was homogenised and filtered, then			
	measurement was done by plasma spectrometry (ICP).			
K ₂ O, Mg, Na ₂ O	Spectrometry according to NF X 31-108.			
	NF X 31-120: 5 g fine earth was mixed with 50 mL EDTA extraction solution. The			
Cu, Fe Mn, Zn	mixture was shaken for 2 hours and centrifuged. Measurement was then made			
	by ICP.			
	NF ISO 13 878: Ground soil was burned in the presence of oxygen in an oven at			
N	950 °C. After the combustion gases were purified, the N produced was			
	determined by scatterometry.			
D	NF ISO 11 263: Extraction was carried out using a 0.5 M sodium bicarbonate			
r	solution.			
S	Aqueous sulphur (SO ₄ ²⁻): 1/5 extraction and IPC dosage.			

3.6.3. Biological properties

Biological properties were measured whether on-field or by the certified "Auréa Agrosciences" laboratory.

As part of the Biofunctool® kit, lamina baits (Laminas) were analysed in year 1. Lamina baits were settled during the first spring sampling campaign and removed during the second spring campaign. In total, they stayed in-field for 21 days on average. The indicator was replaced in year 2 by Litter bag analyses (Litter_bags). Litter bags were handmade using organic cotton (cellulose 100%) squares of 10*8 cm, previously dried at 70 °C for 48 hours. Each cotton square was weighed before insertion in small PVC cages of 5 mm mesh. Litter bags were inserted in soils with two replicates for each of the three sampling positions (A, B, C) on the first spring sampling session and removed in the summer sampling session. Overall, they were incubated on average for 70 days (Figure 8). After soil removal, litter bags were stored in a cool place and frozen after arrival at the Toulouse laboratory at -18 °C. Cotton squares were then removed from the cages, slightly cleaned with water, and dried at 90 °C for 48 hours. Cotton squares were then weighed. Litter bags values corresponded to the difference in weight before – and after incubation, expressed in grams. Soil basal respiration at 0-10 cm was also measured in-field after 24 hours of incubation (SituResp24) after the protocol developed by Thoumazeau et al. (2017). Since the SituResp[®] protocol was originally developed in tropical countries and our measurements were performed in a temperate climate and in spring, we also measured SituResp after 48 hours of incubation (SituResp48), to ensure a colour difference in gels. All gels were prepared at the laboratory in Toulouse a few days before the field campaign and were stored at ambient temperature in a hermetic box filled with soda lime until their use.



Figure 8. Fabrication process for litter bags analysis.

Litter bags were made by cutting cotton squares of 10*8 cm, dried at 70° C for 48h, weighed and inserted in small PVC cages of 5 mm mesh. They were ten incubated in soils at 10 cm depth at the first sampling session and removed about 70 days later in the summer sampling session, just before harvest. They were then stored at 4°C to stop degradation, cleaned with water, and dried in the oven at 90° C for 48 h before weighing.

For enzymatic activities, soil samples were taken at a 0-10 cm depth during the second spring sampling session of years 1 and 2 (Table 1). Once taken, samples were immediately placed in a cool place. Samples taken in year 1 were stored in a freezer at -20 °C from the end of the sampling campaign in early May 2022, and the samples taken in year 2 were stored in a cold room at 4 °C on their return to the laboratory in mid-May 2023. Activity analysis of N-acetylglucosaminidase (*NAG*), β -glucosidase (*Beta_Glu*), and phosphatase (*Phosphatase*) was carried out in Toulouse CNRS from June 1 to July 11, 2023, according to the protocol proposed by Jassey *et al.* (2011, 2012).

The other biological soil properties were measured by Aurea laboratory after soil sampling at 0-20 cm depth.

Carbon mineralisation after 3, 7, 14, 21 and 28 days of incubation was measured based on standard NF EN ISO 16072. Soil samples were incubated at 28 °C, at optimum humidity (80% of field capacity humidity), for 28 days in the dark. Soil was incubated in a closed container in a flask containing a sodium hydroxide solution. The CO₂ produced during incubation was absorbed into this solution. The quantity of C-CO₂ produced was measured by UV spectrometry. The cumulative amount of C-CO₂ released on each measurement date was used to calculate the carbon mineralised over 28 days, expressed in mg of C-CO₂/kg of dry soil (minC_3d_OC, minC_7d_OC, minC_14d_OC, minC_21d_OC, minC_28d_OC) or as a percentage of total OC (minC_3d, minC_7d, minC_14d, minC_21d, minC_28d). N mineralisation after 0, 7, 14, 21 and 28 days of incubation was measured following ISO 14238 (2012). Similarly, soil samples were incubated at 28°C, at optimum humidity (80% of field capacity humidity), for a period of 28 days in the dark. Five sub-samples of the same soil were incubated in pots (for the five extraction dates, the analysis was destructive). After incubation, mineral N was extracted by shaking in a KCl solution and then measured by continuous flow colourimetry. Mineralised N includes nitric N (N-NO₃) and ammoniacal N (N-NH₄). The difference between the mineral N measured after 28 days of incubation and that measured at the start of incubation constituted the quantity of potentially mineralisable N. The result was expressed as mg of mineral N (N-NH₄ + N-NO₃)/kg of dry soil ($minN_7d$, *minN_14d, minN_21d, minN_28d*) or as a percentage of total N (*minN_7d_Ntot, minN_14d_Ntot, minN_21d_Ntot, minN_28d_Ntot*). The database does not include N mineralisation at 0 days since minN_0d = 0.

Total DNA was extracted according to standard NF EN ISO 11063 (2020), adapted by Terrat *et al.* (2015). On receipt at the laboratory, soil samples were sieved fresh to 2 mm and then air-dried. Microbial DNA was extracted from 1 g of dried soil and quantified by agarose gel electrophoresis. The molecular microbial biomass (*MMB*) was then estimated from this quantity of DNA. *MMB* is expressed in μ g of DNA/g of soil. Similarly, Total Microbial Carbon (*TMC*) was analysed based on standard NF ISO 14240-2. Soils were sieved to 2 mm; then, a sub-sample was brought into contact with chloroform vapour (fumigation) to lyse the microbial cells leading to C dissolution. Dissolved C was extracted using a K₂SO₄ solution and then measured by UV spectrometry. The difference with the C extracted from another non-fumigated sub-sample was used to calculate *TMC*, expressed in mg of C per kg of dry soil (*TMC*) or as a percentage of the total OC concentration (*TMC_OC*).

The abundance of bacteria (*AbundBact*) and fungi (*AbundFungi*) was measured following the protocol NF EN ISO 16072, similar to Djemiel *et al.* (2023). Total DNA was extracted in the same way as for measuring *MMB*. The DNA was then purified to eliminate pollutants. A specific DNA sequence was then amplified by qPCR (Polymerase Chain reaction). This PCR made it possible to determine the initial quantity of targeted DNA (16S rDNA for bacteria and 18S rDNA for fungi) from the DNA produced during PCR amplification. Bacteria and fungi abundance was expressed in copy numbers. The abundance ratio of fungi and bacteria was also calculated (*FBRatio*). Similarly, the diversity of bacteria (*DivBact*) and fungi (*DivFungi*) were measured. After extraction and purification of the total DNA, the 16S and 18S specific DNA sequences were amplified by PCR. The amplicons obtained were sequenced using a massive sequencing technique. This sequencing resulted in several tens of thousands of targeted gene sequences. Data was processed by bioinformatics to filter, sort, classify, group, and link sequences from databases. Results are expressed as a number of Operational Taxonomic Units (OTUs).

<u>3.7. Plant health</u>

3.7.1. Pest and disease

Plant disease and pests were monitored in Year 2 on twenty-four plots in the P and C zones. For each monitored disease or pest attack, their frequency of occurrence on leaves or ear were counted, and for each occurrence of disease or pest attacks, the disease or pest intensity of damage was assessed. To ensure to avoid any experimenter bias, all the plant disease and pest monitoring were performed by the same observer. Diseases analyses included *Septoria tritici* blotch, Yellow (stripe) rust and leaf brown rust, powdery mildew, eyespot, and *Fusarium* spp.. Pest analyses included slugs, leaf beetles and leaf miners attacks.

In each zone, twenty plants were randomly selected (Figure 9). Only the three youngest fully developed leaves (L1, L2 and L3) were observed for foliar diseases, and ears were observed when diseases were observable at plant maturity. On each plant organ (L1, L2, L3 and ear), the presence of disease or pest attack was counted and summed to obtain a disease frequency at the zone scale. A frequency of 60 for a given foliar disease or pest attack indicated that all leaves were affected, while a frequency of 0 meant that no leave was affected (*fqSept*, *fqMil*, *fqIminers*). Brown and yellow rust were gathered and defined as "Rust", since on-field, it was not always simple to distinguish the difference between a leave affected by brown or yellow rust (*fqRust*). Likewise, *slug plus leaf beetle damage* were gathered, since

their damage on leaves were hardly distinguishable (*fqslugs_lbeetles*). For ear-observable diseases, a frequency of 20 meant that all ears were affected by disease (*fqFusa*, *fqEyespot*). The total frequency of diseases counted was summarised in *fqTotDis*, while the total frequency of damage was summarised in *fqPests*.

Eyespot and *Fusarium* damage intensity were not recorded, since when an ear was affected, we considered it was automatically affected with an intensity of 100%. For each affected leave the intensity of damage (by diseases: *Septoria*, powdery mildew, rust, or pests: slugs and leaf beetles and leaf miners) was recorded as indicated in Figure 9 (*intSept*, *intMil*, *intRust*, *intslugs_lbeetles*, *intlminers*). The average intensity of disease (*intAvDis*) or pests (*intPests*) per zone was calculated considering *Septoria*, powdery mildew and rust for diseases, and slugs/leaf beetles and leaf miners for pests.

3.7.2. Growth

The average wheat height just before harvest was measured on three positions (A, B, C) of the P zone with three sub-repetitions at each location (*Height*).



Figure 9. In-field protocol for plant pest and disease analysis adapted from (Grudé, 2023).

<u>3.8. Grain quality</u>

3.8.1. Nutritional and sanitary quality

Farmers were asked to keep aside 5 and 10 kg of grain wheat from the P zone at harvest. Grains were collected in early August 2022 (for year 1 monitored plots) as well as in August 2023 (for year 2 monitored plots) and stored at ambient temperature before analyses a few weeks later in 2022 and in 2023.

A first series of analyses was conducted in a certified laboratory (<u>https://www.phytocontrol.com/</u>). Total N concentration in grain (*g.TN*) was measured following NF EN ISO 16634-1 by combustion according to the Dumas principle. As for soils, Fe, Mn and Zn concentrations (*g.Fe, g.Mn* and *g.Zn*) were determined by inductively coupled plasma mass spectrometry (ICP-MS) adapted from EN NF 15763. K and P concentrations (*g.K* and *g.P*) were measured by ICP-MS following a COFRAC (French Committee for Certification) certified method. Eventually, vitamin B9 (*B9*) was determined by High-Performance Liquid Chromatography (HPLC) and immunoaffinity column, with a limit of quantification of 10 µg/kg.

The Valorex company (https://www.valorex.com/) analysed the contents of starch (*Starch*), cellulose (*Cellulose*), and protein (*Prot*) using Near-Infrared spectroscopy (MPA II, Brucker device). Valorex also measured antioxidants (*Antiox*) and polyphenols (*Polyph*) concentrations in year 1 while the James Hutton Institute (Scotland) measured *Antiox* and *Polyph* in year 2. Both partners used the same FRAP (Ferric Reducing Antioxidant Power Assay) methodology for antioxidant analyses and the Folin-Ciocalteu (FC) method for polyphenol analyses as described in Bionutrient Institute (2020).

The concentration of the amino acid Ergothionein (*Ergo*) was measured at the University of Pennsylvania according to the protocol described in Beelman *et al.* (2022) using a Sciex 4000 Q Trap mass spectrometer coupled with a Waters ACQUITY UPLC separation system.

Sanitary quality indicators were also analysed by the certified laboratory *Phytocontrol* (https://www.phytocontrol.com/). Three types of mycotoxins were analysed: (1) Deoxinivalenol (*DON*), (2) HT2 (*HT2*), and (3) Zearalenone (*ZEA*). All were analysed by liquid chromatography coupled with a mass spectrometer (LC-MS/MS) following a COFRAC method, with a limit of quantification of 50 µg/kg. In addition, residues of glyphosate and Aminomethylphosphonic Acid (AMPA) in grain were analysed by LC-MS/MS, using a QuEChERS method (*i.e.* "quick, easy, cheap, effective, rugged, and safe") which corresponds to solid phase extraction method for detection of biocide residues in food. These pesticide residue analyses were performed on forty samples corresponding to grain samples of farmers who used glyphosate just before the wheat growth campaign (year 1 or 2). We did not analyse glyphosate residues in grains of farmers who did not apply glyphosate in the year preceding the monitored campaigns.

3.8.2. Yield and technological quality

During the summer sampling of years 1 and 2, at wheat physiological maturity, ear samples were taken from points A, B, and C on each P zone. Three replicates per point were taken on each plot using the ring method. Rings with a diameter of 50 cm were laid out randomly around each sampling point, and the ears whose stems originated in the ring were cut and placed in kraft bags (Photo 2). Ears were then dried in a greenhouse for at least a week after collection. Once dried, the bags of ears were stripped, and the number of ears per bag was counted (*Grain_ear_n*). Ears were then threshed using a fixedstation thresher (model LD 350, Wintersteiger), and the threshed grains were again dried at 70 °C for 48 hours to ensure a homogeneous and minimal moisture content. Once dried, grain samples were weighed to obtain the value of experimental yield (*ExpYield_n*). Then, from each sample, 500 grains were taken and counted with a grain counter (Numigral model) and weighed to determine the thousand kernel grain (*TKW*). Samples from the same sampling point were then pooled to determine the specific weight (*ExpSW_n*). SW was measured using a Dickey-John GAC500 XT instrument. Each SW measurement was repeated three times for the same sample and averaged to obtain a value per plot. Farmers also provided the SW value obtained after harvest (*FarmerSW_n*) as well as the yield obtained for their whole plot (both values obtained either from their combine harvester or by the grain collector) (*FarmerYield_n*).



Photo 2. Ear sampling with ring methodology in June 2023. ©Clara Lefèvre

3.9. Flour and bread

Bread-making tests were run on a sub-sample of plots (10 plots in year 1 and 10 in year 2) on the wheat harvested by farmers in summer 2022 and 2023. Grain was stored at ambient temperature, and bread-making tests were run in early fall 2022 and 2023 by the flour mill "Moulins Girardeau" (https://www.minoterie-girardeau.com/en/about/). Grains were milled on a test mill with steel grinding wheels. A subsample of flour was incinerated at 900 °C for 1:30 hours and the quantity of ashes was measured (*f.Ashes*). With the remaining flour, the mill yield (*MillYield*) and Hagberg falling time were measured (*f.Hagberg*), as well as the percentage of flour hydration (*f.Hydration*). Breadmaking tests were then run and several variables were measured on dough, such as the elasticity index (*b.IE*), the toughness:extensibility ratio (*b.P_L*) and the baking force (*b.W*). Finally, bread technological

parameters were measured, *i.e.* its length (*b.Length*), volume (*b.Volume*), and the baking score (*Baking_Score*). All analyses were run following the BIPEA³ criteria and protocols.

3.10. Field socio-economic and environmental performances

The ratio between *minN_n* and *ExpYield_n* was calculated, providing information on the efficiency of mineral N (*minN_eff_n*).

Field socio-economic and environmental performances were computed using Systerre[®] methodology. Systerre[®] is a performance assessment tool developed by the French Agricultural Institute "Arvalis-Institut du végétal" which calculates scientifically-based performance indicators of cropping systems from an exhaustive description of their cultivation practices including machinery and input use and outputs including grain yield and biomass production (Iocola *et al.*, 2020; Soulé *et al.*, 2023; Viguier *et al.*, 2021; Weber, Jouy and Angevin, 2019). Different assumptions were made to perform the model:

- The machinery pool is the same for every farmer (*e.g.* one same no-till drill for CONV farmers, one same combined seed drill for CONV farmers, etc.),
- All farmers bought their inputs at the same price,
- All farmers sold their wheat at the same price,
- Plots were not irrigated

These enabled us to monitor only the effects of practices on farm performances, without taking into account the farmer's economic strategy. Amongst Systerre[®] outputs, we considered six specific indicators that provide information on:

- (i) Field economic performance, with:
- the production cost (*ProductionCost*) to produce 1 ton of winter wheat in each P zone accounting for inputs and mechanisation costs,
- the input expenses (*InputExpenses*), *i.e.* the ratio between *ProductionCost* and yield (*farmerYield_n*), and
- The semi-net margin (*SNMargin*) is calculated as follows:

Semi_net margin = gross income - operating costs - mechanisation costs

With gross income = Yield* Selling price * harvested area (1 ha) And Operating expenses = Input expenses * Quantities supplied (inputs = fertilisers, seeds, crop protection products, etc.) * area treated (1 ha)

- (ii) Field social performance:
- working time (*WorkingTime*) was calculated as the work rate on the plot in hours per ha, only taking into account in-field spent time, thus not including time spent on administrative management, crop observation, etc.

³ BIPEA is a European NPO ISO 9001 certified by the Lloyd's Register Quality Assurance. It provides proficiency testing programs and reference materials for laboratories concerned with control and quality. Their services cover different fields: cereals, grains, feed, food, beverages, air, waters, soils and cosmetics. It is ISO/IEC 17043 accredited by COFRAC for the organisation of proficiency testing programs. Also see: https://www.bipea.org/milling/

- (iii) environmental performance:
- Gas consumption by machinery (*GasConsumption*) during field operations. Additional consumption (maintenance, etc.) is not included. And,
- greenhouse gases total emissions (GHGtotEmiss) in CO₂eq/ha accounting for all emissions from fuel, inputs, from fabrication to use (calculated after life cycle assessment).

4. Limitations

We noticed a high intraplot variability between the three replicates for in-field assessed data. In addition, on unchanged plots from year 1 to year 2, we noticed an interannual unexpected variability for some soil data supposed to be stable over time (*e.g.* soil texture, VESS), highlighting the high intraplot intrinsic variability. Although more replicates and a larger size of sampled farms would likely have supported better variability management, a compromise had to be found with time, workforce, and financial resources to ensure the study feasibility (these items are also discussed later on in the manuscript).

Finally, data on cropping practices refers to information provided by farmers. The reliability and accuracy of this data are based on the trust relationship between our research team and farmers (also see **Annexe 5, section 3**).

Ethics statement

The authors have read and followed the <u>ethical requirements</u> for publication in *Data in Brief* and confirm that the current work does not involve human subjects or animal experiments. The authors confirm that data provided by farmers are shared after informed consent is obtained from the study participants and after anonymisation of all participant data.

CRediT AUTHOR STATEMENT

Clara Lefèvre: Conceptualisation, Methodology, Software, Formal analysis, Writing – Original draft, Editing. Olivier Husson: Supervision, Writing – Review & Editing, Validation. Léa Lugassy: Funding acquisition, Supervision. Jean-Pierre Sarthou: Supervision, Writing – Review & Editing, Validation

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

This chapter presented data measured in 44 farms covering a range of cropping practices, soil, and production parameters under contrasted types of crop management systems: conventional and conservation agriculture. Eighty-six winter wheat fields in Northwestern France were monitored for two growing seasons (2021–2023). The dataset encompasses data about cropping practices (tillage, soil cover, rotation, pesticide use, nutrition), soils (chemical, biological, and physical parameters, including texture), and grain production (nutritional, technological, and sanitary indicators). This is to our knowledge one of the first application of a systemic on-farm study of the food production system, aiming to adopt a "One Health" perspective of the crop production system. These data and associated results are discussed in the following chapters of the manuscript.

CHAPTER 3: SETTING UP A ROBUST ON-FARM MONITORING NETWORK

<u>Keywords</u>: Cropping systems, conservation agriculture, conventional agriculture, on-farm experiment

This chapter is presented as Part A of an article ready for submission to the European Journal of Agronomy.

The detailed characterisation of cropping systems is an essential and necessary step to reach robust conclusions on their effects on the rest of the production chain and specifically the soil-production continuum. On-farm experimentation (OFE) has increasingly been recognised as a relevant means to assess the diversity of cropping practices as farmers implement them in real life. In this article, we provide a framework for the rigorous implementation of OFEs to allow the comparison of two contrasted cropping systems: Conservation agriculture (CA) and Conventional agriculture (CONV). We provide a detailed characterisation of cropping practices allowing us to go beyond the simple dichotomy between systems. Finally, we provide feedback on setting up and maintaining an OFE. This article is the first two-part article and will be completed by assessing practices' effects on the soil-grain production continuum from a One Health perspective.

1. Introduction

Conservation Agriculture (CA) is increasingly recognised as a promising alternative to "Conventional" agriculture (CONV) as it provides a good compromise between the promotion of soil health and the need to maintain productivity (Derpsch *et al.*, 2024). However, results on the effects of CA practices on soil and production vary inconsistently (Bajgai, Lal and Lorenz, 2024). These inconsistent results might derive from two main issues. First, the imprecise description of CA and CONV cropping systems, which in reality embrace an extensive range of practices (Derpsch *et al.*, 2014; Fairweather *et al.*, 2009; Sumberg and Giller, 2022), and second, the difficulties in reflecting farm conditions in current studies. Indeed, the large majority of studies looking at management effects on soil properties use small plot trials, which tend to be oversimplified in terms of system complexity (Bajgai, Lal and Lorenz, 2024).

To overcome these two issues, calls are being made to perform more systemic studies of cropping systems, to accurately describe farming practices, and avoid any confusion in the conclusions made on the effects of farming practices on different compartments of production. Recent efforts have been made to work on improved frameworks to allow for better identification of the diversity of CA (Ferdinand and Baret, 2024; Ruiz-Espinosa *et al.*, 2024), CONV, or other so-called "alternative" systems (Büchi *et al.*, 2019).

Analysis methods to describe cropping practices are already well-known. The use of multivariate analysis such as Principal Component Analysis (PCA) is widely used to describe agricultural practices (Armengot *et al.*, 2011; Büchi *et al.*, 2019; Nkurunziza *et al.*, 2017; Yvoz *et al.*, 2020). In addition to PCA, typologies are widely used to discriminate farming systems (*e.g.* Akakpo *et al.* (2021); Alignier, Carof and Aviron (2024); Alvarez *et al.* (2018); Mutyasira (2020); Renaud-Gentié, Burgos and Benoît (2014); Yvoz *et al.* (2020)). Categorisation methods show several advantages since they allow to describe the diversity of the combination of practices. They can also detect contrasts in management and representativity of the management by farmers and allow to select groups and compare them in analyses, going beyond denominations (Ferdinand and Baret, 2024).

In parallel, on-farm studies have been identified as more suitable and representative for assessing farming systems' effects on soils (Wood and Bowman, 2021). The interest in On-Farm Experiments (OFE) has therefore been growing in recent years (Giannini and Marraccini, 2023; Lacoste *et al.*, 2022; Toffolini and Jeuffroy, 2022, 2022), since OFE seem to be appropriate to evidence and highlight the diversity of cropping practices that may exist (Lacoste *et al.*, 2022). However, OFE are difficult to implement due to increased confounding factors, and poor recognition by institutional research, which hamper recognising these research results (Giannini and Marraccini, 2023). Researchers may also be reluctant to work on OFE since they are seen as time-consuming and resource-demanding (financial, workforce) (Giannini and Marraccini, 2023; Pagliarino *et al.*, 2020).

To date, few OFE have been set up in France, allowing to make fine comparisons of cropping systems (Alletto, Vandewalle and Debaeke, 2022; Chabert, Marchand and Sarthou, 2020). Generally, few research articles clearly describe their OFE setting and methodology.

Here we present results from an OFE study conducted in France on two consecutive campaigns (2021-2022 and 2022-2023) on winter wheat fields belonging to two cropping systems, if we follow a dichotomic separation, *i.e.* CA and CONV systems. Winter wheat was chosen as it is the most widely cultivated crop in France and Europe. Following Lacoste *et al.* (2022) framework on OFE, we gathered information on cropping practices at both field and farm scales on 86 fields. Based on these data, thirty-

two indicators were computed to cover the main cropping systems' characteristics. In this chapter, we investigate the robustness of a newly established OFE network and propose a fine analysis of its farmers' cropping management practices. We also provide a methodological framework and feedback on the setting up of an OFE, to foster the wide development of OFE studies by the research community.

2. Materials and methods

Our study followed the criteria of a scientist-driven OFE and complied with the framework of application proposed by Lacoste et al. (2022) (Figure 10). Forty-four farms (one field per farm) distributed over the French West oceanic coast were selected for this on-farm study conducted from 2021 to 2023. A wide range of farm sizes was covered (80-800 ha). On these farms, we studied two cropping systems, i.e. conservation agriculture (CA) and conventional systems (CONV), with twentytwo farms selected for each. The CA group consisted of farmers who did not implement any soil tillage except occasional use of strip-till, applied maximum soil cover (residues or living soil cover) and increased crop diversification, according to the definition of CA provided by the Food and Agriculture Organisation of the United Nations (FAO, 2014). The CONV farms were composed of farmers who implemented tillage on a regular basis and most of them did not use cover cropping in intercrop periods. In total, we monitored forty-four fields cultivated with winter wheat on the agricultural campaign 2021-2022 (hereafter called "Year 1") and forty-two fields cultivated with winter wheat on the agricultural campaign 2022-2023 ("Year 2"). Additional details on the methodology and map of farms' locations were presented in Chapter 2. In line with Lacoste et al. (2022), the study gathered knowledge and participation from different stakeholders, *i.e.* farmers, industrials, agriculture chambers and research. Regular meetings were organised between all the stakeholders to present results and get feedback on the results obtained (Figure 10).



Figure 10. The On-Farm Experimentation (OFE) process was adapted to our study following Lacoste et al. (2022). Steps included in the circles were repeated twice for the 2 successive years of data acquisition.

2.1. Farms and fields selection

2.1.1. Study zone

The study was conducted in two administrative regions of Northwestern France (Pays de la Loire and Nouvelle-Aquitaine) of similar climatic conditions, both classified as Cfb in the Köppen classification (Mild temperate, fully humid with warm summers). The study was performed during wheat growing campaigns 2021-2022 ("Year 1") and 2022-2023 ("Year 2"), which were two extreme years in terms of climate. 2022 was marked by a severe drought, with mean precipitations averaging 500 mm, while 2023 precipitations were almost 20% higher than normal values with mean precipitations averaging 870 mm. Both years were warmer than normal with mean temperatures averaging 14°C for both years (DRAAF Nouvelle-Aquitaine, 2023, 2024). The study zone is mainly flat. The studied farms were mainly situated on loam (28 plots), silt loams (24 plots) and silty clay soils (14 plots). Other represented soils were clay (7 plots), clay loam (5 plots), sandy clay loam (4 plots), sandy loams (3 plots) and one silty clay loam plot (1 plot) according to the USDA classification (Table S1). Slightly more than two thirds of plots (65%) were located on non-calcareous soils (<10% CaCO₃), and more than a third of them (35%) were situated on calcareous soils, with concentrations going up to 70% CaCO₃ (more details are provided in Chapter 4). This was representative of the diversity of soil types in the region (DREAL Nouvelle-Aquitaine, 2016). Winter wheat was the main crop grown in the study zone (DRAAF Nouvelle-Aquitaine, 2023, 2024).

2.1.2. Farm selection

The selection was completed in two main steps. First, in autumn 2021, we selected twenty-two farms conducted in CA. We selected farmers who had practised CA for at least five years in order to monitor already-in-place systems and avoid any transitioning CA system (Derrouch et al., 2020; Ferdinand and Baret, 2024). We considered farmers who followed the CA criteria such as defined in the UN FAO definition of CA (FAO, 2014), *i.e.* (i) Minimal soil tillage or absence of soil tillage, (ii) Crop diversification in space and/or time (rotation), and (iii) Permanent soil covering through introduction of cover cropping or mulching with crop harvest residues. CA farmers were selected based on local experts' knowledge, including the support of local Agricultural Chambers. A call was also sent and re-shared on social networks in September 2021 (Facebook and Instagram). A first verification of CA practices was conducted through telephone interviews. Most CA farmers were members of APAD ("Association for the promotion of sustainable agriculture", non-profit organisation - NPO), as well as part of farmers' innovation networks called "Groupes 30 000". These networks are an initiative from the French Ministry of Agriculture launched in 2021 whose goal is to support farmers in the agroecological transition based on chemical input diminution through the constitution of multi-actor groups (French Ministry of Agriculture and Food Sovereignty, 2021). Of the twenty-two CA farmers selected, eight were part of the 30 000 group "Agrosol" (Vienne Agricultural Chamber, 2021) and four were part of the 30 000 group "Sol Vivant" ("Living soil") (Deux-Sèvres Agricultural Chamber, 2024), both supported by the Agricultural Chambers of the Vienne and Deux-Sèvres departments respectively. Once selected, each CA farmer was requested to select one CONV neighbouring farm according to five requested criteria:

- <u>Geographic proximity</u>: the CONV farm had to be geographically close to the CA farm (less than 10 km maximum),
- 2. <u>Similar pedoclimatic conditions</u>: the CONV farm had to have similar pedoclimatic conditions if not equal to the CA farm (this condition was verified afterwards with soil analyses),
- 3. <u>Same winter wheat variety</u>: the neighbour farmers had to accept to grow the same wheat variety or mix for two consecutive campaigns (varieties may be changed from one year to another but they may always remain the same within a pair of farmers),
- 4. <u>Soil tillage:</u> the CONV farmers must practice regular deep tillage (more than 10 cm deep) on their field and if possible have ploughed their selected field at a minimum of two years before,
- 5. <u>2-year commitment</u>: the neighbouring farmers commit to take part in the study and share their information for the two consecutive years of the study.

Second, once the pairs of farmers were formed and to finalise farm selection, in winter 2021, we ran in all preselected farms an agronomic diagnosis, the "Regeneration Index" (RI) (Lugassy *et al.*, submitted in *Sustainable Futures*). The RI is an online tool (<u>https://agricultureduvivant.org/leviersdaction/lindice-de-regeneration/</u>) developed by the French NPO "Pour une Agriculture Du Vivant" together with its Scientific Committee⁴. The RI is now used by thousands of farmers, cooperatives, and industries in France to structure agroecological value chains and finance the transition. This index was chosen since it is available in open source for research purposes, it is ergonomic and rapid to implement. It aims at providing systemic assessments of farmers' cropping systems performances based on seven axes which are: (1) Soil cover, (2) Soil tillage, (3) Carbon cycle, (4) Nitrogen fertilisation, (5) Pesticide use, (6) Biodiversity and trees, (7) Capacity building and knowledge sharing. This separation into different compartments (whose interactions are considered) of the cropping system provides a systemic vision of cropping practices that go beyond CA pillars. This agronomic diagnosis at the farm scale provides a farm agronomic performance score over 100.

2.1.3. Fields selection

Fields were chosen together with farmers after their selection for the first year of study according to the previously described criteria. For the second year, since most farmers did not grow wheat for two consecutive years, fields were changed. Their selection started from spring 2022 onwards. To monitor interannual variations in the same field, we asked some farmers to grow two consecutive wheats on the same field to repeat all analyses for two consecutive years. Six farmers (*i.e.* three pairs of farmers: 1, 2 and 10) used the same plot in the two monitoring campaigns. In addition, one pair of farmers was unable to propose two fields fitting the requirements, so these farmers dropped the study in year 2 (pair n°9). Therefore, forty-four plots were monitored in year 1 and forty-two in year 2. General information on the pairs of neighbouring farms is shown in Table S1, including fields' location, wheat-grown varieties, soil texture and previous crops. Each selected farmer received a financial retribution of 300 € per year and per hectare.

⁴ https://agricultureduvivant.org/le-mouvement/le-conseil-scientifique/
2.2. Cropping practices indicators

2.2.1. Indicators selection and construction of the minimum dataset

The RI farm was measured only once, based on farmers' data from the growing campaign 2019-2020. Additional indicators at the field scale were needed to provide a fine description of farmers' cropping practices at the field scale.

Figure 11 describes the methodology used to define the set of studied indicators. Firstly, a quick literature review was performed to define all the possible indicators to be included in a systemic assessment of cropping management (Table S2). To this end, only papers dealing with annual cropping systems in temperate or Mediterranean regions, and when possible, specifically cereals, were selected, since management may differ according to the type of crop grown. Secondly, from the set of reviewed indicators, we selected the easiest to obtain or to calculate at the field scale when the indicators were presented at the farm scale. Supplementary indicators were added to check for any influence of confounding factors in the cropping practice, such as farm location, previous wheat crop, or soil texture. In total, the literature review evidenced 74 possible cropping practice indicators either because their scale did not fit our study or to facilitate their calculation, or when the use of a more synthetic indicator was possible. In total, 51 indicators were selected (Table S3). All the collected data were gathered into six thematic subcategories of cropping practices complemented by a subcategory on farm and field characteristics, respectively:

- 1. Soil tillage,
- 2. Soil cover,
- 3. Pesticide use,
- 4. Crop nutrition including mineral fertilisation and organic fertilisation,
- 5. Crop diversification,
- 6. Biodiversity

All collected data were organised in a Microsoft Excel[®] spreadsheet and analysed using R Statistical Software (v4.1.2; R Core Team, 2021).

To reduce the size of the dataset, Spearman correlations were computed using the {Hmisc} R package (Harrell Jr and Harrell Jr, 2019). When significant correlations of 0.8 or more occurred, one of the two correlated indicators was removed from the dataset (Figure 12, Table S3). In addition, since our objective was to provide a fine analysis of cropping practices, we set as a supplementary indicator in the rest of the analysis any indicator that was not a proper cropping practice, such as the presence of semi-natural habitats, and all indicators calculated at year scale, to favour those indicating an average value at the rotation scale in the principal analysis. We made an exception when we had no available information at the rotation scale (*e.g.* for pesticide use), where yearly data were kept as such. Concerning pesticide use, we set additional indicators that provided most minor discriminant information (*e.g. nbHerbi_n, nbFungi_n*) as supplementary variables for pesticide use. Information on wheat varieties and previous crops to the wheat monitored, were set as supplementary indicators in the analysis since farmers were asked within a pair of CA/CONV to grow the same wheat variety, and to have similar previous crops. Therefore, these did not reflect single farmers' choices. The RI was included as a supplementary variable to assess the relevance of keeping a farm performance index in a detailed field assessment of cropping practices. We chose not to exclude these indicators, since they

may provide additional insight into different cropping practices. Finally, based on the selected indicators, a tentative to adapt the RI farm to the field scale was made, but the RI field was not kept in the rest of the analysis since it was highly correlated with RI farms. All details around the calculation of RI field are shown in **Annexe 3**⁵.

Subsequently, the minimum dataset was composed of 17 core indicators complemented by 24 supplementary variables (Table 1, Table S3).



Figure 11. Data selection and analysis methodology.

⁵ We advise readers to consult this Annexe after reading this chapter, since it points out elements that have not yet been discussed at this stage of the chapter.



Figure 12. Correlation matrix between the different preselected indicators.

The table shows only significant correlations. This correlation matrix shows only the correlation between quantitative variables (45 indicators on the total 50 indicators that include five qualitative variables, i.e. cropping system type, farm location, soil texture, wheat variety, and previous crop to monitored wheat).

2.2.2. General characteristics

Each plot was identified according to its pair of neighbouring farms (*Pair*), their type of cropping system which may be CA or CONV (*Type*) and the year in which the plot was monitored (*Year*). The plots' location corresponds to their position in the administrative department (*Location*). For each year, we noted the date of wheat seeding and harvest (*d.seed* and *d.harv*) and the number of days of wheat growth (*d.growth*). We provide information on the type and size of each farm (*Farmtype, FarmSize*), and the size of the field studied (*FieldSize*). The grown wheat varieties were recorded yearly (*Wheatvar* and *Var_mix*) although it did not reflect the farmers' own crop management choices, since farmers were asked to share the same wheat variety with their paired farmer. Dates of wheat seeding and harvest were also indicated as the number of days between the 1st January of the harvesting year and wheat harvest (*d.harv*). The RI score (*RI_farm*) also served as a general descriptor of each farm.

In addition, composite soil samples were collected in spring 2022 and spring 2023 at 0-20 cm depth by a certified laboratory (Auréa Agrosciences, <u>https://aurea.eu/</u>), and soil 5-fractions granulometry was analysed after decarbonation. Texture was then determined according to the USDA referential of soil textures (*Texture_USDA*). For fields that had not changed between year 1 and year 2 (pair n°1, 2 and 10), two farmers (1_CA, 2_CONV) were concerned by soil texture changes from year 1 to year 2 (Table S1). For these two farmers, the sampling points slightly changed between year 1 and year 2 although we remained in the same fields. Indeed for 1_CA, the original sampling points were positioned in a zone affected by resistant rye-grass weeds. In year 2, pair n°1 had one textural class difference, which did not hinder their comparison and inclusion in our analyses. For 2_CONV, the field was heterogenous, and the texture of 2_CONV did not match the texture of 2_CA in year 1 (they had two textural classes difference), therefore moving to another spot of the field allowed to make textures more alike in year 2, reducing to one textural class difference. Texture measurement protocols are precisely described in **Chapter 2**, while more detailed analyses of soil textures are presented in **Chapter 4** and **Annexe 4**.

2.2.3. Cropping practices

2.2.3.1. CA pillars

2.2.3.1.1. Tillage

Similar to Ferdinand and Baret (2024), we defined "tillage" as any mechanical operation that fragments the soil, and "ploughing" as a mechanical operation that inverts the soil horizons. In a slightly different approach than Ruiz-Espinosa et al. (2024), we separated light tillage from heavy tillage considering that a 0 to 10 cm tillage with strictly less than 3 machinery operations for seeding was "light" tillage. Heavy tillage corresponded in at least one operation of more than 10 cm depth or more than three machinery operations for seeding. Decompaction was not considered a tillage operation. This separation was made in consistency with the RI farm indicator of tillage intensity (see Table S2 and Annexe 3 for detailed RI farm indicators) and Ruiz-Espinosa et al., (2024). For each year in the five-year rotation and the year studied, the seeding method was rated from 0 to 3. Each year, a score of 3 was given when the principal crop was implanted with direct seeding, 2 when the implantation was light tillage, 1 when the implantation was made with heavy tillage and 0 when it was made after ploughing. Each yearly score was summed for the five-year historical management and the monitored year, leading to a maximum score of 18 (*Tillage_intensity_rot*). The score of the year studied was noted as *Tillage_n*. We also counted the number of years since the last ploughing, which can give an idea of the age of the system for CA farmers (LastPlough) (Chabert, Marchand and Sarthou, 2020; Chabert and Sarthou, 2017). The total number of machinery operations (for seeding, fertilisation, weeding or crop protection) was also noted for the year monitored only (*nbOperations_n*).

2.2.3.1.2. Soil cover

Similar to Büchi *et al.* (2019) and Dupla *et al.* (2022) who considered the number of times residues were exported over five years, we counted the number of times residues of the principal crops (excluding cover crops) were returned at the rotation scale (*i.e.* five-year historical management and the monitored year) (*RestitRes_rot*), and at the year scale, focusing only on winter wheat residues management *RestitRes_n*). For *RestitRes_rot*, a score of 6 indicated that all residues of principal crops were returned, while a score of 0 indicated that crop residues of main crops were never returned to the soil. However, focusing solely on crop residues is insufficient, since soil cover can also be achieved using living plant ground cover. The most precise approach to quantifying soil cover lies in calculating the percentage of soil cover on a crop sequence over one or several years (Ruiz-Espinosa *et al.*, 2024),

but this may be challenging to implement. To facilitate calculations, Ferdinand and Baret (2024) proposed calculating the number of days covered by living or dead mulch. However, in our case, this was also complicated since farmers did not always remember the specific coverage dates when going back into the past years of rotation. Also, such calculations (percentage of coverage or number of days covered) would have discriminated failures in growing cover crops, since farmers may seed cover crops. However, due to lack of water (especially in summer), cover crops may fail to grow correctly, leading to a limited soil coverage. Hence, like Büchi *et al.* (2019), we calculated the efficiency of soil cover as the ratio between the number of cover crops (CC) inserted during the interculture period (*nbCC_rot*) and the number of opportunities to insert a cover crop. Rapeseed volunteers were considered as an opportunity taken to insert a cover only when there was no-tillage after rapeseed harvest. We considered that there was no opportunity to insert a cover crop. The ratio ranges from 0 (of all the opportunities to plant a cover crop, none were taken) to 1 (of all the opportunities to plant a cover crop, all were taken) (*EffCC_rot*).

2.2.3.1.3. Diversification

The number of different crop species and legume occurrences as cash or cover crops, was counted at the rotation scale (*CropDiv_rot, nbLeg_rot*). Similar to the cereal ratio calculated by Armengot *et al.* (2011), we counted the number of years between the wheat monitored and the last wheat cropped of the rotation (*Time_return_wheat*). Since we focused on the five-year past management, when no wheat had been cultivated in that timeframe, we indicated six years as the maximum return time for wheat.

2.2.3.2. Beyond CA practices

2.2.3.2.1. Pesticide use

The treatment frequency indexes for each monitored year, were calculated for total herbicides (*TFIh_n*) and total fungicides, insecticides and molluscicides, excluding herbicides (*TFIeh_n*). Seed treatments were not included since farmers used the same wheat variety within a pair of farmers and thus seed treatments did not represent farmers' choices. TFI were calculated based on the French calculator of TFI developed by the French Ministry of Agriculture (<u>https://alim.agriculture.gouv.fr/ift/</u>). For one cropping campaign, the TFI count started from the harvest of the preceding main crop until the harvest of the wheat studied, meaning that cover crop or residue chemical destruction when applicable are included in the TFI calculations. In addition, we counted the number of different herbicides, fungicides, insecticides used based on the commercial treatment name (*nbHerbi_n*, *nbFungi_n*, *nbIns_n*, *nbMoll_n*) for the campaign studied. We also specifically considered the number of succinate dehydrogenase inhibitors (SDHI) used (*nbSDHI_n*) which has to our knowledge never been investigated in any systemic study before, although an increasing number of publications evidence worrying consequences on human, animal and environment health (Bénit *et al.*, 2019).

2.2.3.2.2. Crop nutrition, including mineral and organic fertilisation

Like most similar studies, average yearly mineral fertilisation was calculated for N, P, K elements at the rotation and year scale (*minN_rot, minN_n, minP2O5_rot, minP2O5_n, minK2O_rot, minK2O_n*) (Table S3). We also considered mineral sulphur fertilisation at both rotation and year scales (*minSO3_rot, minSO3_n*), since sulphur fertilisation may have implications for N use efficiency in wheat cultivation (Salvagiotti *et al.*, 2009; Tabak *et al.*, 2020). Regarding organic fertilisation, like in other studies we counted the number of organic inputs along the rotation (*nbOrginput_rot*) (Table S3). Average organic N and C inputs were estimated through restitutions by crops (aerial or root), cover crops and organic fertilisation based on the Simeos-AMG model (*C_entries_rot, orgN_rot*) (Agro-Transfert Ressources et Territoires, 2024). N, P and K inputs from organic amendments for the sole years monitored were also recorded (*orgN_n, orgP2O5_n, orgK2O_n*).

2.2.3.2.3. Biodiversity

Biodiversity and landscape indicators are mostly missing in systemic assessments of cropping practices. Indeed, these are not directly linked with farmers' practices although they might influence cropping practices (Chabert and Sarthou, 2017). Therefore, we chose to use easy-to-get and simple landscape indicators to test their possible links with practices. First, the presence of a hedge at less than 100 m of the studied zone was noted as 1, and the absence of a hedge was noted as 0 (*Hedge*). Also, each field studied was given a score for the presence of SNH, from 0 to 3 defined as follows: 3 points if there is a forest (as defined by the Corine Land Cover 2021) and/or a watercourse at less than 200 m from the study area, 2 points if a forest AND a watercourse are present at more than 200 m from the study area but less than 1 km from the study area, 1 point if a forest OR a watercourse is present at more than 200 m from the study area but less than 1 km of the study area (*SNH*). These calculations were made using QGis software (QGIS Development Team, 2009). Agroforestry was not included since none of the plots were located on agroforestry systems.

2.3. Cropping practices analysis

Due to the non-normality of the repartition of several variables, we ran Wilcoxon paired-rank tests on each pair of farmers to evidence differences in the network between CA and CONV farmers using the wilcox_test of the {rstatix} package (Kassambara, 2023). Then, a principal component analysis (PCA) was run using the minimum dataset and the associated supplementary variables. Missing values were imputed on the first four dimensions of the PCA. The PCA and imputation of the missing values were run using the PCA and imputePCA function of the {FactoMineR} package (Lê, Josse and Husson, 2008). When imputed data was leading to inconsistencies, data were imputed based on expert knowledge. Based on PCA results, a Hierarchical Ascendant Classification (HAC) was run on the first four dimensions of the PCA for more in-depth description of the management routes. The number of clusters was decided visually using the gain of extra-cluster variability for successive clustering and confirmed using a K-means clustering. Clustering and K-means consolidation were computed respectively using the HCPC functions of the {FactoMineR} package (Lê, Josse and Husson, 2008). Clusters were then described using the 17 principal components of the PCA and the 24 supplementary variables. Due to the non-normality of variables repartition in the clusters, the variation between clusters was quantified using the Kruskal Wallis test, with Dunn adjustment to allow for multiple comparisons (dunnTest function of the {FSA} package (Ogle et al., 2023)).

3. Results

3.1. Farmers' pre-selection and CA vs. CONV differences

Based on data from campaign 2019-2020, *RI_farm* was significantly higher in CA systems than in CONV systems (p=1.8E-8) (Figure 13A, Table 3). Mean *RI_farm* scores are 74/100 and 39/100 in CA and CONV systems respectively. Also, scores were more homogeneous in CA systems than in CONV systems, going from 64 to 86/100 in CA systems, and from 6 to 69/100 in CONV systems. Despite the score heterogeneity in CONV systems and the possible overlaps between CA and CONV scores when CONV systems reach high *RI_farm* scores, the difference in scores between the same pair of farmers remained always positive, going from 13 points to 64 points of difference (mean *RI_farm* difference = 35) (Figure 13B).



Figure 13. Results of farm pre-selection with the Regeneration Index at the farm scale. (A) Regeneration indexes at the farm scale according to the type of cropping system (B) Differences of Regeneration index scores between pairs of farmers.

3.2. Evidencing farmers' strategies through hierarchical clustering

The first four axes of the PCA explained 61.3% of the total variability (Figure 14A). The contribution of the indicators to the construction of the first four axes is presented in Table S4. The first axis explained 28.9% of the total variation. Variables that contributed the most to the first axis were those related to tillage interventions, efficiency of soil cover, and diversification of the rotation, meaning that the first axis explained 16% of the total variability. Variables that contributed the most to the second axis were those related to pesticide use and crop nutrition (specifically mineral SO₃ and N), therefore rather differentiating farmers with higher reliance on synthetic pesticides and mineral N-S fertilisation. The third axis explained 8.9% of the total variability and mostly differentiated farmers with little return time of wheat in the rotation, as well as farmers that provided the most organic inputs along the rotation. These were well correlated with the type of farm (*Farmtype*, correlated at 38% to the third dimension). Lastly, the fourth axis explained 5.9% of the total variability and mostly differentiated at mostly differentiated

farmers with the greatest crop diversity from those with the lowest rate of residue restitution across the crop rotation (Table S4).

Four clusters were evidenced after the HAC and K-means partitioning, corresponding to four different cropping strategies (Figure 14C). Each cluster gathered from 17 to 26 fields. Individual farmers were found in one (N=34) or two (N=8) cropping practice clusters (Table 3).

Cluster 1 was only composed of CONV farmers. Cluster 2 was mainly composed of CONV farmers while it included three CA farmers (7_CA_2023, 2_CA_2022 and 21_CA_2022). Cluster 3 was mainly composed of CA farmers while it included four CONV farmers (3_CONV_2023, 6_CONV_2023, 9_CONV_2022 and 12_CONV_2023). Cluster 4 was only composed of CA farmers (Table 3). When a CONV plot was found in a CA cluster (*i.e.* cluster 3 or 4), the corresponding paired CA plot was always found in a CA cluster, and *vice-versa*, when a CA plot was found in a CONV plot, the paired CONV plot was always in a CONV cluster (1 or 2).

Farm location, soil texture, previous crop to studied campaign, were spread homogeneously amongst clusters (Figure S1). Monitored year was also spread homogeneously amongst clusters (mean year in all clusters = 2022.5). Farm types (*Farmtype*) and sizes (*FarmSize*), and wheat harvesting dates (*d.harv*) did not significantly differ between CA/CONV groups, nor between clusters. Field sizes of CA farmers were significantly higher than those of CONV farmers (p=0.005). Specifically, fields of cluster 4 farmers were bigger than fields of cluster 1 farmers (p=0.01). Wheat seeding dates (*d.seed*) differed between CA and CONV groups. CONV wheat seeding was on average three days after CA wheat seeding (p=0.03). However, no difference of wheat seeding dates was highlighted between clusters. As highlighted in the previous section, RI scores were significantly higher in CA than in CONV. Specifically, RI scores were higher in clusters 3 and 4 than in clusters 1 and 2 (p= 1.6E-8 between cluster 1 and 3, p=5.9E-7 between cluster 2 and 3, p=1.6E-6 between cluster 1 and 4, p=4.9E-5 between cluster 2 and 4).

75% of farmers remained in the same cluster between year 1 and year 2 (Figure S2). Five CONV and six CA farmers changed cluster between year 1 and year 2. Two out of the five CONV farmers who changed category between the two years shifted from cluster 1 to cluster 2, *i.e.* remained in a "CONV" cluster. Two other farmers shifted either from cluster 2 to 3 (two farmers) or from cluster 1 to cluster 3 (one farmer). Four CA farmers shifted from cluster 3 to cluster 4 between years 1 and 2, therefore remaining in a CA cluster. One out of these four farmers was part of the group of six farmers who kept the same field between the years 1 and 2. Two other CA farmers shifted from cluster 2, therefore downshifted to a CONV cluster. Amongst these two, one farmer used the same plot for year 1 and year 2. Finally, on the six plots that were unchanged between year 1 and year 2, only these two CA farmers changed cluster, the other four remained in the same cluster anyway



Figure 14. Graphs of variables and of individuals according to Type or after clustering. (A) Variables repartition on the first two dimensions of the PCA. (B) Individuals repartition coloured according to their Type (CA or CONV). (C) Representation of the four clusters, showing different management strategies adopted by farmers after Hierarchical Clustering Analysis (n = 86 fields) on the first four dimensions of the PCA.

3.3. Contrasted farmers' practices based on CA pillars

All the results presented in this sub-section are displayed in Table 3.

3.3.1. Tillage

Average tillage scores (Tillage_intensity_rot) at the rotation scale were higher in CA than in CONV systems (on average 16.7/18 in CA and 4.8/18 in CONV, p=1.10E-8). The highest Tillage intensity rot scores were found in cluster 3 and 4 compared with cluster 2 (p=7.4E-8 between cluster 1 and 3, p=3.4E-7 between cluster 2 and 3, p=8.3E-8 between cluster 1 and 4 and p=4.3E-7 between cluster 2 and 4). We observed an identical pattern for tillage scores at the year scale (*Tillage_n*), with *Tillage_n* scores higher in CA than in CONV (p=2.2E-08), and scores of cluster 3 and 4 significantly higher than scores of cluster 1 and 2 (p=1.4E-7 between cluster 1 and 3, p=2.3E-7 between cluster 2 and 3, p=8.8E-8 between cluster 1 and 4 and p=1.8E-7 between cluster 2 and 4). These results were confirmed by the average number of years since last ploughing (lastPlough), which was significantly different between CA and CONV (p=2.8E-8). On average in the network, CA farmers stopped ploughing their plot 13.4 years before monitoring, while last ploughing of CONV plots was done 1.7 years before the monitoring, despite a high variability in the CONV group (coefficient of variability -CV- of 139.9%), indicating that some CONV farmers had not ploughed for a longer time. Indeed, in the CONV group, eleven plots had been ploughed more than three years before the wheat campaign (from 3 to 9 years). However, all these eleven plots had a *Tillage_intensity_rot* score lower than 10/18, indicating that at the rotation scale, these farmers applied regular heavy tillage. One CONV farmer (9_CONV_2022) had a high Tillage_intensity_rot score (9_CONV_2022, 17/18), explained by his rotation composed of five years of alfalfa cultivation before winter wheat cropping. LastPlough values of cluster 3 and 4 were significantly higher than those of cluster 1 and 2 (p=3.6E-6 between cluster 1 and 3, p=4.1E-6 between cluster 2 and 3, p=8.8E-8 between cluster 1 and 4 and p=2.2E-7 between cluster 2 and 4). The number of in-field operations (*nbOperations_n*) was higher in CONV than in CA (on average 11.5 in CONV and 10.3 in CA, p=0.007). However, the only differences between clusters were those between cluster 2 and 3 (p=6.7E-6) and cluster 3 and 4 (p=4.2E-3). Indeed, *nbOperations_n* was significantly higher in cluster 2 (12.5 operations on average) than in cluster 3 (9.1 operations on average), and significantly higher in cluster 4 (11.4 operations on average) than in cluster 3.

3.3.2. Soil cover

The efficiency of soil cover with cover cropping (*EffCC_rot*) was significantly higher in CA compared with CONV (80% in CA and 20% in CONV on average, p=6.7E-7). Clusters 3 and 4 plots had a significantly higher mean *EffCC_rot* of 80% compared with cluster 1 and 2 (10 to 20% on average), with p=1.6E-8 between cluster 1 and 3, p=5.0E-6 between cluster 2 and 3, p=8.6E-7 between cluster 1 and 4 and p=1.5E-4 between cluster 2 and 4. At the rotation scale, no differences were observed between CA and CONV farmers in their residue restitution strategy (*RestitRes_rot*). In both CA and CONV systems, residues were returned to soil about 4 to 4.7 times over the six studied campaigns. When looking at the *RestitRes_rot* values repartition between clusters, we observed two different strategies in the CONV clusters (cluster 1 and 2), with a higher rate of residue return in cluster 2 (4.6 times on average) than cluster 1 (3.1 times on average, p=0.04). Two different strategies in CA systems were also observed with cluster 4 and *RestitRes_rot* = 4 in cluster 3, p=3.7E-3). We observed no difference of *RestitRes_rot* between cluster 1 and 3, cluster 2 and 3, and between cluster 2 and 4, but a significant difference between cluster 1 and 3, cluster 4 (p=1.5E-5).

3.3.3. Diversification

Rotations were twice more diversified in CA than in CONV ($CropDiv_rot = 9.4$ in CA and 4.6 in CONV, p=5.1E-8). $CropDiv_rot$ significantly differed between cluster 1 ($CropDiv_rot = 4.6$) and cluster 3 and 4 ($CropDiv_rot = 8.9$, p=1.4E-3 and $CropDiv_rot = 10.5$, p=1.7E-4 respectively), such as between cluster 2 ($CropDiv_rot = 4.2$) and cluster 3 and 4 (p=4.6E-6 and p=5.4E-7 respectively). Similarly, CA farmers grew significantly more legumes in the rotation ($nbLeg_rot$) than CONV farmers. On average $nbLeg_rot$ was 3.3 for CA vs. 1.1 in CONV (p=2.9E-6). Like $CropDiv_rot$, we observed no difference in $nbLeg_rot$ results between the CONV clusters (1 and 2) and no differences between the CA clusters (3 and 4), but differences between cluster 1 vs. cluster 3 and 4 (p=2.4E-6 and p=5.3E-4 respectively), and between cluster 2 compared with cluster 3 and 4 (p=4.5E-7 and p=3.0E-4 respectively). The return time of wheat ($Time_return_wheat$) and the number of wheat varieties grown in-field (Var_mix) did not significantly differ between CA and CONV systems, and neither between clusters indicating that winter wheat was likely to be grown every two to three years in the fields on average over the rotation.

3.4. Going beyond CA pillars

3.4.1. Pesticide use

The treatment Frequency Index for herbicides (*TFIh_n*) was significantly higher in CA than in CONV (on average 2.7 in CA and 1.9 in CONV, p=2.3E-4). However, we observed different patterns within the CONV clusters and the CA clusters. Indeed, *TFIh_n* was higher in cluster 2 compared with cluster 1 (on average 1.4 in cluster 1 and 2.2 in cluster 2, p=0.02), and higher in cluster 4 compared with cluster 3 (3.2 in cluster 4 and 2.2 in cluster 3, p=0.02). Also, *TFIh_n* of cluster 1 was significantly lower than in cluster 4 (p=5.6E-6). *TFIh_n* of cluster 1 vs. cluster 3 were not significantly different, such as *TFIh_n* of cluster 2 vs. cluster 3 and 4. CA used twice as many herbicide products (*nbHerbi_n*) between the harvest of the last principal crop and the harvest of wheat than CONV (on average 4.1 in CA vs. 2.6 in CONV, p=1.2E-4). Regarding the repartition of *nbHerbi_n* in the clusters, we observed no significant difference between cluster 1 and cluster 2, and no significant difference between cluster 3 and 4 (p=9.4E-3 and p=2.1E-6). There was no significant difference between cluster 2 and 4 (p=9.8E-2). Only one of the eighty-six monitored plots was concerned by an absence of herbicide use during the wheat campaign (18_CONV_2023).

TFI of fungicides, insecticides and molluscicides, therefore excluding herbicides (*TFleh_n*) was significantly lower in CA than in CONV systems (on average 2.1 in CONV vs. 1.3 in CA, p=2.3E-4). *TFleh_n* was significantly higher in cluster 2 than in cluster 3 (1.1 in cluster 3 vs. 2.3 in cluster 2, p=2.9E-4), but no additional difference was observed between clusters. We observed no difference between the number of insecticides (*nblns_n*) and molluscicides (*nbMoll_n*) used between CA and CONV systems. We observed no significant difference between clusters regarding insecticide use (*nblns_n*). We noted that globally over the 2 successive campaigns, insecticides were used by 56% of CA farmers, and 51% of CONV farmers. For *nbMoll_n*, we observed a difference between cluster 3 and 4 (p=0.04). None of the farmers in cluster 1 and cluster 3 used molluscicides. In total, eleven out of the eighty-six plots monitored received a molluscicide in fall (12.7% of plots), in which five were CONV plots (8_CONV_2022, 16_CONV in 2022 and 2023, 21_CA in 2022 and 2023 and 22_CA_2022). Six out of these eleven plots were found in cluster 2 and five in cluster 4, leading to high coefficient of variations in clusters 2 and 4 (cv = 181.6% in cluster 2 and 165.9% in cluster 4). Previous crops to plots concerned

by molluscicide applications were for the most rapeseed (six plots), pea (three plots), oilseed flax (one plot) and silage maize (one plot). In total, 21 farmers had rapeseed as previous crop, 6 farmers had pea, 3 had oilseed flax, and 7 had silage maize. In most cases, farmers using molluscicides used them both in year 1 and year 2 (16_CONV, 19_CONV, 19_CA and 21_CA). Others used them only in year 1 (8_CONV and 22_CA) or in year 2 (4_CA). The number of fungicides (*nbFungi_n*) used during wheat growth was significantly different between CA and CONV systems (on average 1.7 in CA vs. 2.9 in CONV, p=7.53E-5). The difference was not significant when looking at the specific SDHI fungicide type use (*nbSDHI_n*). Forty plots received SDHI fungicides, *i.e.* 46.5% of plots. Specifically, *nbFungi_n* was significantly lower in cluster 3 than in cluster 1 and 2 (1.7 in cluster 3, 2.8 in cluster 1 and 2.9 in cluster 2, p=0.03 and p=0.02 respectively), and the other clusters comparisons showed no differences. Five farmers did not use any fungicide during the campaign, all were CA farmers (1_CA_2022 and 2023, 5_CA_2022, and 11_CA_2023 in cluster 3, 8_CA_2022 in cluster 4 and 21_CA_2022 in cluster 2). Only one CA farmer managed to avoid the use of fungicides in the two years of study. This farmer used the same plot for the two successive years. On his plot, wheat was grown in co-culture with alfalfa as a perennial crop.

3.4.2. Crop nutrition

3.4.2.1. Mineral fertilisation

Mineral N, P, K fertilisation rates at the rotation scale were lower in CA than in CONV.

First, the average yearly mineral N fertilisation (*minN_rot*) at the rotation scale (5 years) was 115 kgN/ha/year in CA *vs.* 129 kgN/ha/year in CONV, *i.e.* 11% lower in CA systems than in CONV (p=0.03). Specifically, cluster 3 was significantly different from cluster 1, 2 and 4. On average mineral N fertilisation was lower iin cluster3 with 97.1 kgN/ha/year and 139.7 kgN/ha/year in cluster 1 (p=8E-4), 127.7 kgN/ha/year in cluster 2 (p=0.01) and 134.6 kgN/ha/year in cluster 4 (p=0.006). However, at the year scale, no difference in mineral N fertilisation (*minN_n*) was measured between CA and CONV systems, and neither between clusters.

Second, the average mineral P fertilisation at the rotation scale (*minP2O5_rot*) was lower in CA compared with CONV, with on average 13.8 kgP/ha/year in CA and 27.8 kgP/ha/year in CONV (p=1.1E-3). Cluster 3 values were significantly lower than cluster 1 and cluster 2 values, *i.e.* 8.4 kgP/ha/year in cluster 3, 42 kgP/ha/year in cluster 1 (p=2.2E-8) and 21.4 kgP/ha/year in cluster 2 (p=7.2E-3). *minP2O5_rot* in cluster 4 (on average 18.7 kgP/ha/year) was significantly lower than in cluster 1 (p=4.1E-3). The same trend was observed at the year scale (*minP2O5_n*), with lower P fertilisation rates in CA (on average 10.5 kgP/ha) than in CONV (on average 24 kgP/ha), despite a high CV in the CA group (167.3%). In the clusters, *minP2O5_n* was significantly higher in cluster 1 (on average 34.2 kgP/ha) than in cluster 3 (7 kgP/ha, p=5.3E-5) and cluster 4 (10.6 kgP/ha, p=2.03-3).

Third, the average mineral K fertilisation at the rotation scale ($minK2O_rot$) was lower in CA than in CONV, with an average of 4.1 kgK/ha/year in CA and 21 kgK/ha/year in CONV (p=2.1E-4). $minK2O_rot$ was significantly higher in cluster 1 compared with all the other clusters, with an average $minK2O_rot = 41.5$ kgK/ha/year in cluster 1, while $minK2O_rot = 8.5$ kgK/ha/year in cluster 2 (p=2E-3), 3.5 kgK/ha/year in cluster 3 (p=3.9E-6) and 4.8 kgK/ha/year in cluster 4 (p=1.0E-4). At the year scale, mineral K fertilisation ($minK2O_n$) was also lower in CA than in CONV (on average 10.5 kgK/ha in CA, vs 24 kgK/ha in CONV, p=0.004). $minK2O_n$ was significantly higher in cluster 1 (23.3 kgK/ha on average) than in cluster 4 (1.7 kgK/ha on average, p=0.01). The intra-cluster variability of cluster 4 was

very high (CV=424%), because only one out of the eighteen plots of cluster 4 received mineral K_2O fertilisation during winter wheat growth (2_CA_2022, 31 kgK/ha).

Mineral S fertilisation at the rotation scale ($minSO_3$ _rot) followed a different pattern since it was higher in CA (on average 41.5 kgSO₃/kg/year) than in CONV (on average 29.2 kgSO₃/ha/year, p=0.02). Specifically, $minSO_3$ _rot was significantly higher in cluster 4 (on average 57.2 kgSO₃/ha/year) than in all the other clusters (on average 28.3 kgSO₃/ha/year in cluster 1 with p=0.02, *vs.* 31.4 in cluster 2 with p=0.02 and 28.6 in cluster 3 with p=0.01). At the year scale, SO₃ fertilisation ($minSO_3$ _n) was not different between CA and CONV systems. However, despite a high intra-cluster variability, $minSO_3$ _n was higher in cluster 4 (on average 63.2 kgSO₃/ha/year) than in cluster 3 (on average 29.3 kgSO₃/ha/year, p= 0.04).

3.4.2.2. Organic fertilisation

At the rotation scale, OC restitutions to soil (*C_entries_rot*) were significantly higher in CA than in CONV (on average 3.5 tC/ha/year in CA vs. 2.7 tC/ha/year in CONV, p=9.6E-5). C entries rot was significantly lower in cluster 1 compared with all the other clusters, with an average OC return of 1.9 tC/ha/year in cluster 1 compared with 3.1 tC/ha/year in cluster 2 (p=1.8E-3), 3.1 tC/ha/year in cluster 3 (p=3.2E-3) and 4.0 tC/ha/year in cluster 4 (p=1.5E-7). On the contrary, C entries rot in cluster 4 were significantly higher than in cluster 1 (p=1.5E-7, as said previously) and cluster 3 (p=4.7E-2). Still at the rotation scale, the cumulated number of organic inputs (*nbOrginput rot*) was higher in CA than in CONV, with on average 1.9 additions in CA, and 1.1 additions in CONV (p=0.01). nbOrginput_rot was three times higher in cluster 3 compared with cluster 1 with one organic amendment brought every three years in cluster 3 on average against less than one in six years in cluster 1 (p=0.02). At the year scale, N additions from organic amendments (orgN_n) were not significantly different between CA and CONV systems. This result was associated with a high intra-group variability (CV=223.5% in CA and 457.6% in CONV), explained by the fact that only three CONV plots received N from organic amendments before winter wheat growth (3_CONV_2023, and 2_CONV_2022 and 2023), and eleven CA plots received N from organic amendments (1 CA in 2022 and 2023, 3CA 2023, 4 CA 2023, 10 CA in 2022 and 2023, 11 CA 2023, 12 CA 2023, 13 CA 2023, 16 CA 2023 and 21 CA 2023). Nine out of these fourteen plots were on mixed farms. Although no statistical differences were evidenced between clusters, no farmers from cluster 1 added any organic N to their plots during the monitoring year.

3.4.3. Biodiversity

The presence of hedges (*Hedge*) around the studied plots was not different between CA and CONV. Despite high intra-cluster variability in cluster 4 (CV=424.3%), we found fewer hedges on cluster 4 plots (only one farmer with a hedge in cluster 4: 21_CA_2023) compared with cluster 2 (81% of plots with a hedge, p=0.01) and 3 (83% of plots with a hedge, p=0.003). Finally, the presence of semi-natural habitats varied significantly between CA (*SNH*=2.0 on average) and CONV plots (1.6 on average, p=0.024). These differences were not significant when looking at the clusters repartition.

In a nutshell, at the rotation scale, soil tillage, average mineral N, P and K fertilisation decreased, while soil cover efficiency, crop diversity, OC returns to soils and mineral SO₃ fertilisation increased in CA systems compared with CONV systems. These differences came with increased use of herbicides and decreased use of fungicides in CA. Several variables showed no differences between the two systems, such as rate of crop residue returns to soil, the frequency of wheat cropping in the rotation, insecticide and molluscicide use, as well as mineral N fertilisation on wheat. Overall, clusters 1 and 2 were more representative of a CONV farming system while clusters 3 and 4 were more representative of CA systems. Differences within the CONV and CA systems were observed. Indeed, for the CONV clusters we observed an increased crop residue return, and an increased OC return to soils at the rotation scale, associated with increased use of herbicides in cluster 3 and 4 mostly lied in increased residue return and more generally increased OC returns to soils in cluster 4 compared with cluster 3, associated with an increased reliance on herbicides and molluscicides in cluster 4 compared with cluster 3. Lastly, mineral N and S fertilisation was higher in cluster 4 than in cluster 3 (Figure 15).



Figure 15. Radar chart representing the repartition of the principal components used to build the HAC. All values were normalised to build the radar chart. Dotted values represent the mean value for each indicator.

Table 3. Clusters description.

Green-coloured lines correspond to the principal components used in the HAC, while white lines correspond to the supplementary indicators included in the PCA and clustering, meaning that they were not used to build the clusters. cv= coefficient of variation, sig = significance, ns = Non-significant (p > 0.05). Between-cluster differences were identified with Kruskal Wallis and a Dunn adjustment. Differences between CA and CONV systems were identified with a Wilcoxon paired-rank test based on farmers pairs appraisal.

	Cluster Number of farms		1 n=10		2 n=16			3			4 n=12			CA			CONV	,	CA vs.		
	Number of fields		n=17 i CON	including V and 0 C	17 A	n=25 i CON	ncluding V and 3 C	22 A	n=26, in and	cluding 22 d 4 CONV	2 CA	n=18 in and	cluding 18 d 0 CONV	B CA		CA			CONV		CONV
Va	ıriables	Unit	mean	cv (%)	sig	mean	cv (%)	sig	mean	cv (%)	sig	mean	cv (%)	sig	n	mean	cv (%)	n	mean	cv (%)	sig
	Farmtype	-	0.4	139.6	а	0.5	106.2	а	0.5	102.0	а	0.2	230.1	а	43	0.4	119.2	43	0.4	131.4	ns
General	FarmSize	ha	169.4	64.9	а	190.1	48.1	а	174.0	48.7	а	192.6	41.8	а	43	184	45.7	43	180	54	ns
	FieldSize		4.6	61.9	а	8.0	75.6	ab	6.6	69.0	ab	10.1	65.0	b	43	8.6	67.1	43	6.1	80.8	0.005
	Tillage_ intensity_rot	score	3.8	87.4	а	5.6	66.3	а	16.0	22.2	b	16.9	9.8	b	43	16.7	12.2	43	4.8	74.1	1.10E-08
Tillage	lastPlough	yr	1.2	165.7	а	2.2	146.1	а	11.0	49.0	b	15.9	51.3	b	43	13.4	51.2	43	1.7	139.9	2.78E-08
Thidge	Nb Operations_n	-	10.7	18.9	ab	12.5	19.5	а	9.1	18.7	b	11.4	16.2	а	43	10.3	23.0	43	11.5	20.0	0.007
	Tillage_n	score	0.8	108.7	а	1.0	91.3	а	2.8	20.2	b	3.0	0.0	b	43	2.9	12.6	43	0.9	97.8	2.23E-08
	EffCC_rot	%	10	1.1	а	20	1.6	а	80	0.3	b	80	0.3	b	43	80	33.9	43	20	158.8	6.70E-07
Cover	RestitRes_ rot	score	3.1	42.5	а	4.6	33.3	bc	4	39.7	ab	5.7	14.7	с	43	4.7	33.7	39	4.0	39.8	ns
	TFlh_n		1.4	60.3	а	2.2	33.9	bc	2.2	46.7	ab	3.2	33.4	С	43	2.7	43.2	43	1.9	47.0	3.20E-04
	TFleh_n	-	1.9	40.2	а	2.3	43.9	а	1.1	59.5	b	1.6	82.6	ab	43	1.3	77.1	43	2.1	43.8	2.31E-04
Posticido	nbHerbi_n		1.9	64.3	а	3.0	40.8	ab	3.5	45.4	bc	5.1	35.6	с	43	4.1	44.3	43	2.6	52.5	1.17E-04
IISP	nbFungi_n		2.8	38.1	а	2.9	54.3	а	1.7	70.4	b	1.9	72.4	ab	43	1.7	74.3	43	2.9	46.1	7.53E-05
use	nbIns_n	-	0.4	139.6	а	0.8	95.2	а	0.5	102.0	а	0.9	101.3	а	43	0.7	106.2	43	0.6	114.9	ns
	nbMoll_n		0.0	0.0	ab	0.2	181.6	ab	0.0	0.0	а	0.3	165.9	b	43	0.1	251.3	43	0.1	278.9	ns
	nbSDHI_n		0.4	123.2	ab	0.7	85.2	а	0.3	178.5	b	0.6	92.0	ab	43	0.4	136.8	43	0.6	96.4	ns
	minN_rot		139.7	19.4	а	127.7	25.2	a	97.1	33.6	b	134.6	21.4	a	42	115	29.4	41	129	26.9	0.032
	minP2O5_rot	kg/ha/yr	42.0	44.6	a	21.4	63.9	b	8.4	103.3	C L	18.7	/1.2	DC h	42	13.8	93.5	41	27.8	69.3	1.11E-03
Mineral	minK20_rot		41.5	104.3 54.1	a	8.5 21 /	60.6	0	3.4	189.1	0	4.8 57.2	124.5 52.9	D b	42	4.1	130.4	41	21	152.9	2.07E-04
fertilisa-	minN n		173.8	12.8	a	164.7	21.1	a	151.0	31.5	a	165.6	17.7	a	42	156	27.6	41	169	16.4	0.015
tion	minP2O5 n		34.3	56.4	a	20.9	96.5	ab	7.0	201.5	c	10.6	171.8	bc	43	10.5	167.3	42	24	87.3	0.004
	minK2O n	kg/ha/yr	23.3	122.3	a	6.5	225.8	ab	2.0	252.7	ab	1.7	424.3	b	43	2.7	282	42	12.3	183.2	0.048
	 minSO3_n		49.0	86.8	ab	45.7	76.5	ab	29.2	120.0	а	63.2	73.7	b	43	45.4	97.2	42	44.4	82.3	ns
	C_entries_rot	tC/ha/yr	1.9	36.5	а	3.1	27.3	bc	3.1	28.9	b	4.0	23.0	С	43	3.5	28.6	41	2.7	38.2	9.55E-05
fertilisa-	nbOrginput_ rot	-	0.7	180.7	а	1.4	128.0	ab	2.5	101.6	b	1.2	133.3	ab	43	1.9	111	42	1.1	158.1	0.011
tion	orgN_n	kg/ha/yr	0	0.0	а	12	346.1	а	22.2	212.2	а	14.4	258.5	а	43	19.5	223.5	43	7.0	457.6	ns

	Cluster Number of farms Number of fields		n=17 i CON	1 n=10 including V and 0 C	17 A	n=25 i CON	2 n=16 including V and 3 C	22 A	n=26, in and	3 n=18 icluding 2 d 4 CONV	2 CA	n=18 in and	4 n=12 cluding 18 d 0 CONV	B CA		СА		CONV		CA <i>vs.</i> CONV	
Vc	riables	Unit	mean	cv (%)	sig	mean	cv (%)	sig	mean	cv (%)	sig	mean	cv (%)	sig	n	mean	cv (%)	n	mean	cv (%)	sig
Diversifi-	CropDiv_rot nbLeg_rot	-	4.6 0.8	34.0 135.0	a a	4.2 0.9	33.7 146.9	a a	8.9 3.8	42.4 49.3	b b	10.5 2.9	38.1 37.7	b b	43 43	9.4 3.3	40.3 44.8	43 43	4.6 1.1	48.4 160.7	5.09E-08 2.86E-06
cation	wheat Var_mix	yr -	2.6 0.5	53.3 103.1	a a	2.5 0.5	51.9 97.8	a a	3.3 0.4	49.4 129.6	a a	2.9 0.6	41.1 93.3	a a	43 39	3.1 0.5	46.3 109.4	42 40	2.6 0.5	52.7 101.3	ns ns
Biodi- versity	Hedge SNH	score score	0.2	185.8 47.7	ab a	0.5	98.1 63.2	a a	0.6	87.3 42.0	a a	0.1	424.3 42.5	b a	43 43	0.4 2.0	131.4 41.05	43 43	0.4 1.6	125.1 56.9	ns 0.024
Wheat campaign	d.seed d.harv	days	299 188	4.3 2.2	a a	298 187	3.1 3.2	a a	296 186	3.7 2.9	a a	294 185	3.0 2.9	a a	41 32	295 185	3.5 2.8	41 38	298 187	3.5 2.9	0.033 ns
Index	RI_farm	score	33.4	50.0	а	44.4	31.2	а	72.8	15.0	b	73.1	5.1	b	43	74.4	7.2	43	39.2	37.4	1.77E-08

4. Discussion

<u>4.1. CA and CONV: two contrasted groups with a high variability of practices along a</u> <u>continuum</u>

CA farmers selection was supported by the fact that most CA farmers were already involved in farmer innovation groups led by local agricultural chambers. This limited the risk of selecting CA farmers who were not really implementing CA on their farms. On the other hand, CONV farmers pre-selection relied mostly on the CA farmers' vision of a CONV farmers' practices. In most cases, the CONV and CA groups of farmers were well identified with lower RI scores attributed to CONV farmers and higher RI scores attributed to CA farmers at the pre-selection step. The RI confirmed that paired fields had contrasted cropping management and provided us with a holistic vision of the different farmers' management at the farm scale. It also evidenced within-group heterogeneity of practices. Overall, it was useful to make a first validation of the network. However, since the RI was performed on 2019-2020 growing campaign data, it could not properly identify CONV farmers transitioning to more agroecological practices.

These were better identified after the PCA and HAC steps. Most CONV farmers were correctly identified as belonging to the same "CONV" group in the PCA, such as most CA farmers were well identified as belonging to the same "CA" group. However, some farmers that we initially identified as CA or CONV farmers appeared as outliers of their groups after the analyses, explaining the presence of "CA" farmers in cluster 2, or of "CONV" farmers in cluster 3. In addition, some CA and CONV farmers changed cluster while other remained in the same cluster over the two years, showing that farming practices are dynamic and not static over time, confirming observations from other authors (Ferdinand and Baret, 2024).

The situation we met with pair n°9 in year 1 was a good example of a misidentification of a CONV farmer that was more to be considered as a CA farmer. Indeed, the CA farmer of pair 9 had been implementing CA on his farm for almost twenty years and considered 9_CONV as a CONV farmer when selecting him, though he was transitioning to a CA system. According to 9_CA, 9_CONV was still a "CONV" farmer because he kept performing superficial tillage on several plots of his farm (9_CA, pers. comm., 2021). Similarly, the HAC associated some other CONV farmers with CA-clusters. As previously said, it was the case for 9_CONV in 2022, but also 3, 6 and 12_CONV in 2023, who were found in cluster 3.

On the contrary, the HAC associated some CA farmers with CONV clusters. It was the case for 21_CA in 2022 and 2 and 7_CA in 2023, who were associated with cluster 2. In the case of the four CONV farmers found in CA clusters, we knew from the interviews that three of them were initiating a transition to reduced or no-tillage systems (3, 6 and 9_CONV), while the fourth one was already implementing reduced tillage (12_CONV) on his farm, although he was not willing to shift to no-till. In the case of the three CA farmers found in CONV clusters, the explanation for 2_CA is likely to be linked to his use of the same field between the two successive years of study, where no intercrop was set between the two wheats. For the other two CA farmers who used the same plot for the two successive years of study, both grew crops between the two wheat crops (Alfalfa for 1_CA, and sunflower for 10_CA). For the other two CA fields belonging to CONV cluster, the first plot (21_CA_2022) was on a mixed farm and the farmer proposed two fields with different rotations in the two years of study, the first being based on winter cereals with a relatively low efficiency of soil covering (the farmer missed opportunities to cover soil) and the other based on an alternation between wheat-maize and a cover

crop in between. OM inputs across his rotation also differed, with two OM inputs on his year 1 plot against six on his year 2 plot. The first field was also a recently transitioned plot (6 years after last ploughing), whereas the second plot was more ancient (17 years after last ploughing). For 7_CA_2023, his rotation was mainly based on a succession of spring crops without cover crop implantation in between. These spring crops were mainly implanted through heavy tillage, and direct seeding was performed on winter crops (which represented a low percentage of his rotation).

Some indicators showed high coefficient of variations within the CA and CONV groups, confirming the intrinsic heterogeneity of cropping practices within the two groups. Although the HAC did not increase variability inside clusters, it did not drastically decrease it either, testifying again of the diversity of cropping practices, the existing trade-offs between them, and the likely associated diverse system responses (soil-production continuum), as already evidenced by Rillig and Lehmann, (2019) regarding cropping practices. In that sense, clusters should not be opposed to the CA vs. CONV comparison, but rather seen as bringing additional information on the differences between groups, by accounting for supplementary indicators that are not included in the CA and CONV definition of practices. Although it is difficult to conclude on differences in agronomic performances between clusters 1 and 2 (for the CONV clusters) and clusters 3 and 4 (for the CA clusters), we noted that cluster 2 showed tillage-based systems with increased residue and OM returns to soil compared with cluster 1, resulting in an increased reliance on herbicide products, without any decrease of tillage intensity. This suggested that herbicides used on cluster 2 plots were used for insurance purposes rather than others. Similarly, between clusters 3 and 4, in addition to the CA pillars being respected in the two clusters, residues and OM returns to soils restitutions were higher in cluster 4 than in cluster 3, associated with an increased herbicide use. This result came in opposition to the idea that increased mulches foster weed control (Bhaskar et al., 2021), suggesting that plots of cluster 4 may have issues with herbicide resistance leading to herbicide use increases.

Finally, recent research argued on the need to better categorise CA systems and proposed new indexes and means to classify them (Ruiz-Espinosa *et al.*, 2024). Our work indicates that classifying CA systems only on the basis of the three CA pillars is not sufficient to make systemic conclusions and links with these systems' sustainability as they may differ due to divergences in the other interacting compartments of the cropping practices (*e.g.* fertilisation, pesticide use) (Craheix *et al.*, 2016).

4.2. Cropping practice analysis

4.2.1. CA pillars

First, soil tillage indicators showed that CONV systems were implementing ploughing or tillage on a regular basis, while CA farmers did not plough soils and for the most, were mostly establishing their crops through direct seeding. However, our analyses showed that almost half of CONV farmers (11/22) in the network had not ploughed their plot for more than two years before the study, showing that CONV farmers do not always rely on yearly ploughing, but tend to adopt tillage as an alternative to ploughing, and plough their fields on a long-period basis. Also, the absence of ploughing and very limited soil tillage in the CA group may suggest a decrease in the number of in-field operation for CA farmers compared with CONV farmers. These can be partially explained by the seeding and termination of cover crops for CA farmers, but not only. Indeed, our analyses showed a difference within the CA group, with cluster 3 fields showing less in-field operations than field of cluster 4. These could be linked to the higher *TFlh_n* in cluster 4 compared with cluster 4, although this should be verified to understand the precise reason for each in-field operation for these farmers.

Second, soil cover analyses confirmed that farmers of the CONV group had limited use of cover cropping in the intercrop periods (or use of perennial crops) at the rotation scale compared with CA farmers. However, results were more heterogeneous regarding the rate of residue return within the CA and CONV groups. Indeed, we noted two different strategies either in the CONV group and the CA group. For the CONV group, farmers of cluster 2 tended to return most crop residues to soil (76% at the rotation scale), while farmers of cluster 1 had a lower rate of residue return at the rotation scale (50%). Similarly, we observed two different strategies in the CA group, with farmers of cluster 4 systematically returning residues to their plots, and farmers of cluster 3 who returned residues in 66% of cases, which is even less than farmers of cluster 2.

Rotations were twice as diversified in CA compared with CONV systems and included more legumes. However, in both CA and CONV systems, winter wheat represented an important part of the rotation since it was grown on average every two years in every rotation.

4.2.2. Beyond CA pillars

TFI of herbicides in the network were higher in CA than in CONV, with twice as many herbicide products used in CA than in CONV. This increase in herbicide use may be mainly explained by cover crops termination which largely relies on herbicide use. We observed two different schemes in the CONV clusters with cluster 1 having lower TFI herbicides than cluster 2, and similarly between the CA clusters, with cluster 3 showing lower TFI herbicides than cluster 4. The increase in herbicides use in cluster 2 compared with cluster 1 cannot be explained by a potential increase in cover crop cultivation in cluster 2, since on average they missed 80% of the opportunities to insert a cover crop when possible. Differences in herbicides use were not significant between cluster 1 and cluster 3, suggesting that CONV farmers used as many herbicides as CA farmers, while they did not grow any cover crop prior to winter wheat. This suggests that CA systems may be more efficient than CONV systems in herbicide use. Some CA farmers therefore managed to limit herbicide use, although it is a challenge in CA systems (Cordeau, 2022). Only one farmer managed to grow winter wheat without any herbicide use. TFI of fungicides, insecticides and molluscicides was lower in the CA group than in the CONV group. However, the differences between clusters were limited to a significantly lower TFI in cluster 3 compared with cluster 2. Specifically, we observed no difference in insecticide use between CA and CONV, with in any case, about half of the farmers in each group using insecticides. Regarding molluscicide use, we did not observe higher rates of molluscicide use in CA compared with CONV plots, though CA systems are referenced as using more molluscicides, due to the reduction of soil tillage and an increased presence of crop residues resulting in the creation of favourable conditions for slugs (Douglas and Tooker, 2012). However, we noted a difference within the CA group, with plots of cluster 4 who received a molluscicide, while no molluscicide was applied on plots of cluster 3. This could be linked with the increased quantity of residue returned on cluster 4 (Douglas and Tooker, 2012). Most farmers (CONV and CA) who applied a molluscicide in fall had whether rapeseed, pea or oilseed flax as a previous crop. Molluscicide application concerned only 30% of plots with rapeseed as previous crops, 50% of plots with pea as a previous crop and 33% of plots with oilseed fax as a previous crop. Finally, fungicide applications were higher in CONV than in CA, although SDHI use was identical between the two groups. The absence of difference in SDHI use might be due to their high efficiency coupled with a lack of farmers' awareness on the potential risks associated with their use (For health, biodiversity and the environment, Duarte Hospital et al., 2023). Cluster 3 showed to be more efficient in limiting fungicide use. The case of the one farmer who managed to avoid the use of fungicide over the two years is interesting. This farmer was the only one in the network who grew wheat in co-culture with a perennial crop (alfalfa). Indeed, intercropping with perennial crops may support a drastic decrease in fungicides needs since it creates a physical barrier to fungal spores dispersal between wheat plants (Boudreau, 2013; Stomph *et al.*, 2020).

At the rotation scale, we observed on average that mineral N, P and K fertilisation rates were lower in CA systems compared with CONV systems, contrarily to mineral S fertilisation which was more important in CA than in CONV systems. Specifically, the average yearly rate of mineral N fertilisation was 11% lower in CA than in CONV. Within the CA group, cluster 3 had the most efficient use of mineral N fertilisation, using less than 100 mineral N units on average per hectare and per year. We could assume that this would be linked with a higher crop diversification and increased proportion of legumes in the rotation, but these are not significantly different from cluster 4, while cluster 4 mineral fertilisation rates are significantly higher than cluster 3. Although cluster 4 showed more residue returns than cluster 3, cluster 3 had more organic inputs than cluster 4 at the rotation scale. However, this is not confirmed by our analyses, since the average return of organic N was not significantly different between clusters (results not shown, since *orgN_rot* was excluded from the analysis because of a high correlation with *orgN_n*).

A possible explanation would be that organic N return calculations were made based on a model (Simeos-AMG), that considers standard compositions for organic amendments, and therefore may not completely fit reality and lead to misestimations, especially when legumes are involved. In our analyses, we considered the total number of legumes grown cumulating main crops and cover crops. However, a different proportion of legumes grown as main crops between cluster 3 and 4 could also explain such differences in N fertilisation.

Another hypothesis is that farmers of cluster 3 could be voluntarily more cautious than farmers of cluster 4 in their use of mineral fertilisation. In other words, farmers of cluster 4 may use more N fertilisation than needed on their plots on average. However, these results on N fertilisation are not confirmed at the year scale on wheat, since we could not evidence any difference of fertilisation rates between CONV and CA systems.

This N fertilisation also evidenced the high importance of wheat as a major high added-value crop. Indeed, on such crops, farmers are likely to be more reluctant to decrease N fertilisation rates, by fear of decreasing yields (see Chapter 4). Regarding mineral P fertilisation, on average CA farmers used each year 14 kg min P/ha less than CONV farmers. Similar to N results, cluster 3 was evidenced as the most efficient in mineral P fertilisation than Clusters 1 and 2. Regarding mineral K fertilisation at the rotation scale, cluster 1 had a higher fertilisation rate than all the other clusters. Fang et al. (2023) evidenced that optimal concentrations of N, P, and K had a positive indirect influence on the available soil nutrient content and efficiency of nutrient use by plants by increasing the abundance of Proteobacteria, decreasing the abundance of Actinobacteria, and enhancing the potential functions of N metabolism pathways (also see Case study n°2). Finally, we observed the opposite pattern for mineral S fertilisation, which was higher in CA than in CONV systems. SO₃ fertilisation was the highest in cluster 4 compared with all the other clusters. According to other authors, coupled N-S fertilisation would support wheat yields (Järvan, Edesi and Adamson, 2012a, 2012b). Biomass returns to soils were higher in CA than in CONV. Cluster 1 showed the lowest rates of biomass returns, and cluster 4 higher returns than cluster 3. Cluster 2 has equivalent returns rates as cluster 3, also showing that CONV farmers may also be efficient in returning biomass to soils. However, when looking only at OM amendments, cluster 3 showed the higher rate as any other cluster.

Finally little evidence was made on the biodiversity variables between CA and CONV. This might be explained by the fact that within a pair, CA and CONV farmers were located closely from one another, therefore they were situated in very similar geographical environments. The only difference that cluster 4 had less hedges than cluster 3, therefore with more open systems than cluster 3.

4.2.3. Validation of the approach to discriminate cropping practices

A key point of this chapter was to define a minimum set of indicators based on many variables commonly studied in the literature to study cropping practices. Starting from a general farm scale composite score (the RI), we selected relevant cropping practices variables from a large set of variables available in the literature. After statistical analyses, we reduced this set of indicators to a reduced one allowing to cover all the compartments of cropping practices with a high level of precision, also including fields' historical management. This reduced set of indicators eventually provided concordant results with the RI, while giving more insights and precise information.

The data presented here is not necessarily representative of all farmers of the region. However, it provided meaningful insights into the diversity of cropping practices between two contrasted and rarely studied systems: CA and CONV. Although we observed some CONV farmers with similar practices as CA farmers, all CONV plots showed similar characteristics, such as their regular tillage, little crop diversity and low soil cover rates, while all CA plots had rotations based on little or absence of tillage, high crop diversity and high soil cover rates. Fertilisation and pesticide use were important factors to consider since they allow to evidence differences in strategies for CONV and CA farmers, leading to highlight the presence of more efficient systems and evidencing existing trade-offs between the different compartments of cropping practices.

The two-steps approach used to select CONV farmers was the best manner we identified to allow CONV farmers to participate to such a study without too much reluctance or mistrust. Indeed, so-called CONV farmers may not be interested by such studies, especially because they might think that they are biased towards the promotion of agroecological practices. Even while adopting such selection strategy, two CONV farmers told us about their doubts in the study objectivity towards defending CA systems during informal discussions. Four CONV farmers expressed during the different interviews and discussions willingness to shift practices or had already started the shift towards CA. These farmers were well identified by the clustering since they appeared in cluster 3 in the second year of the study. Concerning CA farmers, the comparison showed that although all of them respected the CA pillars (it was a prerequisite condition), their differences in the use of pesticide and fertilisation led some to be associated with CONV-clusters.

4.3. Setting and maintaining stakeholders' dynamic in an OFE

The social component of OFE studies is key. Researchers need to keep a neutral and objective behaviour to enable every type of farmers to join OFE. We initially feared that CONV farmers would not be interested in participating in such a study because of a reluctance to be judged or compared with their paired neighbouring farmer, representative of an alternative and "sustainable" way of growing crops. Two elements helped us to overcome this risk. First, enabling each CA farmer to select his own paired farmer allowed to ensure that the collaboration would run well over the two years of study. Indeed, most of the time both farmers knew each other, or even were friends or close enough to each other to enable data sharing and open discussion. Second, taking the time to discuss with farmers without judgment helped to overcome CONV reluctance and doubts on the orientation and

the aim of the study. Keeping a very neutral, pedagogical position, regularly visiting farmers' plots and giving space to many discussions with all farmers allowed to generate trust between the researchers and the farmers, as also highlighted by other authors working on OFE (Palomo-Campesino, García-Llorente and González, 2021).

In addition, the financial reward to farmers for their participation was also an important parameter to consider when establishing the OFE. Farmers, and especially innovative farmers are often solicited, spending time to support different trials or to reply to interviews. For a farmer, taking part in such study means that they will spend time for interview, for gathering documents needed by researchers, for visits or other requests made by researchers. We consider that doing such, farmers take part of a research & development work which needs to be acknowledged by Research institutions.

Another key to success of such study was our flexibility. Indeed, our aim was not to interfere in farmers' practices but to set a win-win relationship, where farmers provided us the information we needed to do research, and in exchange we provided relevant analyses and information. It was therefore very important to work timely with farmers when providing them guidelines, asking them to come to their fields for samplings (also see **Chapter 4**), or to perform the interviews at the best moment of the year (mainly in winter since they had more time to dedicate to us).

Finally, the organisation of regular meetings, workshops and keeping farmers and stakeholders updated about the study advances was also important to maintain the interest and willingness to share information. The role of digital technologies (Regular Whatsapp communications with farmers, calls, online visio-conferences) was also very important to maintain the dynamics and the involvement of all stakeholders in the field. No farmer or other study partner dropped the study between the two years, except one pair of farmers who was not able to propose a field that would match our requirements in the second year. Indeed, the CONV farmer of this pair was also transitioning to CA and had stopped tillage in almost all his plots. This was an evidence of success in our approach, showing the interest of all partners in the successful outcome of the study.

5. Conclusion

In this article, we proposed a methodology and framework to set up a robust OFE allowing on-farm comparison of management practices. The figures and insights brought in this article may be site specific and more systemic studies of cropping systems may help giving the full picture of French agricultural systems and thus strengthen our conclusions.

Nevertheless, CA is promoted as a sustainable farming system. Our study showed that nuances need to be brought for the analysis of system sustainability, especially when looking at non-CA specific parameters such as fertilisation or pesticide use. Similarly, no specific definition of conventional agriculture exists. Indeed, we argue that CA systems and all cropping systems, including CONV systems, should always be described based on a systemic vision of the cropping practices and their impact on the ecosystem to avoid confusing conclusions. This requires describing them with sufficient accuracy and precision at a reasonable cost. Therefore, defining groups of practices (CA or CONV) as uniform entities is not sufficient and these groups should be regarded more as a continuum. Comparing practices according to the whole practice compartments, also including fertilisation and pesticide use, would allow to generate global frameworks enabling the comparison of all types of systems (CA, CONV, organic, agroecological, regenerative, etc.) based on a full picture of practices.

Data availability

Data and metadata are available at: <u>https://doi.org/10.18167/DVN1/SI026U</u>

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Supplementary materials

Table S1. Main information on participating famers.

Yr= Year. Figures indicated in the "Location" column correspond to French department administrative numbers, i.e. department, 16 = Charente, 17 = Charente-Maritime, 37 = Indre-et-Loire, 49 = Maine-et-Loire, 79 = Deux-Sèvres and 86 = Vienne. Farms' location within each department are indicated according to geographic coordinates ("south", "west", "north", "south" and middle ("mid"). CA= Conservation agriculture, CONV = Conventional agriculture. Soil texture is expressed following the USDA classification and was determined after soil sampling in spring 2022 and 2023.

Pair	Туре	Loca- tion	Yr	Wheat variety	Texture (USDA)	Previous crop	Yr	Wheat variety	Texture (USDA)	Previous crop
1	СА	79		KWS ultim	silt loam	Meslin dominated by legumes		KWS ultim	loam	Winter wheat
	CONV	west				Rapeseed			silt loam	
2	CA	16		complice, oregrain, KWS	silt loam	Sunflower		oregrain, providence, KWS	silt loam	Wintor whoat
2	CONV	south		ultim	sandy loam	Silage maize		ultim	loam	Winter Wileat
3	СА	86		KWS ultim	clay	Alfalfa		abilene	silty clay	Meslin dominated by legumes
	CONV	south				Sunflower			clay	Sunflower
	CA	86		absalon cesario complice	clay			absalon, complice, filon,	sandy clay loam	Rapeseed + legumes
4	CONV	mid		macaron, sacramento	loam	Sunflower		letsgo, macaron, sacramento	loam	Rapeseed
E	CA	79		absalon, armada, cesario,	cilty clay	Lentil		ascott, cesario, complice,	silty clay loam	Sunflower
5	CONV	south		complice	Silly Clay	Winter wheat		oregrain, providence	silty clay	Sulliowei
6	CA	86	022	absalon, armada, complice,	silt loam	Sunflower	023	absalon, complice, gerry,	silt loam	Sunflower
	CONV	south	5	providence, KWS ultim	Sile Iouin	Реа	5	prestance, KWS ultim	Sile loan	Sumower
7	CA CONV	86 south		absalon	sandy clay loam	Sunflower		montecarlo	loam	Sunflower
	CA	86				Rapeseed +				
8	0	north		absalon	silt loam	legumes		absalon	silt loam	Sunflower
	CONV	north				Rapeseed				
	CA	16			clav	Rapeseed +				
9		north		prestance		legumes	_	withdrew		
	CONV				sandy clay loam	Alfalfa			1	
	CA	16		absalon, cellule, cesario,		Meslin dominated				
10		north		filon, nemo	silty clay	by legumes		absalon, advisor	silty clay	Winter wheat
	CONV			- ,		Реа				
11	CA				silty clay	Silage maize		NA	silty clay	Sunflower

	CONV	17 north	absalon, amstrong, azzezo, oregrain, descartes, calumet, filon, nemo		Winter barley			Pea
12	CA	79	absalon, advisor, sillon,	loam	Silage maize	amstrong, pillier, prestance,	loam	Silage maize
12	CONV	west	KWS sphere	IOdili	Rapeseed	KWS ultim	IUain	Grain maize
13	СА	79 wost	advisor	silt loam	Rapeseed + legumes	absalon, advisor	silt loam	Rapeseed + legumes
	CONV	west			Grain maize			Rapeseed
14	СА	37 porth	advisor, apache, cellule, oregrain	loam	Grain sorghum	apache, prestance, providence	loam	Rapeseed + legumes
	CONV	north	macaron	silt loam	Seed maize	NA		Rapeseed
15	CA	86	absalon, amstrong, pillier,	loam	Buckwheat	ΝΔ	loam	Sunflower
15	CONV	mid	sepia, unique	Ioann	Grain maize		Ioani	Rapeseed
	CA	86		sandy loam	Grain maize	aigle, fillon, forcali,	loam	Rapeseed + legumes
16	CONV	mid	NA	loam	Rapeseed	macaron, mondio, KWS sphere, syllon, tallendor	sandy loam	Rapeseed
17	CA	37	KAA/C ultim	alay loom	Grain maize	chovignon	alay laam	Grain maize
17	CONV	west	KWS UIUM	ciay ioani	Sunflower	chevignon	ciay loan	Sunflower
10	CA	49	absalon, apache, pillier,	loam	Sunflower	absalon, sy adoration,	loam	Sunflower
10	CONV	east	stromboli	IOdili	Grain maize	veloski	IUain	Grain maize
19	CA	17	KWS ultim	silty clay	Oilseed flax	KW/S ultim	silty clay	Pea
	CONV	north		clay	Реа		clay	100
20	СА	37	NA	loam	Meslin dominated	NA	loam	Rapeseed + legumes
	CONV	north		silt loam	Grain sorghum		clav loam	Rapeseed
	CA	49			Rapeseed			
21	CONV	west	absalon	silt loam	Silage maize	KWS ultim	silt loam	Silage maize
	<u> </u>			İ.	Rapeseed +			
22	CA	79	nemo	loam	legumes	nemo	silt loam	Oilseed flax
	CONV	east			Rapeseed			

Table S2. Step 1 of data selection.

Quick review of different systemic studies and indicators used to characterise cropping practices. at farm or field scale. This review allowed to define the pool of initial indicators for the systemic analyse of cropping practices within the on-farm network, after running the Regeneration Index to all farmers. Note that the Regeneration Index indicators were inserted in the table, since some indicators may be relevant for an analysis at the rotation scale. Indicators used by Ferdinand and Baret (2024) and Ruis-Espinosa et al., (2024) although mentioned in the text are not shown in this table since they worked on CA indicators only and not properly on the whole cropping practices. OM: organic matter.

1 = (Büchi et al., 2019), 2 = Regeneration Index (Lugassy et al., submitted), 3= (Chabert, Marchand and Sarthou, 2020; Chabert and Sarthou, 2017), 4= (Dupla et al., 2022), 5=(Akakpo et al., 2021), 6=(Armengot et al., 2011). *tillage and stubble, ** Timescale not specified.

Indicator	Scale	1	2	3	4	5	6	Sum	Decision	Justification or comment
Farm structure	•									
Useful Agricultural Area	Farm	Х	Х		[1	2	Kept	
Farm type (cereal grower or breeder)	Farm			х				1	Kept	
Number of labour units	Farm	х	х					2	Disregarded	Focus on FarmSize
Number of livestock unit	Farm	х	х					2	Disregarded	Focus on FarmType
Farmer's agronomic approach	1									<u> </u>
Participation in agroecology training/experience-sharing networks	Farm		x					1	Disregarded	Not adaptable to field scale
Crop Diversification	I							1		
Crop diversity at the field scale		Ι	[[<u> </u>		[
over 5 years	Field	х					X	2	Kept	
Number of years in meadow	Field	х						1	Adapted	
Fraction of temporary meadow										-
cover, averaged over 10 years	Field				х			1	Adapted	
Crop diversity at the farm scale	Farm	v	v					2	Adapted	
on 1 campaign	Tann	^	^					2	Adapted	
Mean number of species in the rotation	Field				х			1	Adapted	
Diversification index over 5 years (Indigo method)	Field	х						1	Adapted	years
Mean number of species in the	Field	x			х			2	Adapted	
Number of spring crops over 10										-
years	Field				х			1	Adapted	
Number of crops seeded in line over 5 years	Field				х			1	Adapted	
Simpson diversification index on seeded crops	Not specified			X* *				1	Adapted	
Number of occurrence of	Field	v						1	Kent	
principal crop) over 5 years	rielu	^						T	керс	
% of production area covered by legumes over 1 year	Farm			х				1	Adapted	Number of occurrence of legumes over 5 years
% of production area covered by cover crops over 1 year	Farm			х				1	Adapted	Number of cover crops over 5 years
Mean length of crop rotation	Not specified			х				1	Disregarded	Focus on the 5 preceding years
Cereal ratio over 5 years	Field						x	1	Adapted	Years passed between monitored wheat and last cultivated wheat

Indicator	Scale	1	2	3	4	5	6	Sum	Decision	Justification or comment
Number of machinery passages over 1 year	Field	х						1	Kept	
Number of weeding operations over 1 year	Field	x						1	Disregarded	Focus on total operations over 1 year
Number of ploughings + weeding operations over 1 year	Field	х						1	Disregarded	Focus on seeding and total operations
Number of years since last ploughing	Field			х				1	Kept	
Number of years since last tillage operation	Field			х				1	Disregarded	Focus on last ploughing
Cumulative tillage depth	Field			X* *				1	Adapted	
number of tillage operations over 1 year (ploughing, stubble ploughing, harrowing, etc.)	Field	x			Х*			2	Adapted	
Soil protection index over 5 years (linked with soil tillage, IDEA method)	Field	x						1	Adapted	Tillage intensity over 1 and 5 years
Soil Tillage Intensity (STIR) over 1 year	Field	х			х			2	Adapted	
Tillage practices on the farm in % of cultivated area	Farm		х	х				2	Adapted	
Tractor power	Farm				х			1	Disregarded	Farmers may have more tractors
Intensity of weed control	Field						Х	1	Disregarded	
Mechanical weeding (Early/late/no mechanical weeding)	Field					x		1	Disregarded	Focus on TFI and total operations
Soil cover										
		1	1	1	<u> </u>	<u> </u>	1			Number of time
Number of times residues were exported over 5 years	Field	x			x			2	Adapted	residues were returned over 5 years + monitored year
Crop residue management	Field					х		1	Disregarded	Lack of precision of the indicator
Number of cover crops occurrence during the five-year crop rotation	Field	x						1	Kept	
Intercropping (residue, bare, intercrop)	Field			х				1	Adapted	Efficiency of soil cover during intercrop over
1-year mean soil cover rate	Field		Х					1	Adapted	5 years
Fertilisation										
Total quantity of organic inputs	Field				v	v		2	Kant	
over 5 years	FIEld	^			^	^		5	Kept	
Quantity of organic inputs by soil amendments over 5 years (kgOM/ha)	Field	x						1	Adapted	Annual carbon inputs
Quantity of organic inputs from residues over 5 years (kgOM/ha)	Field	x			x			2	Adapted	from residues, cover crops and organic fertilisation over 5
Annual C inputs (organic amendments, roots, restored aerial parts) (tC/ha/yr)	Farm		x					1	Adapted	years

Indicator	Scale	1	2	3	4	5	6	Sum	Decision	Justification or comment
Min N min fertilisation over 1	Field	x		х				2	Kept	
year (kgN/ha) Org N fertilisation over 1 year										
(kgN/ha)	Field	х		х				2	Kept	
Total N fertilisation over 1 year	Field	v				v		2	Kont	
(kgN/ha)	Field	^				^		2	керс	
Total N fertilisation over 5	Field						х	1	Kept	
Total P fertilisation over 1 year										Separated between
(kgN/ha)	Field					Х		1	Kept	organic and mineral
Total K fertilisation over 1 year	Field					v		1	Kont	
(kgN/ha)	Tield					^		1	керс	
Total Ca fertilisation over 1	Field					х		1	Disregarded	
year (kgiv/na)										Focus on main
vear (kgN/ha)	Field					х		1	Disregarded	elements
Ternary fertilizer leaf										
application	Field					Х		1	Disregarded	Too specific
Synthetic biostimulant used	Field					Х		1	Disregarded	
										Number of legumes
Area of farm that received a	Farma		v					1	Adamtad	over 5 years + mean
organic nitrogen	Farm		X					1	Adapted	5 years and studied
										year
Crop protection (phytosanitary m	anagement)									
Number of herbicide	Field	x						1	Kent	
treatments over 1 year		^						-	Керс	
Number of fungicide	Field	х						1	Kept	
										Separated by number
Total number of particidas										of herbicides,
applications over 1 year	Field	х				х		2	Adapted	fungicides,
										molluscicides,
Treatment Frequency Index										Insecticides and SDHI
(TFI) herbicides over 1 year	Field	х	х	х				3	Kept	
TFI except herbicides over 1	Field	v	v					2	Kont	
year	FIEIU	^	^					2	кері	
				V*						Separated into TFI
Total TFI	Field			X* *				1	Adapted	fungicides and TFI
										des/insecticides
TFI in comparison with the										Complexity and
average TFI in the same	Farm		х					1	Disregarded	robustness of
geographic region										indicator
Farmer's strategy to reduce TFI	Farm		Х					1	Disregarded	Overlap RI farm
Seeding		1	1	1			1			
Crop variety/Seed origin	Field					Х	X	2	Kept	Only wheat variety
Planting strategy (early, season,	Field					x		1	Adapted	seeding and narvest
off season, regrowth)								-		campaign
Variety choice and crop spatial di	stribution								l	
Association with legumes (no,	Field					v		1	Disregarded	Only one in notwork
association, relay)	FIEIU					^		1	ופט באמי הפט	Only one in network

Indicator	Scale	1	2	3	4	5	6	Sum	Decision	Justification or comment
Association with trees (no tree, extensive density, intensive density)	Field					x		1	Disregarded	Not applicable to our network
Association with Cucurbitaceae	Field					Х		1	Disregarded	
Irrigation								•	•	
Water stress (yes, no)	Field					x		1	Disregarded	Winter wheat usually not irrigated in this region of France
Landscape and biodiversity										
%Area of ecological interest on farm	Farm		x					1	Disregarded	No longer included in the common agricultural policy
Agroforestry/tree planting project	Farm		х	х				2	Adapted	Presence of hedge
% of hedge area within a 1.5 km radius	Farm			х				1	Adapted	plot
% of woodland area within a 1.5 km radius	Farm			x				1	Adapted	Presence of woodland in a 200 m radius around the studied plot
% of cultivated area within a 1.5 km radius	Farm			х				1	Disregarded	Focus on troos and
% of fallow land area within a 1.5 km radius	Farm			х				1	Disregarded	watercourse as semi
% of human-modified area within a 1.5 km radius	Farm			х				1	Disregarded	
% of water area within a 1.5 km radius	Farm			x				1	Adapted	Presence of watercourse in a 200 m radius around the studied plot
					То	otal Di	srega	rded	25	
					Total Kept			18		
					Total Adapted		31			
		Total indicators 74			ors	74				

Table S3. Step 2 of data selection. Definition of the minimum dataset of analysis after Spearman correlation analysis.

Compa-	Sub-						Orienta-
rtment	compa- rtment	Indicator	Correlated to	Decision	Description	Unit	tion
		nbCC_rot	EffCC_rot	removed	Number of cover crops seeded in the intercrop period between 2017-2022 or 2018- 2023. Value ranging from 0 (no cover planted) to 5 (cover planted at each intercrop). The presence of rapeseed regrowth in the intercropping period, in the case of no-tillage, is counted as a plant cover.	-	
	Cover	EffCC_rot	-	kept	 Ratio between the number of opportunities to plant a cover crop and the number of cover crops planted in the rotation. Number of opportunities to implant a cover crop in the rotation, to be considered as an opportunity, there must be : 1) A period >= 8 weeks between the harvesting of one crop and the seeding of the next. 2) There is no opportunity if the soil is permanently covered by a perennial crop (<i>e.g.</i> alfalfa) or by a meadow, or by a catch crop. The ratio ranges from 0 (of all the opportunities to plant a cover crop, none were taken) to 1 (of all the opportunities to plant a cover crop, all were taken). 	-	More the better
		RestitRes_n	Restit_rot	removed	1 if the residues of the previous crop have been restored, and 0 if the residues of the previous crop have been exported.	-	
ractice		RestitRes_rot	-	kept	Number of times main crop residues were returned over the 5 years preceding the campaign under study as well as the campaign under study.	-	
_		minN_n	-	Supp.	Inorganic nitrogen units used to grow winter wheat in 2022 or 2023.	kg/ha/yr	
		minP2O5_n	-	Supp.	P_2O_5 inputs by the farmer in the harvest year 2022 or 2023 in kg/ha.	kg/ha/yr	
		minK2O_n	-	Supp.	K ₂ O inputs by the farmer in the harvest year 2022 or 2023 in kg/ha.	kg/ha/yr	
	uo	minSO3_n	-	Supp.	SO_3 inputs by the farmer in the harvest year 2022 or 2023 in kg/ha.	kg/ha/yr	
	rtilisati	minN_rot	-	kept	Average mineral N inputs by the farmer in the 2022 or 2023 harvest year and the 5 years preceding these harvest years in kg/ha/year.	kg/ha/yr	Less the
	neral fe	minP2O5_rot	-	kept	Average P_2O_5 inputs by the farmer in the 2022 or 2023 harvest year and the 5 years preceding these harvest years in kg/ha/year.	kg/ha/yr	better
	Mii	minK2O_rot - kept Average K ₂ O inputs by the farmer in the 2022 or 2023 harvest year and the 5 years preceding these harvest years in kg/ha/year.					-
		minSO3_rot	-	kept	Average SO ₃ inputs by the farmer in the 2022 or 2023 harvest year and the 5 years preceding these harvest years in kg/ha/year.	kg/ha/yr	
	Organic fertilisatio n	C_entries_rot	-	kept	Annual inputs of organic carbon on the plot, via organic amendments, roots and aerial parts returned, either by crops or by cover crops over the 5 years preceding the year of study, <i>i.e.</i> 2017-2022 or 2018-2023.	tC/ha/yr	More the better

Compa- rtment	Sub- compa- rtment	Indicator	Correlated to	Decision	Description	Unit	Orienta- tion
		orgN_rot	nbOrginput_rot	removed	Annual organic nitrogen inputs on the plot, via organic amendments over the 5 years preceding the study year, <i>i.e.</i> 2017-2022 or 2018-2023.	kg/ha/yr	
		nbOrginput_rot	-	kept	Number of applications of organic fertiliser over the 5-year historical management and the study campaign (six campaigns in total).	-	
		orgN_n	orgP2O5_n, orgK2O_n	Supp.	Quantity of organic nitrogen provided by organic fertilisation over the 2021-2022 or 2022-2023 campaign.	kg N/ha	
		orgP2O5_n	orgK2O_n	removed	Quantity of organic phosphorus provided by organic fertilisation over the 2021-2022 or 2022-2024 campaign.	kg P₂O₅/ha	
		orgK2O_n	-	removed	Quantity of organic potash provided by organic fertilisation over the 2021-2022 or 2022-2025 campaign.	kg K₂O/ha	
		CropDiv_rot	-	kept	Number of different crops grown in the 5 years prior to the study year (cover crops or main crop).	-	
	ation	nbLeg_rot	-	kept	Number of times a legume was planted (crop or cover) between 2017-2022 or between 2018-2023.	-	More the better
	versific	Time_return_wheat	eturn_wheat - kept Length of time winter wheat has been back in the rotation. This number may be higher than 5 if no winter wheat has been grown since 2017. It is then noted as 6.				
	Ō	nbVar	Var_mix	removed	Number of wheat varieties grown in field in year 1 or year 2.	-	
		Var_mix	-	Supp.	Wheat grown is a monospecific variety (0) or a mixture of varieties (1).	-	-
		Prec_crop_n	NA	Supp.	Previous crop harvested in 2021 or 2022.	-	-
		Hedge	-	kept	Presence (1) or absence (0) of a hedge at the edge of the plot studied in year 1 or 2.	-	
	Bio- diversity	SNH	-	Supp.	Score from 0 to 3 defined as follows: 3 points if forest (as defined by the Corine Land Cover 2021) and/or watercourse less than 200 m from the study area, 2 points if forest AND watercourse more than 200 m from the study area but less than 1km from the study area, 1 point if forest OR watercourse more than 200 m from the study area but less than 1 km from the study area, 0 if neither forest nor watercourse within 1 km of the study area.	-	More the better
		TFIh_n	nbHerbi_n	kept	Sum of the ratios of applied herbicide dose over recommended dose.	-	
	a	TFleh_n - kept			Sum of the ratios of applied treatment dose (except herbicide) over recommended dose. Seed treatment excluded.	-	
	cide us	nbHerbi_n - Supp.	Number of herbicide applications between the destruction of the previous crop (or intercrop) and wheat harvest in 2022 or 2023.	-	Less the		
	Pesti	nbFungi_n	-	Supp.	Number of fungicide applications between the destruction of the previous crop (or intercrop) and wheat harvest in 2022 or 2023.	-	
		nblns_n	-	Supp.	Number of insecticide applications between the destruction of the previous crop (or intercrop) and wheat harvest in 2022 or 2023.	-	

Compa- rtment	Sub- compa- rtment	Indicator	Correlated to	Decision	Description	Unit	Orienta- tion
		nbMoll_n	-	Supp.	Number of molluscicides in the 2022 and 2023 study years.	-	
		nbSDHI_n	-	Supp.	Number of SDHIs used in the 2022 and 2023 study years.	-	
		Tillage_intensity_rot	LastPlough, Tillage_n	kept	Score out of 18 representing the sum of Tillage_n scores over the last 5 years prior to the study year (<i>i.e.</i> 2017-2022, or 2018-2023)	Score	
	υ	lastPlough	Tillage_n	kept	Number of years since the last ploughing on the plot. For plots studied in 2022, the reference year is 2021 (2021 = 0, corresponding to autumn ploughing before winter wheat seeding), and for plots studied in 2023, the reference year used is 2022 (2022=0, corresponding to autumn ploughing before winter wheat seeding).	year	More the
	Tillag	Tillage_n	GasConsumption	Supp.	Type of tillage for seeding in 2022 or 2023: 0 = Direct seeding; -1=Light tillage; -2 = Heavy tillage; -3= Ploughing. We consider that light tillage = less 10 cm deep and strictly less than 3 machinery operations. Heavy tillage = at least one operation is > 10 cm depth or more than 3 machinery operations. Decompaction is not considered as a tillage operation.	Score	- Detter
		nbOperations_n	-	kept	Total number of machine operations during the crop year.	-	Less the better
	Index	RI_farm	Tillage_intensity_rot	Supp.	Score of Regeneration Index. Score /100 that provides information on the farm's agronomic performance.	Score	More the better
		Pair	-	removed	Farmer's associated pair. 22 pairs of farmers participated in the experiment in 2021-2022 and 21 in 2022-2023.	-	-
	Farm Identificat	Туре	NA	Supp.	Type of management practice: CA = Conservation agriculture, CONV= Conventional agriculture.	-	-
	ion	Year	-	removed	Monitored campaign 2021-2022 (year 1) or 2022-2023 (year 2).	-	-
		Location	NA	Supp.	Position of the field within the département in mainland France. Departments are described by their administrative number.	-	-
eral		d.seed	d.growth	Supp.	Number of days between 1/01/2021 or 01/01/2022 and the wheat seeding date in 2021 or 2022.	days	-
Gene	Date	d.harv	-	Supp.	Number of days between 1/01/2022 or 2023 and the wheat harvest date in 2021 or 2022.	days	-
		d.growth	-	removed	Number of days of wheat growth in 2022 or 2023.	days	-
	Farm	Farmtype	-	Supp.	Type of farm: 1 = mixed farms or animals' presence on farm (some farmers establish exchanges with other farmers to graze cover crops), 0 = cereal grower with no animals on farm.	-	-
		FarmSize	-	Supp.	Useful agricultural area.	ha	-
	Field	FieldSize	-	Supp.	Total size of the studied field. Measurements were held on a 1ha square of each field.	ha	-

Compa- rtment	Sub- compa- rtment	Indicator	Correlated to	Decision	Description	Unit	Orienta- tion
Soil	Soil	Texture_USDA	-	Supp.	Soil texture classified in the USDA classification.	-	-
Total init	ial variables	50					
Tota	al kept	17					
Total	removed	9					
Total Sup	p. variables	24					
N	/IDS	17					

	Dim. 1		Dim. 2		Dim. 3		Dim. 4	
	Contrib.	Corr.	Contrib.	Corr.	Contrib.	Corr.	Contrib.	Corr.
Hedge	0.00285064	0.011838	8.74541451	-0.488401	9.14846834	0.3726729	2.50218307	-0.177069
EffCC_rot	13.4781036	0.8139919	0.00282027	-0.008771	0.01624977	0.0157064	2.14391933	0.1639033
RestitRes_rot	2.09007953	0.3205436	5.21042101	0.3769842	6.8004485	-0.321309	35.086211	-0.663058
minN_rot	3.62679934	-0.422248	16.0195914	0.6610167	2.00170107	-0.174322	4.16584113	0.228473
minP2O5_rot	7.9764394	-0.626196	1.2534797	0.1849036	9.80905257	-0.385893	7.41123902	0.3047397
minK2O_rot	5.2087969	-0.506028	0.00071782	-0.004425	0.92971799	-0.118804	3.19680719	0.2001436
minSO3_rot	1.59601175	0.2801069	16.8548204	0.6780298	1.49898222	-0.150852	0.25798204	0.0568563
C_entries_rot	6.09799989	0.5475193	8.22692986	0.4737024	0.89183738	0.1163581	1.17907245	-0.12155
nbLeg_rot	10.9469095	0.7335867	2.11365529	-0.240106	0.46420771	0.0839479	0.53972594	-0.082238
NbOrginput_rot	2.70579654	0.3647147	0.78262844	-0.146105	25.5772652	0.623133	3.93919518	0.222171
CropDiv_rot	7.98819946	0.6266574	1.14255106	0.1765324	0.83984139	-0.112915	23.42339	0.5417619
Time_return_								
wheat	1.41653736	0.263888	2.38753393	-0.255189	15.0740479	-0.478375	7.19212879	-0.300201
TFIh_n	2.86410646	0.3752323	10.8006632	0.5427651	3.63092801	0.2347808	1.03187949	-0.11371
TFleh_n	3.66712328	-0.424589	8.56319241	0.4832864	12.6469474	0.4381739	0.14390715	-0.042464
Tillage_								
intensity_rot	16.1131228	0.8900114	0.00412711	-0.01061	1.1192704	-0.130353	0.17667679	0.0470515
lastPlough	11.9251824	0.765664	3.65938195	0.3159298	0.46197598	-0.083746	2.59053883	0.1801684
Nb								
Operations_n	2.29594124	-0.335959	14.2320717	0.623047	9.08905823	0.3714609	5.01930255	-0.250787

Table S4. Contributions and correlations of main variables to the first four dimensions of the PCA. In red: Most-contributing variables, in blue: Least-contributing variables.



Figure S1. Repartition of (A) Location, (B) Texture and (C) Previous crop in the different clusters.

(A) Location is separated into four main geographical zones, allowing to gather fields that are close despite their location in different administrative departments. Zone_1 gathers plots from Charente department (16) and the south of Vienne department (86), Zone_2 gathers plots from the north of Charente-Maritime (17) department and the south of Deux-Sèvres department (79), Zone_3 gathers plots from Indre-et-Loire (37) department and the north and middle of Vienne (86) department, Zone_4 gathers plots from east and west Deux-Sèvres (79) and east and west Maine-et-Loire (49). (B) Textures are expressed with the USDA classification based on soil samples analyses made in spring 2022 and spring 2023. (C) Previous crops to studied wheat are classified as follows: Spring_Cereal = Buckwheat, Grain maize, Grain sorghum, Seed maize and Silage maize; Legume = Alfalfa, Lentil, Meslin dominated by legumes and Pea, Winter Cereal = Winter barley and Winter wheat; Oilseed= Oilseed flax, Rapeseed, Rapeseed + legumes (co-culture) and Sunflower.



Figure S2. Pairs of farmers' occurrences in clusters.

Cluster 1 gathers only CONV farmers. Cluster 2 is also mainly made of CONV farmers, except 1 CA farmer in 2023. Cluster 3 is mainly composed of CA farmers except 4 CONV farmers, and cluster 4 is only composed of CA farmers. Pairs 2, 6,12_2023, and pair 9_2022 belonged to the same clusters, therefore their dots slightly overlap on the graph.
In Chapter 3 we described the setting of a robust OFE network of eighty-six CA and CONV fields over two successive monitoring years. The study provided a fine description of farmers' cropping practices, evidencing different strategies. Such fine multivariate analyses are key to unveiling farmers' cropping management strategies, beyond the classic terminology (*e.g.* CA *vs.* CONV). We showed that CA pillars are necessary but not sufficient to conclude on the full CA systems' sustainability. Similarly, we showed that CONV systems included a wide variety of practices. This systemic analysis of cropping practices was a necessary step towards the next chapter, which proposes an in-depth investigation of the effects of these identified cropping practices on the soil-production nexus, including social, economic and environmental performance indicators in a One Health perspective.

CHAPTER 4: EFFECTS OF CROPPING PRACTICES ON THE SOIL-PRODUCTION NEXUS

Keywords: Conservation agriculture, On-farm experiment, System approach

This chapter is presented as Part B of an article ready for submission in the European Journal of Agronomy.

This study aims to compare two cropping systems, Conventional (CONV) and Conservation Agriculture (CA) in real conditions. After having finely analysed each cropping practice of the system (**Chapter 3**), this chapter assesses the practice effects on soil health, crop production quality, and overall sustainability using a comprehensive set of indicators.

While agroecological or regenerative systems are often linked to better soil health as well as crop nutritional quality, these assumptions have rarely been tested in real-world conditions. In this study, we analysed on 86 winter wheat fields in Northwestern France from 2021 to 2023, the effects of CA and tillage-based practices in a One Health perspective, looking for different benefits or trade-offs for the health of soil, plants, environment and consumers.

The summary of measured indicator abbreviations and short descriptions is available in **Annexe 1** of the manuscript.

Graphical abstract



Figure 16. Graphical abstract of Chapter 4 showing the diverse effects of CA practices on One Health. *When a more efficient use of inputs (Cluster 3).

1. Introduction

The crop production system strongly influences the environment, soil, plant, animal and people health, also known as the One Health concept (also see **Chapter 1**). Through their influence on soil health, plant health and biodiversity, cropping practices can have positive or negative effects on the environment. Cropping practices also affect animal and human health through their ability to ensure feed/food security and feed/food safety and can contribute to the well-being of farmers when they ensure sustained livelihoods and decent revenues. Important research has been carried out on the topic. However, almost no application has been set up to try to demonstrate these links based on real-world data.

Winter wheat is the most widely cultivated crop in the world, and contributes to about 20% of the total dietary calories and proteins worldwide (Erenstein *et al.*, 2022; Shiferaw *et al.*, 2013). It therefore represents an interesting crop to study from a One Health perspective, since winter wheat is likely to be included in most farmers' cropping rotations. Wheat grain quality depends on the content of organic compounds (carbohydrates, proteins, lipids), mineral nutrients (P, K, Ca, and microelements), vitamins, antioxidants, and antinutritional compounds (Mallick *et al.*, 2013). These specific compounds mainly found in bran might explain why the consumption of wholegrain cereals has been associated with multiple beneficial health effects and the current recommendations support an increase of wholegrain consumption as part of a healthy diet for the prevention of chronic diseases (Hu *et al.*, *al.*, *al*

2020; Tammi *et al.*, 2024). Positive impacts on human gut microbiota associated with the consumption of intact cereal grain fibres, including wheat bran, were also reported (Jefferson and Adolphus, 2019). However, different factors may alter wheat quality, such as phytopathogenic fungi and the presence of mycotoxins in grains (Nada *et al.*, 2022), as well as the use of pesticides and possible pesticide residues in grain (Fantke *et al.*, 2011; Xu *et al.*, 2019). The environmental effects of current production systems (Crippa *et al.*, 2021) and questions around an apparent food nutrient content decline also arose in recent years, causing public health concerns (Marles, 2017; Mayer, Trenchard and Rayns, 2022).

From the crop production point of view, a shift to more agroecological practices would be likely to support positive nutrition and food security outcomes (Bezner Kerr *et al.*, 2021; Frison and Clément, 2020). In this regard, CA systems have increasingly been seen as a relevant alternative to conventional or traditional systems towards sustainable and productive agriculture (Derpsch *et al.*, 2024). However, although these systems have been widely studied, little information exists on the effects of CA and more generally of cropping systems on the environment, soil health and crop production quality. Information is even scarcer when looking at results coming from real-condition studies. Regarding CA systems only. Also, most studies considered them based on systemic approaches, rather focusing on no-till systems only. Also, most studies considered conventional systems with the lens of tillage-based systems only (Fairweather *et al.*, 2009; Palomo-Campesino, García-Llorente and González, 2021). These sometimes incomplete approaches lead to diverging conclusions on the effects of a given system on crop production and performances (Miner *et al.*, 2020; Pittelkow *et al.*, 2015). In addition, as discussed in **Chapter 1**, methodologies and indicators of soil health or production quality assessment, are also debated.

On-Farm Experiments (OFE) and systemic approaches to cropping system assessments are considered to be a valuable solution to obtain robust and meaningful results (Lacoste *et al.*, 2022).

A lot of research focuses on setting up in-field soil health assessments accessible to farmers, since these may foster the training and raise the awareness of farmers on the importance of soils, and therefore support the scaling-up of sustainable soil management practices by farmers (Hughes *et al.*, 2023). From a soil health assessment perspective, Biofunctool© was created as a cost-efficient and infield applicable tool (Thoumazeau *et al.*, 2019a, 2019b). Although it has been used mostly in tropical settings (Kulagowski *et al.*, 2021; Pheap *et al.*, 2019; Thoumazeau *et al.*, 2023), in research (Escande, Metral and Lévèque, 2021), and also has been included in agronomy graduate study programs (*e.g.* in Toulouse, Montpellier or Bordeaux Schools of Agronomy, pers. comm., 2024). Biofunctool[®] was therefore included in our set of soil health indicators.

After having set up the OFE and finely analysed the farmers' cropping practices within the newly established network, four cropping strategies were identified (**Chapter 3**). Two CONV cropping strategies were evidenced, with one more input-efficient and one less input-efficient (Clusters 1 and 2), and two CA cropping strategies also with one more input-efficient and the other less input-efficient (Clusters 3 and 4). Based on these results, the objectives of this study were to:

(1) Provide robust evidence of the cumulated effects of cropping practices on One Health components, through the analysis of soil health, wheat grain quality and system

performances, while limiting the risk of making confusing conclusions due to confounding cropping practice factors that we would not have considered,

- (2) Identify soil health, grain production or performance indicators that are more robust, reliable or sensitive to cropping practices by comparing:
 - a. indicators robustness and relevance to perform reliable assessments of soil health, grain quality and performance assessments of cropping systems, and
 - b. farmers' provided information versus measured indicators of crop performances, as a means to validate the robustness of the overall farmers' provided information.

To do so, we analysed an unprecedentedly large set of indicators to evidence cropping system effects on soil health, wheat grain and system performance during the two-year field monitoring.

2. Materials and methods

2.1. Study site, data collection and sample analyses

The study was conducted between 2021 and 2023 on 86 farmers' winter wheat fields in Northwestern France. The study zone extends on a North-South axis going from Tours to Angoulême and on a West-East axis going from La Rochelle to Poitiers cities. Farmers' selection was conducted in two steps according to strict criteria described in **Chapter 3**. All data was collected during campaigns 2021-2022 ("year 1") and 2022-2023 ("year 2"). Part of soil data at 0-10 cm depth was obtained across three sessions of sampling (SD_1, SD_2 and SD_3, as described in **Chapter 2**). Specifically, indicators corresponding to those included in the Biofunctool[®] kit and of the WET sensor (*i.e.* soil temperature, moisture, conductivity and permittivity) were measured directly in-field at positions A, B and C of the "W" pattern. Grain was collected in the summer of 2022 and 2023 on the principal study zone (P zone), as well as at positions A, B, and C of the "W" pattern (Figure 7, **Chapter 2**). These soil and grain samples were analysed whether directly in-field or at the research centre laboratory in Toulouse. The rest of the soil samples were collected by the certified laboratory (https://aurea.eu/) and sampled at 0-20 cm (or at soil maximal depth for the shallowest soils) across the "W" pattern and pooled to obtain one composite sample per field. All the methods and specific protocols are available in **Chapter 2**.

2.2. Selection of studied indicators

Our objective was not to define a new set of indicators, but rather to test well-known and newer soil health indicators (Lehmann *et al.*, 2020; Obriot *et al.*, 2016; Stott, 2019), and grain quality indicators while covering at best the main soil properties (physical, biological and chemical) and grain quality indicators (nutritional, technological and sanitary). The list of all tested indicators and their interest in assessing soil health and production quality is described in the following sub-sections. In total, we considered eighty-nine soil health parameters (including soil texture), twenty grain quality indicators and eight performance indicators. This sub-section does not provide details on laboratory and field protocols since they have already been detailed in **Chapter 2**.

2.2.1. Soil health indicators

This chapter focuses on soil textures measured after decarbonation (*Clay, Sand, Silt, DM* and *CaCO*₃), since textures without decarbonation were measured only in 2023 (see **Annexe 4**).

Soil physical properties indicators selected for this study were soil moisture (Moist s1, Moist s2) and temperature (Temp_s1, Temp_s2) at 0-10 cm depth, the crusting index (CIndex) at 0-10 cm depth, the Biofunctool measurements of soil aggregate stability at 0-10 cm (Agg), speed of infiltration of water in soil (Beerkan), and the visual evaluation of soil structure (scoreVESS) at 0-30 cm depth. As already explained, each Biofunctool® soil physical indicator was measured at three different positions of the W pattern on each studied field. As an interesting soil structural stability indicator (Johannes et al., 2017), we calculated the OM:clay ratio at 0-20 cm depth for all soils in the network based on information on texture measured after decarbonation. However, we obtained anormal results specifically for calcareous soils with CaCO₃ concentrations above 40% at 0-20 cm depth. The ratio was re-calculated in 2023 with clay concentration measurements without decarbonation, leading to different results. The correlation between the OM:clay ratio with or without decarbonation showed a strong discrepancy for soils with more than 40% CaCO₃, and for those with less than 40% CaCO₃, a regression line indicated that OM:clay ratio after decarbonation was overestimated as compared with those without decarbonation, as shown in Annexe 4 of this manuscript. As this ratio was developed on non-calcareous soils without decarbonation, we could not use it in the rest of the study, and it was therefore discarded.

The considered soil chemical properties were Organic Carbon (*OC*) and Organic Matter (*OM*) concentrations, C/N (*C_N*), soil water pH (*pH*), Metson cation exchange capacity (*CEC*), as well as all soil total and bioavailable elements analysed by the Aurea laboratory, *i.e.* total N (*s.TN*), available P₂O₅ (*s.AP*), total and available Mg (*s.TMg* and *s.AMg*), total and available K₂O (only in 2023, *s.TK₂O* and *s.AK₂O*), total and available Na₂O (*s.TNa₂O* and *s.ANa₂O*), total and available Zn (*s.TZn* and *s.AZn*), total and available Mn (*s.TMn* and *s.AMn*), total and available Fe (*s.TFe* and *s.AFe*), total and available B (*s.TB* and *s.AB*), total and available S (*s.TS* and *s.ASO4*), total and available Mo (*s.TMo* and *s.AMo*), total and available Cu (*s.TCu* and *s.ACu*), as well as the ratios of bioavailable: total elements (*s.ratioMg*, *s.ratioK2O* -2023 only-, *s.ratioNa*, *s.ratioZn*, *s.ratioMn*, *s.ratioFe*, *s.ratioB*, *s.ratioS*, *s.ratioMo* and *s.ratioCu*). All these elements were measured on soil samples taken at 0-20 cm depth.

Three supplementary WET Sensor measured variables were added, namely pore water conductivity (*ECp_s1* and *ECp_s2*), soil bulk electrical conductivity (*ECb_s1* and *ECb_s2*) and soil permittivity (*Perm_s1*, *Perm_s2*) at 0-10 cm depth.

Biologically mineralisable nitrogen (BMN) expressed as a percentage of total N (*BMN_Ntot*) and in mg/kg of dry soil (*BMN_tot*), as well as concentrations of KMnO₄ carbon (also known as Permanganate Oxidisable Carbon – POXC, or labile carbon) expressed as a percentage of total OC (*s.POXC_OC*) or as mg/kg of dry matter at 38°C (*s.TPOXC*) were also analysed, from soil taken at 0-20 cm depth. KMnO₄ carbon measurements were also performed at 0-10 cm depth using the Biofunctool® protocol in year 1 and confronted with the certified laboratory measurements. Biofunctool® POXC measurements were aborted in year 2 due to important divergences between the results of the two protocols. All details are explained in **Annexe 5**.

In addition, we measured granulometric fractionation of OC (C_0_{50} , C_{200}_{2000} , C_{50}_{200} , C_{50}_{2000}), N (N_0_{50} , N_{200}_{2000} , N_{50}_{200} , N_{50}_{2000}) and C/N ($C_N_0_{50}$, $C_N_{200}_{2000}$, $C_N_{50}_{2000}$, $C_N_{50}_{2000}$, $C_N_{50}_{2000}$) in the 0-50, 50-200, 200-2000 and 50-2000 µm fractions at 0-20 cm depth.

Regarding biological soil properties, as part of the Biofunctool[®], we used lamina baits (*Laminas*) in 2022 and Litter bags in 2023 (*Litter_bags*), and soil basal respiration after 24 and 48 hours of incubation (*SituResp24, SituResp48*). We also considered soil N-acetylglucosaminidase (*NAG*), β -glucosidase (*Beta_Glu*), and phosphatase (*Phosphatase*) enzyme activities. These analyses were run from samples taken at 0-10 cm depth.

As part as Aurea's analyses on samples taken at 0-20 cm depth, C mineralisation after 3, 7, 14, 21 and 28 days of incubation expressed in mg of C-CO₂/kg of dry soil (*minC_3d, minC_7d, minC_14d, minC_21d and minC_28d*) or as a percentage of total OC (*minC_28d_OC*), N mineralisation after 28 days of incubation, expressed as mg of mineral N (N-NH₄ + N-NO₃)/kg of dry soil (*minN_28d*) or as a percentage of total N (*minN_28d_Ntot*), microbial molecular biomass (*MMB*), total microbial carbon expressed in mg C /kg dry soil (*TMC*) or as a percentage of the total OC concentration (*TMC_OC*), abundance of bacteria (*AbundBact*) and fungi (*AbundFungi*), the abundance ratio fungi:bacteria (*FBRatio*), and the diversity of bacteria (*DivBact*) and fungi (*DivFungi*) were measured.

Finally, an attempt was made to include soil macrofauna measurements based on small Berlese installations (Edwards, 1991), with soil sampled at 0-10 cm depth including the following adaptations : (i) Berlese had to be stored at farmers' places since we could not transport them back to the laboratory, (ii) farmers were asked to store the Berlese traps in a dry and bright room (*i.e.* window front) for 1.5 months. We installed three Berlese traps per plot. Unfortunately, after 1.5 months, we could not observe any insects in most of the traps, and a very limited number in some of them. The Berlese analysis was therefore aborted.

2.2.2. Grain quality

Nutritional grain quality indicators were selected according to their importance in grain composition as well as their importance for human nutrition and health.

Analyses of total N (*g.TN*), Fe, Mn, Zn, K and P concentrations (*g.Fe*, *g.Mn* and *g.Zn*) were performed in a certified laboratory. Starch (*Starch*), cellulose (*Cellulose*), and protein (*Prot*) contents in grain were also analysed. For minor elements, vitamin B9 (*B9*), antioxidants (*Antiox*), polyphenols (*Polyph*) and ERGO (*Ergo*) concentrations were measured. Selenium (Se) concentrations were also analysed, since cereals are also one of the dominant food sources of Se (Combs Jr, 2001), and Se is an important micronutrient, essential for a number of enzymes that perform important metabolic functions necessary for human and animal health, but all values fell systematically under the laboratory detection rate and therefore values were not kept in the analysis.

Sanitary quality indicators considered in the analysis were the three types of mycotoxins deoxynivalenol (*DON*), HT-2 toxins (*HT2*), and zearalenone (*ZEA*), as well as glyphosate and AMPA residues in grain.

As technological grain quality indicators, we considered the number of grains per ear (*Grain_ear_n*), the thousand kernel grain (*TKW*), the experimentally measured specific weight (*ExpSW_n*), as well as the farmers' provided specific weights (*FarmerSW_n*).

2.2.3. Performance

We considered the efficiency of mineral nitrogen (*minN_eff_n*), as well as the experimentally measured yield (*ExpYield_n*) and farmers' provided yield (*FarmerYield_n*). In addition, Systerre[®] environmental, economic and social performance indicators were analysed and added to the analysis, *i.e.* the semi-

net margin (*SNMargin*), the total input expenses (*InputExpenses*) and the total production cost (*ProductionCost*) for the economic indicators, gas consumption (*GasConsumption*) and greenhouse gas emissions (*GHGtotEmiss*) as environmental indicators, and the in-field working time per hectare (*WorkingTime*) as representative of a social performance indicator.

2.3. Statistical analysis

All the analyses were run with R studio (R Core Team, 2021). Data were visualised and manipulated with the tidyverse environment (Wickham *et al.*, 2019). Since the repartition of results did not always follow a normal law, we ran Wilcoxon paired-rank tests to compare soil health, grain quality and performance results within a pair of CA/CONV farmers. Wilcoxon tests were run using the wilcox_test function of the {rstatix} package (Kassambara, 2023).

Soil textural classes did not always result to be identical within a pair of farmers, thus for the comparison of soil health data, we kept in the CA *vs.* CONV analysis farmers whose textural classes were identical or up to 1 soil class difference according to the USDA reference (Figure S3, Table S1 of **Chapter 3**). This excluded three pairs of farmers, *i.e.* pairs n°2, 4 and 9 in year 1. Pair n°2 kept the same plot between year 1 and year 2 but was not excluded from the analysis in year 2. Indeed 2_CONV plot was sloppy and heterogeneous, so we changed position in year 2 and managed to find a position where soil texture was almost equivalent to 2_CA.

Similarly, as regards to grain analyses, adjustments were made for six pairs of farmers. For two pairs in year 1, the neighbour farmer dedicated one part of their plot to the study and seeded the rest of their plot with their own wheat, of different variety than the one used for the study. However, at the time of sampling in April, we could not differentiate the different varieties, and farmers did not remember where precisely they had sown the right variety (pairs 4 and 8). Therefore, we excluded them from the grain technological analyses. We did not exclude them from the nutritional grain analysis, since at harvest, they all gathered grain of the same variety for the analysis (therefore not directly coming from the same part of the field, but which was managed identically). For four other pairs of farmers, the whole plot was seeded without respecting the rule of seeding the same wheat variety within the same plot. Therefore, these four pairs were excluded from all the grain quality analyses (pair 13 and 14 in 2022, 11 and 14 in 2023).

Due to non-normality of variables repartition in the clusters, the variation between clusters was quantified using the Kruskal Wallis test, with Dunn adjustment to allow for multiple comparisons (dunnTest function of the {FSA} package (Ogle *et al.*, 2023)). All plots were included in these comparisons.

Principal Component Analyses (PCA) were run with the PCA function of the {FactomineR} package (Lê, Josse and Husson, 2008) to evidence any effects of covariates on soil health, grain quality and system performances. Missing values were imputed with the imputePCA function of the {missMDA} package (Josse and Husson, 2016), based on the first five dimensions of the PCA.

3. Results

For each indicator, we analysed the results of the dichotomic CA-CONV means comparison and of the four clusters comparison, and classified them into four groups, as presented in Table 4. About three-quarters of the indicators showed no difference according to the type of management, regardless of the comparison made (Group A, 62% of the performance indicators, 75% of the grain quality indicators and 66% of the soil indicators). The remaining indicators showed differences according to cropping management, either when analysed by cluster (Group B), by CA-CONV comparison (Group C) or for both modes of analysis (Group D) (Table 4, Figure 17).

-							
Is there a difference	Is there a difference	Numbe	Number of concerned				
between CA and	between clusters?	indicat	indicators				
CONV systems?		Soil	Grain	Performance			
NO	NO	63	15	5	А		
NO	YES	5	2	1	В		
YES	NO	14	3	0	С		
YES	YES	12	0	2	D		
Total indicators		89	20	8			

Table 4. Repartition of results according to the simple CA/CONV comparison, versus the cluster comparison.



Figure 17. Repartition of soil health, grain quality and performance indicators within the different comparison groups.

3.1. Soil health indicators

3.1.1. Possible co-factors affecting soil health

The repartition of clay, silt, sand and CaCO₃ concentrations between the two compared groups and between clusters was homogeneously spread at 0-20 cm depth (Group A, Table S5). Considering all studied soil health indicators, the first two axes of the PCA explained 35.1% of the variability (Figure S4A). It was difficult based on the PCA to evidence any Type or Cluster effect on soil health indicators taken all together (Figure S4B, C). Based on the PCA results, we could not find evidence of any year, previous crop or pair effect (Figure S4D, E and G). However, the PCA evidenced a little zone effect, specifically on plots located in zone 2 (the south-western zone of the study), which are well correlated to dimension 2 (Figure 4F).

3.1.2. Soil biological indicators

Total Microbial Carbon (*TMC*) and Molecular Microbial biomass (*MMB*) at 0-20 cm depth were on average 15% and 28% higher in CA than in CONV systems (p=4.61E-03 and p=7.23E-04 respectively). Specifically, cluster 3 had the highest concentrations of TMC (+50%, p=0.046) and MMB (+43.5%, p=0.029) compared with cluster 2 (Group D, Table S16). Total C and N mineralisation after 28 days at 0-20 cm (*minC_28d, minN_28d*), and soil basal respiration after 24 hours at 0-10 cm (*SituResp24*) were 10%, 26% and 50% higher in CA than in CONV systems (p=0.016, p=0.044 and p=0.002 respectively), but when comparing these results between clusters, no differences were evidenced (Group C, Table S14). An additional discussion on C mineralisation measurements is available in Annexe 5. However, TMC and mineralised C after 28 days expressed as a percentage of OC at 0-20 cm (*TMC_OC* and *minC_28d_Ntot*) showed no differences between CONV and CA systems nor between the different clusters of practices. In general, TMC represented about 3% of OC, and minC after 28 days represented 1.25% of OC, while minN (NH₄ and NO₃) after 28 days stood for about 0.7% of total N (Group A, Table S6).

Bacteria abundance (*AbundBact*) and diversity (*DivBact*) at 0-20 cm depth were not significantly different between CA and CONV types, nor between clusters (Group A, Table S6). However, CA plots showed a 15.2% lower in fungi abundance (*AbundFungi*) compared with CONV plots (p=0.01), and a 7.7% lower fungal diversity (*DivFungi*) compared with CONV systems at 0-20 cm depth (p=0.014). Abundance ratio fungi:bacteria (*FBRatio*) was also lower in CA than in CONV at 0-20 cm depth (3.2 in CA and 3.7 in CONV, p=0.005) (Group C, Table S14). For *AbundFungi, DivFungi*, and *FBRatio*, no differences were evidenced between clusters.

Regarding enzymatic activities at 0-10 cm depth, N-acetylglucosaminidase (*NAG*) and β -glucosidase (*Beta_Glu*) enzyme activities were irresponsive to crop management (Group A, Table S6), but phosphatase (*Phosphatase*) activity was 30.6% higher in CA compared with CONV systems (p=7.60E-04), and specifically, its activity was 45.7% higher in cluster 3 compared with cluster 2 (p=0.007, Group D, Table S16).

Lamina baits scores (*Lamina*) did not allow to discriminate crop management in year 1 (Group A, Table S6). On the contrary, litter bag degradation (*Litter_bag*) was 28% higher in CA systems than in CONV systems in year 2, although no differences were evidenced between clusters (p=0.016, Group C, Table S14). A summary of these results is presented in Table 5.

Table 5. Summary of results for chemical elements.

Detailed information is available in Tables S6, S14 and S16. When a difference between clusters was identified, the p-value corresponds to the result of the Kruskal Wallis test with Dunn adjustment. When the difference is between CA vs. CONV, the p-value corresponds to the results of the paired Wilcoxon test.

Biological indicator	Sampling depth	Observed difference	p-value
	(cm)		
ТМС	0.20	+15% in CA compared to CONV;	4.61E-03;
	0-20	+50% in Cluster 3 compared to Cluster 2	0.046
TMC_OC	0-20	No difference	>0.05
ММВ	0.20	+28% in CA compared to CONV;	7.23E-04;
	0-20	+45% in Cluster 3 compared to Cluster 2	0.029
SituResp24	0-10	+ 50% in CA compared to CONV	0.002
minC_28d	0-20	+ 9% in CA compared to CONV	0.016
minC_28d_OC	0-20	No difference	>0.05
minN_28d	0-20	+ 26% in CA compared to CONV	0.044
minN_28d_Ntot	0-20	No difference	>0.05
AbundBact	0-20	No difference	>0.05
DivBact	0-20	No difference	>0.05
AbundFungi	0-20	-15% in CA compared to CONV	0.01
DivFungi	0-20	-8% in CA compared to CONV	0.014
FBRatio	0-20	-13.5% in CA compared to CONV	0.005
NAG	0-10	No difference	>0.05
Beta_Glu	0-10	No difference	>0.05
Phosphatase	0.10	+31% in CA compared to CONV	7.60E-04;
	0-10	+46% in Cluster 3 compared to Cluster 2	0.007
Lamina	0-10	No difference	>0.05
Litter_bag	0-10	+28% in CA compared to CONV	0.016

3.1.3. Soil chemical indicators

Soil pH, CEC and C/N showed no significant difference according to cropping practices. The average soil pH was about 7.3, with a coefficient of variation (CV) of 7 to 10% according to the different clusters. Mean CEC was 14 mEq/100g. However, within-group variations were important (CV ranging from 35.9% in cluster 1 to 47.1% in cluster 2). Conductivity (bulk and pore) and permittivity showed no significant difference between the CA and CONV groups, nor between the clusters (Group A, Table S7).

Organic matter (*OM*) and total N (*s.TN*) concentrations were significantly higher in CA than in CONV systems (3.4% in CA vs 2.4% in CONV on average for *OM* with p=3.16E-03, and 0.2% in CA vs 0.1% in CONV for *s.TN* with p=4.82E-03). Specifically, for both indicators, cluster 3 showed higher *OM* and *s.TN* concentration values than cluster 2 (p= 0.036 and p=0.034 respectively) (Group A, Table S12).

Total Fe, Zn, Cu and Mo concentrations were different between clusters while they showed no significant differences in the CA vs. CONV comparison. Total Fe and Zn concentrations were higher in cluster 3 than in cluster 2 and 4 (p=0.01 and p=0.01 for Fe cluster 3 compared with cluster 2 and 4 respectively, and p=0.01 and 0.002 for Zn cluster 3 compared with cluster 2 and 4 respectively). Total Cu was higher in cluster 3 than in cluster 2 (p=0.008) (Group B, Table S13). We noted the presence of

outliers in total Fe, Zn, Cu and Mo total concentrations: four plots had a total Fe concentration higher than 70 000 mg/kg (4_CA_2023, 7_CA and 7_CONV_2022, 15_CA_2023), two plots had a total Zn concentration above 300 mg/kg (5_CONV_2022 and 7_CA_2022), one plot had a total Cu concentration of more than 190 mg/mg (9_CA_2022), and one plot had a total Mo concentration of more than 5 mg/kg (7_CA_2022). Removing these outliers in the clusters multiple comparisons did not influence the results, except for Mo. When removing the only outlier in Mo comparisons, the average concentrations between clusters were no longer significantly different. All the other total element concentrations (K₂O, Mg, Mn, Na₂O, S and B), showed no significant differences both in the CA *vs.* CONV comparison and between clusters. They also had high CVs (on average higher than 50%) (Group A, Table S8). A summary of these results is presented in Table 5.

Most available chemical elements presented no differences between CA or CONV groups or between clusters (K_2O , Mn, Na₂O, Su, B, Fe, P, Cu and Mo) (Group A, Table S8), except available Mg and Zn concentrations that were 35.4% and 45% higher in CA compared with CONV (p=0.002 and p=1.54E-02 respectively). Specifically, available Mg was higher in cluster 3 than in cluster 2 (p=0.002), while available Zn was higher in cluster 3 than in cluster 1 (p=0.04) (Group D, Table S16). A summary of these results is presented in Table 5.

When looking at the proportion of available elements on the total concentration (ratios available:total concentrations), most elements also showed no difference between clusters (K₂O, Mn, Na₂O, S, Fe, Mo) (Group A, Table S8), except for Mg, Zn and B. The relative proportion of available Mg, Zn and B as part of their total concentration was higher in CA systems than in CONV systems: 5% of total Mg was available to plants in CA systems against 4% in CONV systems (p=5.38E-04), 5.4% of Zn was available in CA systems against 3.7% in CONV (p=0.049), and 3.7% of total soil B was available in CA systems compared with 3.1% in CONV (p=0.04) (Group C, Table S14). A summary of these results is presented in Table 6.

Chemical	Total	Available	A/T			
element						
K ₂ O	No difference	No difference	No difference			
Ma	No difference	CA > CONV	CA > CONV			
IVIS		Cluster 3 > Cluster 4				
Mn	No difference	No difference	No difference			
Na ₂ O	No difference	No difference	No difference			
Su	No difference	No difference	No difference			
В	No difference	No difference	CA > CONV			
Fe	Cluster 3 > Cluster 2 & 4	No difference	No difference			
Р	NA	No difference	NA			
Cu	Cluster 3 > Cluster 2	No difference	Cluster 2 > Cluster 3			
Мо	Cluster 3 > Cluster 4*	No difference	No difference			
7n	Cluster 3 > Cluster 2 & 4	CA > CONV	CA > CONV			
211		Cluster 3 > Cluster 1				

 Table 6. Summary of results for chemical elements. For these indicators, sampling depth was 0-20 cm.

 *When removing the outliers, the difference was cancelled. A/T = Ratio of chemical available element/total element.

Total labile carbon concentration (POXC) was 12.5% higher in CA compared with CONV systems (1 162 mg/kg in CA vs. 1 033 mg/kg in CONV, p=0.004). However, the difference was not reflected in

the clusters, where although we observed a 14% difference between cluster 1 and cluster 4, the high CV and lesser number of individuals in the clusters prevented from identifying any significant difference. An additional discussion on POXC measurements is available in **Annexe 5**. Biologically mineralisable N expressed as a percentage of total soil N (*BMN_Ntot*) represented about 2% of total N in all systems, while total BMN (*BMN_tot*) concentration was higher in CA than in CONV in the paired comparisons (p=0.001), although the difference was not reflected in the clusters (Group A, Table S7, and Group C, Table S14).

Most granulometric fractionations of OC showed no difference both in the CA vs. CONV comparison and in the cluster comparisons (Group A). All of them, except the C fractionation in the 0-50 μ m textural class (average CV=6%), showed high CVs ranging from 11% to about 50% per cluster (Group A, Table S9). Differences occurred in the 50-200 μ m textural class where more C was found in CA systems than in CONV (9.8% of total C in CA and 8.5% of total C in CONV, p=0.037). However no significant differences were identified between clusters.

N granulometric fractions were differently spread between CA vs. CONV systems. First, a lower proportion of total N was found in the soil's finest fraction (0-50 μ m) of CA compared with CONV soils (N_0_{50}). On average, N_0_{50} was 87% in CA against 90% in CONV soils (p=9.53E-04). Specifically, cluster 3 showed a lower N_0_{50} (86.8%) compared with cluster 1 (90%, p=0.04) (Group D, Table S16). Inversely, CA systems showed the highest proportion of N in the 50-200 μ m (N_{50}_{2000}) and 50-2000 μ m (N_{50}_{2000}) soil fractions compared with CONV systems. Second, N_{50}_{200} was 8.6% in CA against 6.5% in CONV soils (p=1.46E-04). Specifically, in cluster 3, N_{50}_{200} was 8.8% which was significantly higher than cluster 1 and 2 (6.2 and 6.8% respectively, p=0.006 and p=0.004). Third, N_{50}_{2000} was 13% in CA against 10.4% in CONV soils (p=9.02E-4). A similar pattern as N_{50}_{200} was observed for N_{50}_{2000} where cluster 3 had the highest percentage values when compared with cluster 1 (13.2% in cluster 3 vs. 10.0% in cluster 1, p=0.04) (Group D, Table S16).

Finally, a lower fraction of the C/N was found in CA compared with CONV in the 50-200 ($C_N_50_200$) and 50-2000 µm ($C_N_50_2000$) fractions (p=0.012 for $C_N_50_200$, and p=2.91E-04 for $C_N_50_2000$; Group C, Table S14). These results are summarised in Table 7.

Table 7. Summary of results for granulometric fractionations at 0-20 cm depth. The finest fraction is considered to be the most stable fraction, the 50-200 μ m the labile fraction, while the 200-2000 μ m is considered to be the most fresh fraction (Christensen, 2001).

Fraction	0-50 μm	50-200 μm	200-2000 μm	50-2000 μm
	Most stable fraction	Labile fraction	Fresh fraction	Labile + Fresh fraction
Total C	No difference	CA > CONV	No difference	No difference
Total N	CA < CONV	CA > CONV	No difforance	CA > CONV
	Cluster 1 > Cluster 3	Cluster 3 > Cluster 1 & 2	No unerence	Cluster 3 > Cluster 1
C/N	No difference	CA > CONV	No difference	CA < CONV

3.1.4. Physical soil properties

Only the stability of aggregates (*Agg*) differed between the CA and CONV cropping systems, with CA systems holding higher *Agg* scores compared with their CONV neighbours at 0-10 cm depth (p=1.97E-05). Specifically, cluster 3 and 4 had the highest score values compared with cluster 1 (p=0.003 between cluster 1 and 3, and p=0.008 between cluster 1 and 4), and cluster 3 also had higher scores than cluster 2 (p=0.03 between cluster 2 and 3) (Group D, Table S16). All the other soil physical

properties showed no differences between CA and CONV groups at 0-10 cm depth (temperature, moisture, crusting index, infiltration or VESS). However, coefficients of variation were very high for these indicators (up to 117% for Beerkan values in CA systems) (Group A, Table S10).

3.2. Grain quality indicators

3.2.1. Possible co-factors affecting grain quality

The first two axes of the PCA gathering all grain quality indicators explained 37.4% of the variability (Figure S5A). It was difficult based on the PCA to evidence any Type or Cluster effect on grain quality indicators taken all together (Figure S5B, C). Based on the PCA results, we could not find evidence of any geographic zone, previous crop or pair effect (Figure S5E, F and G). However, the PCA evidenced a year effect, specifically for plots located in zone 2, with grains of year 2 which seemed to have a lower associated B9, antioxidants, P and K concentrations (Figure 5D). It was difficult to evidence any wheat variety effect on grain quality due to the too large number of possible combinations. Indeed, we recorded 28 combinations of different wheat varieties or mixes.

3.2.2. Nutritional grain quality indicators

Most nutritional grain quality indicators showed no difference between CA vs. CONV systems or between the different clusters of practices (*Starch, g.TN, Prot, g.Fe, g.Mn, Polyph, Antiox, B9*) (Group A, Table S11). However, for cellulose and Zn content, although no differences were detected in the CA vs. CONV comparison, we observed differences in clusters repartitions. Cellulose (*Cellulose*) content was higher in cluster 3 than in cluster 2 (p=0.04), while grain Zn content (*g.Zn*) was higher in cluster 3 than in cluster 2 (p=0.04 and p=0.02), but no differences were observed between clusters. Ergothioneine (*Ergo*) concentrations were also higher in CA than in CONV (2.0 ng/mg in CA and 1.8 ng/mg in CONV on average, p=0.047). However, no differences were observed in the cluster comparisons (Group C, Table S15). All results are summarised in Table 8.

Table 8. Summary of results for grain quality indicators.

Detailed information is available in Table S13 and Table S15. When a difference between clusters was identified, the p-value corresponds to the result of the Kruskal Wallis test with Dunn adjustment. When the difference is between CA vs. CONV, the p-value corresponds to the results of the paired Wilcoxon test.

Nutrient	Observed difference	p.value
Starch	No difference	>0.05
Cellulose	Cluster 3 > Cluster 2	0.040
Total Nitrogen	No difference	>0.05
Proteins	No difference	>0.05
Р	CA > CONV	0.036
К	CA > CONV	0.022
Fe	No difference	>0.05
Mn	No difference	>0.05
Zn	Cluster 3 > Cluster 4	0.00009
Polyphenols	No difference	>0.05
Antioxidants	No difference	>0.05
Vitamin B9	No difference	>0.05
Ergothioneine	CA > CONV	0.047

Figure 18 displays the relationships between soil and grain elements measured during the two years. R² are all below 0.1 suggesting an absence of linear relation between soil available nutrients and wheat grain nutrients.

3.2.3. Sanitary quality indicators

No significant difference was found in the presence of mycotoxins between the CA and CONV groups, or between the clusters (Group A, Table S11). Nevertheless, 7 out of 86 samples showed the presence of DON mycotoxins (4 in 2022 and 3 in 2023). Of these seven samples, six came from CA plots and one from a CONV plot. Values ranged from 63 μ g/kg (2_CONV_2022) to 318 μ g/kg (15_CONV_2022). Previous crops associated with the samples concerned by DON presence were either maize (2 samples), sunflower (2 samples), rapeseed (1 sample), buckwheat (1 sample) or pea (1 sample). Only 1 in 86 samples showed the presence of HT2 mycotoxins at 62 μ g/kg (11_CA_2022). 2 out of the 86 samples were concerned by the presence of ZEA mycotoxins, at very low rates (10 and 26 μ g/kg). These two samples came from CA fields (16_CA_2022 and 19_CA_2023).

On the 40 samples analysed for glyphosate and AMPA, residues were detected on one sample only, at a concentration <0.01 μ g/kg (glyphosate) and at 0.014 μ g/kg (AMPA) (10_CA_2023). The concerned farmer had sprayed 2L/ha of glyphosate concentrated at 360 g/L for the last time nine months before wheat harvest and sampling (also see **Case study n°2** for a deeper investigation on pesticide residues in grain).

3.2.4. Technological quality indicators

We observed no difference in specific weight (*FarmerSW_n*, *ExpSW_n*), thousand kernel weight (*TKW*) or number of grains per ear (*Grain_ear_n*) between CA and CONV cropping systems, nor between the clusters (Group A, Table S11).



Figure 18. Relations between soil available nutrients and grain available nutrients.

Soil concentrations were measured in year 2 only for K₂O concentration and all the other elements in both years. Red line corresponds to the regression line following a linear model. Grey area represents the standard error associated with the regression line.

3.3. Performance indicators

The first two axes of the PCA gathering all performance indicators explained 56.6% of the variability (Figure S6A). It was not possible to evidence any zone, previous crop or pair effect based on the PCA results (Figure S6E, F and G). The PCA evidenced a *Type* effect, with CONV plots situated on the upper part of the graph and CA plots on the bottom part, showing a lower working time (*WorkingTime*), gas consumption (*GasConsumption*) and input expenses (*InputExpenses*) in CA plots compared with CONV plots. These differences were also seen in the clusters repartition (Figure S6B and C). We also observed a year effect, with 2023 being more homogeneous than 2022 (Figure S6D).

(semi-net margin; *SNMargin*, and production costs; *ProductionCost*) (Group A, Table S12). Over the two monitored years, three plots showed a negative *SNMargin*, all in 2022 (5 and 11_CONV and 22_CA). These three farmers had relatively low yields in 2022 (respectively 36, 27 and 45 q/ha). On the contrary, seven farmers had yields lower than 45 q/ha with associated positive *SNMargin* ranging from 20.8 €/ha (10_CONV 2022, *farmerYield_n* = 41 q/ha) to 229.8 €/ha (18_CA_2022 with *farmerYield_n* = 40 q/ha). Overall, the *SNMargin* decreased as *ProductionCost* increased regardless of the associated yield and cropping practices (Figure 19). Input expenses (considering expenses to buy fertilisation and pesticides; *InputExpenses*) were not significantly different between CA and CONV systems (a tendency was observed, p=0.07), but were significantly lower in cluster 3 compared with all the other clusters (p=0.004 between cluster 3 and cluster 1, p=0.04 between cluster 3 and cluster 2, p=0.04 between cluster 3 and cluster 4), with an average expense of 429 €/ha in inputs in cluster 3 against 490 to 530 €/ha in the three other clusters (Group B, Table S13).

Production performance indicators showed no difference between CA *vs.* CONV and between clusters (yield; *farmerYield_n* and *ExpYield_n*, and mineral N efficiency; *minN_eff_n*) (Group A, Table S12).

Greenhouse gas emissions (*GHGtotEmiss*) showed no difference between CA and CONV cropping systems nor between clusters, with mean emissions of about 2 300 to 2 500 kg CO₂-eq/ha/year (Group A, Table S12). *GasConsumption* was significantly lower in CA compared with CONV systems (p=1.59E-12), and it was also significantly lower in cluster 3 and 4 with mean *GasConsumption* of less than 40 L/ha/yr compared with cluster 1 and 2 with mean *GasConsumption* of about 57 L/ha/year (between cluster 1 and 3, p= 3.8E-06; between cluster 2 and 3, p=2.7E-09; between cluster 1 and 4, p=1.0E-03 and between cluster 2 and 4, p=1.3E-05) (Group D, Table S16).

Finally, *WorkingTime* was significantly lower in CA than in CONV systems (p=1.21E-06). It was lower in cluster 3 than in cluster 1 (p=5.2E-04) and cluster 2 (p=6.3E-08), and lower in cluster 4 than in cluster 1 (p=1.3E-01) (Group D, Table S16).



Figure 19. Semi-net margin evolution according to production cost. Blue dots represent yields higher than 60 q/ha. Black dots represent yields lower or equal to 60 q/ha. Grey dots were added when we had no information on the farmer's yield. Red lines show regression lines, measured based on yield data (missing data were excluded from the regression calculation).

4. Discussion

4.1. Soil and environmental health

4.1.1. Carbon transformations and nutrient cycling in CA soils

The increase in OM content in CA soils (about 3.4%) compared with CONV soils (2.4%), is in the same order as the loss in soil OM measured in a mountainous French region by Saby *et al.* (2008), with a drop from 3.87% to 2.87% between 1990 and 2004, due to both changes in land use, from permanent grassland to cultivation, and to an increase in temperature during the survey period (1990-2004). This should also be put in perspective with the 4p1000 initiative (Minasny *et al.*, 2017): with 41.7% higher OM in CA than in CONV, seeming gained over an average period of 13.4 years of implementation of CA in our study, the annual gain of 3.1% is far above the objective of 0.4%. In addition, total N (*s.TN*) content was higher in CA than in CONV.

CA systems led to significant positive effects on *POXC_tot* and *BMN_tot*, although there was no evidence of a positive impact on these indicators when expressed as a percentage of OC or total N. This suggests that their concentration in soils increased, together with OC and total N concentrations, so their total quantity in the soils increased as well. The higher N and C mineralisation rates in CA, consolidate the hypothesis of a higher soil microbial presence and activity in CA soils. The highest degradation of litter bags in CA plots in year 2 also suggests a higher biological activity in the superficial soil horizon. These results confirm the findings of Li *et al.* (2018) who evidenced in their meta-analysis an increased BMN by 64% in CA soils.

We did not observe significant effects of CA on pH, CEC or C/N levels in the superficial soil horizon. These results do not confirm Franzluebbers and Hons (1996) assumptions that increased SOM in the topsoil with zero tillage would lead to acidity from decomposition, but would rather confirm Duiker and Beegle (2006) hypothesis that soil pH under no-tilled soils would be buffered due to the higher SOM content. Furthermore, the absence of significant difference in soil pH between CA and CONV systems (soil pH in our study ranged from 5.55 to 8.30) with linear statistical models might be related

to the non-linearity of the processes at stake, OM contributing to the development of neutral to slightly acidic soil pH (Brady and Weil, 2010; Husson, 2013). The lack of difference between CEC values in CA and CONV is surprising given the significant higher OM content in CA but cannot be explained with certainty by the data available. However, it could be linked to the nature of the OM in ACS soils, the OM in the process of humification (revealed by higher *s.POXC_tot* in CA) having lower CEC values than an OM well evolved into humus (Antil *et al.*, 2014).

The differences observed in soil nutrient content concerned principally available Mg, Zn and B whose concentrations were significantly higher in CA than in CONV systems. Increased Zn contents in topsoil under CA were also evidenced by Franzluebbers and Hons (1996), likely to be explained by the presence of mulches. Overall, these differences might be related to changes in soil oxidation-reduction and pH conditions which strongly impact Zn, Mg and B solubility. Indeed, CA systems lead to reduction in the surface horizons and neutralisation of pH, while plant roots can greatly impact pH in their rhizosphere (Husson, 2013; Husson *et al.*, 2018).

Fractionation of C, N and C/N are interesting indicators providing information on the repartition of C, N and the C/N across the different soil fractions. Regarding C, the only difference, in favour of CA, was observed on the 50-200 µm fraction, which is associated with the labile fraction. This higher quantity of C in CA systems might be explained by their more frequent use of cover crops. N was also more concentrated in the 50-200 μ m of CA soils. Inversely, N was more concentrated in the 0-50 μ m fraction in CONV soils. This suggests that different processes are at stake in the different fractions, and that CA systems best concentrate N in OM in the 50-200 µm fraction, while CONV systems tend to concentrate it in the finest fraction. As a result, N was more concentrated in CA soils. Given that the C/N ratio values of soil bacteria and fungi are 4–6:1 and 10–15:1 respectively (Cleveland and Liptzin, 2007; Six et al., 2006), this suggests that organic N is concentrated mainly in bacterial biomass and necromass in the finest fraction of OM (i.e. not bound to particulate OM) in CONV systems, and in labile and fresh particulate OM (N-containing plant cover debris) in CA systems. As for the higher C/N ratio in this labile and fresh particulate OM in CONV systems than in CA systems, it is probably due to the surprisingly higher abundance of fungi (whose ratio is about 3 times higher than that of bacteria) in CONV than in CA systems. This could most likely be linked to the significantly higher use of the herbicide glyphosate in CA systems than in CONV systems (also see Chapter 3 and Case study n°2). It is indeed clearly demonstrated that this herbicide reduces the fungal biomass in the soil of plots regularly treated with it (also reducing the number of fungal arbuscules in wheat roots – Wilkes et al. (2020)).

4.1.2. Enhancement of soil biodiversity

Regarding enzymatic activities, only *Phosphatase* was positively impacted by CA practices. The other two enzyme activities studied systems (*NAG* and *Beta_Glu*) were not significantly different between. An increased phosphatase activity suggests an improved soil P cycle and a better plant nutrition thanks to an improved mobilisation of P from soil to plant (Campdelacreu Rocabruna *et al.*, 2024; Janes-Bassett *et al.*, 2022). Although Olsen P concentrations in soils were not different across cropping systems, some research works suggest that Olsen P may not be the most adapted indicator to predict plant P uptake from soils. Indeed, it might be rather linked to other processes such as the affinity of soil particles for P and to the total adsorption capacity, estimated from the total Fe bound in Fe oxides, the inorganic P released by NaOH and citrate/bicarbonate and the combination of total organic P, phosphatase activity in the rhizosphere, and pH (Recena *et al.*, 2015).

CA systems led to significant positive effects on TMC (+15%) and MMB (+28%) compared with CONV, with even higher differences (up to 50% for TMC and 43.5% for MMB) when comparing cluster 3 with cluster 2. However, abundance (+17%) and diversity of fungi (+8%) were higher in CONV than in CA systems, while we observed no impact of cropping practices on the abundance and diversity of bacteria. As a consequence, the abundance ratio fungi:bacteria was higher in CONV than in CA. These results confirm the findings of Li et al. (2018) who evidenced in their meta-analysis an increased soil TMC by 25% in CA systems. However, our results are not completely in line with those of a recent meta-analysis (Chen et al., 2020). Although our results are consistent with Chen et al.'s meta-analysis findings on MMB (+ 37% increase in conservation tillage systems compared with conventional), the latter revealed that both fungal and bacterial biomasses were higher in conservation tillage systems (+31% for fungi, +11% for bacteria) on the one hand, and the abundance ratio fungi/bacteria was not significantly different between conventional and conservation tillage systems on the other hand. However, Chen et al. (2020) did not include the qPCR biomass quantification method as we used in our study in their meta-analysis, which was only based on results obtained through phospholipid-derived fatty acids (PLFA) and direct microscopic observation methods. However, as highlighted by Djemiel et al. (2023), the few existing comparisons of PLFA measurements with qPCR methods in the estimation of absolute microbial quantities, showed good repeatability. The values obtained for fungi:bacteria ratio fall in the range of the values commonly found in French cropping systems (Djemiel et al., 2023). Djemiel et al. (2023) pointed out the important effects of soil C/N or pH on the fungi:bacteria ratio. However, in our study, we could not evidence any difference in C/N or pH in the different cropping systems, suggesting that the differences are linked to other factors. In this regard, higher values of fungal growth in CONV systems were also found by Rosinger et al. (2025). They assume that this increase is related to the presence of functional pore domains and to microbial physiology. Indeed, they evidenced that bacterial growth increased and inversely fungal growth and fungi:bacteria ratio decreased with increasing percentage of storage pores (0.5-50 μ m). In our case, the higher abundance and diversity of fungi in CONV systems is also coherent with the higher C/N ratio found in the 50-200 μ m fraction (as seen in the previous section 4.1.1.).

4.1.3. Soil structure maintenance in CA soils

Aggregate stability, which favours the physical protection of soil OC and consequently C storage, was the only physical soil indicator that had contrasted results according to management. The fact that no significant difference was observed for Beerkan and VESS raises questions since these two indicators are recognised to be improved in CA soils (Basche and DeLonge, 2019; Olivares *et al.*, 2023). However, these two indicator measurements were associated with high intra-plot variability, making it difficult to measure significant differences. Regarding soil structure, as already mentioned, several CONV farmers have in fact started to shift their practices into soil conservation paradigm, bringing their soils closer to CA soils. We also observed, while performing VESS in-field, that many CA soils had a pretty compacted structure below 10-15 cm, which we believe to be linked to the eluviation/illuviation process (leaching of fine clay and silt particles from soil surface, Sauzet *et al.* (2016)) due to a lack of lime maintenance of soils, as several farmers have acknowledged during our field meetings.

Altogether, these results indicate that CA systems, with higher C inputs, function with a higher turnover of OM than CONV systems, as reflected by the higher C and N mineralisation and the higher microbial activity (higher *TMC*, *MMB*, *Litter_bag* and *s*.*POXC_tot* in this study). This high biological activity in CA system contributes to soil aggregation (especially macro-aggregates) and stabilisation, as reflected by the increased water stable aggregates.

4.1.4. Greenhouse Gas (GHG) emissions

The calculation of GHG emissions can be questionable in Systerre[®] tool. Indeed, the model takes into account all the possible emissions from gas, pesticides and fertilisation. Specifically, regarding fertilisation, the model expresses potential N₂O emissions due to the evaporation of specific fertilisation types (*e.g.* urea). These emissions may occur if farmers do not spray at the right time, and in the right weather conditions. Several publications showed, from various cropping systems around the world, higher N₂O emissions from cultivated fields under CA management than under conventional systems (*e.g.* Ahmad *et al.* (2009), Dendooven *et al.* (2012), Mangalassery *et al.* (2014), Oorts *et al.* (2007)), while others, having focused on well-established and advanced CA systems, demonstrate that lower N₂O emissions in CA systems than in conventional ones are possible (Palm *et al.*, 2014; Six *et al.*, 2004), sometimes in great proportion (between 40% and 57% according to Omonode *et al.* (2011). Beyond the age and advanced stage of CA systems, Li *et al.* (2023) show that in CA systems, N₂O emissions can be mitigated through adapted fertilisation strategies.

4.2. Human and animal health

4.2.1. Food safety and nutrition

We observed an important year effect on grain final composition which suggests that climatic conditions have a major impact on grain composition, as highlighted in the review conducted by Scarpa *et al.* (2020). Another possible factor which may strongly impact grain quality is the variety as shown for rice by Debnath *et al.* (2023). However, it was not possible in our study to evidence such an effect, the design of this OFE including a large number of varieties or combinations of varieties limited the statistical power.

Most elements showed no difference between cropping systems. However, some of them did, such as an increased concentration of P, K and ERGO in CA soils, together with an increased concentration of cellulose, and Zn in cluster 3. ERGO is an antioxidant amino acid derivative of histidine produced exclusively by fungi and some bacterial species. It has recently gained interest in human health research due to its accumulation in human tissues that are subjected to high levels of oxidative stress (Borodina et al., 2020). Recent work has shown that tillage intensity was inversely correlated with plant ERGO concentration in soybeans (Glycine max), maize (Zea mays), and oats (Avena sativa) (Beelman et al., 2021; Carrara et al., 2024). Carbohydrates are very important components of wheat grain (Zhang et al., 2017). Amongst them, cellulose is an insoluble dietary fibre, which intake is associated with several health benefits such as the good functioning of gut microbiota (Ciudad-Mulero et al., 2019). Zn deficiency is the fifth leading risk factor for human diseases and is associated with several disorders and infections, specifically in developing countries (Roohani et al., 2013; Sangeetha et al., 2022). Modern cultivars of cereal have inherently very small concentrations of Zn and cannot meet the human need for this micro-element. Therefore, agricultural strategies known as biofortification are used to improve crop Zn concentration. Biofortification is mostly based on classical plant breeding and genetic engineering for larger nutrient concentrations, and optimised fertiliser applications (Cakmak and Kutman, 2018). Our results suggest that CA systems coupled with efficient use of fertilisation, especially organic, may contribute to an increase in Zn concentration in grain. Overall, CA practices seem to really be capable of improving grain quality from a human health perspective. However, these elements are mostly located in bran, suggesting that these positive effects would be reflected on health only through consumption of wholegrain-based products (Guo et al., 2022).

Also, as shown by He *et al.* (2022) on watermelon concerning P and Fe, the higher grain contents of P and K in CA systems and Zn in cluster 3, might be linked to a greater bioavailability of these elements in these soils. However, the cause-effect relationships between mineral element contents in a soil and in a plant product that it has nourished, could also be seen only with contrasting levels of soil contents (high deficiency *vs.* optimal content), as stated by Montgomery and Biklé (2021). Since all the soils we studied, both in CONV and in CA, appeared to have sufficient mineral contents, they were within a range that did not allow such a demonstration. The increase in Zn content might also be related to the increase in available soil Zn measured in CA systems, although this relation is not linear.

We observed no modifications in the protein (or N) content of grain wheat. Our results only partially confirm the results of Wood, Tirfessa and Baudron (2018), in evidencing that OM increases can improve the nutritional quality of wheat, although wheat quality as such is difficult to define, as it varies with the final destination of wheat grains.

Although it is assumed that CA crops might have a higher probability of having higher mycotoxin contents, there is very little work to support this (*e.g.* Dill-Macky and Jones (2000); Obst, Lepschy-Von Gleissenthall and Beck (1997)). Our results tend to support this assumption, but also showed that DON and ZEA mycotoxin levels were always very low, far below the safety threshold for human consumption of 1 250 μ g/kg for DON, and 100 μ g/kg for ZEA in the 2023 campaign. The only sample which showed the presence of HT2 had a concentration higher than the regulatory threshold of 50 μ g/kg for HT2-T2⁶, making it not suitable for human consumption. In the European Union, estimates of chronic dietary exposure for populations of all age groups to HT2-T2 toxins are below the tolerable daily intake of 100 ng/kg body weight, therefore limiting health concerns for the population (European Food Safety Authority, 2011).

Finally, one sample only presented glyphosate residues, also at a very low level, indicating little transfer of glyphosate from soil or terminated plants to wheat grains, and thus a safe use of glyphosate in the systems studied (also see **Case study n°2** for a deeper discussion on pesticide use and residues in grains).

4.2.3. Human well-being

There is little scientific evidence for an economic impact of CA in the European context, except costs savings in terms of fuel, labour and machinery (Craheix *et al.*, 2016). Our calculations did not account for farmers strategies for production cost (choice of tractor...), which differ greatly within CA prone systems.

Working time was significantly lower in CA than in CONV, confirming the current knowledge on CA on limited time spent in field, as demonstrated by the European Conservation Agriculture Federation (ECAF) which estimates that adopting CA saves between 1 and 4.2 hours of work per hectare (ECAF, 2023).

Overall, we evidenced the multiple benefits of CA practices for the translation of the One Health concept into concrete reality. First, several improvements such as the increase in OM and microbial biomass and activity (except on fungi abundance and diversity), and the decrease in risk of erosion, lead to better soil conditions, and suggest a global increase in soil health. On the climate aspect, the

⁶ regulation (UE) 2023/915 of 25 April 2023 for DON, HT2-T2 and ZEA.

decrease in input consumption in the most efficient CA group (cluster 3), associated with a decreased gas consumption foster climate change mitigation through the lowered dependency on fossil fuels.

Furthermore, food security appears to be maintained, since CA led to yield maintenance and nutrient maintenance or even enhancement (depending on elements) in grains. The absence or very low quantity of mycotoxins and glyphosate residues in grain allow to conclude on the safety of CA products. CA also promotes welfare by maintaining farmers' economic margins and reducing their working hours.

5. Conclusion

Our results show that CA systems, at least the most efficient of them, improve soil health through increased organic matter and reduced erosion risk, while maintaining yields and limiting environmental impacts such as input consumption, improved structural stability of aggregates. Despite concerns over increased herbicide use in CA systems, only one sample showed trace residues and a high concentration of HT2-T2 mycotoxins, although no increased risks from mycotoxins were observed for all the other samples. This study suggests that the observed effects on grain quality are mostly governed by increased plant health in CA systems. These Plant Health aspects were treated in **Case study n°1**. Therefore, after having proven to be the system offering the best potential for bundling ecosystem services (*i.e.* allowing to combine surface productivity and environmental performances - Chabert and Sarthou, (2020); Perego *et al.* (2019)), CA systems, offer a viable, climate-friendly alternative to conventional tillage-based systems, with benefits to the *concept-becoming-reality* One Health.

Data availability

Data and metadata are available at: https://doi.org/10.18167/DVN1/SI026U

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Supplementary materials



Figure S3. Soil textures after decarbonation expressed in the USDA texture triangle in 2022 (left) and 2023 (right). Figures inside dots correspond to pairs of farmers numbers. Colours correspond to CA or CONV farmers. Textures are also available in Table S1 in Chapter 3. These triangles were made using the R studio {ggtern} package (Hamilton and Ferry, 2018).

(A)

(B)

(C)





Figure S4. PCA results for soil health indicators.

(A) corresponds to the PCA graph of variables, (B) corresponds to the graph of individuals coloured according to their type of cropping system (CA vs. CONV), (C) corresponds to the graph of individuals coloured according to their cluster (1, 2, 3 or 4), (D) corresponds to the graph of individuals coloured according to monitoring year (2022 = Year 1 or 2023 = Year 2), (E) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their pair.

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Figure S5. PCA results for grain quality indicators.

(A) corresponds to the PCA graph of variables, (B) corresponds to the graph of individuals coloured according to their type of cropping system (CA vs. CONV), (C) corresponds to the graph of individuals coloured according to their cluster (1, 2, 3 or 4), (D) corresponds to the graph of individuals coloured according to monitoring year (2022 = Year 1 or 2023 = Year 2), (E) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their previous context to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their previous context to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their previous context to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to the graph of individuals coloured according to the graph of individuals coloured accordin





Figure S6. PCA results for performance indicators.

(A) corresponds to the PCA graph of variables, (B) corresponds to the graph of individuals coloured according to their type of cropping system (CA vs. CONV), (C) corresponds to the graph of individuals coloured according to their cluster (1, 2, 3 or 4), (D) corresponds to the graph of individuals coloured according to monitoring year (2022 = Year 1 or 2023 = Year 2), (E) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their pair.

Group A: No difference CA-CONV, no differences between clusters

Table S5. Soil texture indicators.

Sig. = Significance. DM= Dry matter. Letters show results of Kruskal Wallis tests with Dunn adjustment allowing for multiple comparisons. Last row show results of Wilcoxon paired-rank test comparing results based on comparisons between pairs of farmers.

						Coarse_				
Variable		Clay	Silt	Sand	DM	elements	CaCO₃			
UI	nit				%	%				
1	n	17	17	17	17	8	17			
	mean	20.5	42.5	24.0	88.9	22.8	10.2			
	CV (%)	30.5	38.4	66.8	4.1	53.5	182.1			
	sig.	а	а	а	а	а	а			
	n	25	25	25	25	12	25			
2	mean	19.1	41.2	27.2	88.3	22.1	9.7			
2	CV (%)	36.0	40.7	67.5	5.1	50.3	200.6			
	sig.	а	а	а	а	а	а			
3	n	26	26	26	26	13	26			
	mean	23.7	37.9	23.7	88.7	27.1	10.9			
	CV (%)	33.4	36.7	66.3	3.7	44.6	145.8			
	sig.	а	а	а	а	а	а			
	n	18	18	18	18	9	18			
л	mean	19.7	41.1	24.0	88.6	21.9	11.9			
	CV (%)	39.4	40.7	73.3	3.5	47.3	155.3			
	sig.	а	а	а	а	а	а			
	n	43	43	43	43	21	43			
CA	mean	21.5	40.7	23.9	88.3	23.5	10.5			
	CV (%)	38.2	37.5	67.2	4.2	45.7	155.8			
	n	43	43	43	43	21	43			
CONV	mean	20.3	40.1	25.9	88.8	23.9	10.8			
	CV (%)	32.3	40.4	68.0	4.1	50.3	178.9			
	sig.	0.34	0.89	0.66	0.19	0.79	0.44			

Table S6. Biological soil properties.

Sig. = Significance. Letters show results of Kruskal Wallis tests with Dunn adjustment allowing for multiple comparisons. Last row show results of Wilcoxon paired-rank test comparing results based on comparisons between pairs of farmers.

Vari	able	тмс_ос	minC_ 28d_ OC	minN_ 28d_ Ntot	AbundBact	DivBact	NAG	Beta_ Glu	Lamina
UI	nit	% OC		% of total N	DNAr 16S	nb OTU 16S	nmoi g di	l/hour/ ry soil	Score
	n	17	17	17	17	17	17	17	9
1	mean	3.0	1.4	0.7	8.19E+05	1.99E+03	0.6	0.7	2.6
-	CV (%)	28.9	36.8	38.7	12.9	7.4	68.1	46.5	46.4
	sig.	а	а	а	а	а	а	а	а
	n	25	25	25	25	25	25	25	13
2	mean	2.7	1.4	0.8	7.98E+05	2.05E+03	0.6	0.8	2.3
2	CV (%)	30.5	45.0	63.0	18.0	6.7	55.4	58.7	53.8
	sig.	а	а	а	а	а	а	а	а
3	n	25	26	26	26	26	26	26	13
	mean	3.0	1.0	0.7	7.95E+05	1.93E+03	0.8	0.7	2.4
	CV (%)	24.5	34.8	40.1	17.8	8.4	58.7	47.9	37.4
	sig.	а	а	а	а	а	а	а	а
	n	18	18	18	18	18	18	18	9
л	mean	2.8	1.4	0.8	7.75E+05	1.99E+03	0.7	0.8	2.4
-	CV (%)	24.0	30.9	73.7	18.1	11.7	62.7	66.8	49.9
	sig.	а	а	а	а	а	а	а	а
	n	39	39	39	40	40	40	40	19
CA	mean	2.9	1.2	0.8	7.98E+05	1.97E+03	0.7	0.7	2.5
	CV (%)	24.6	41.3	43.0	19.2	9.5	63.1	59.9	41.4
	n	40	40	40	40	40	40	40	19
CONV	mean	2.9	1.3	0.7	8.05E+05	2.02E+03	0.6	0.7	2.3
	CV (%)	29.8	39.7	56.7	14.6	7.1	62.2	54.8	53.8
sig.		0,66	0.44	0.56	0.67	0.07	0.42	0.96	0.30

	Main characteristics			Conductivity and permittivity					Carbon	Nitrogen		
Variable		рН	CEC	C/N	ECp_s1	ECp_s2	Perm_s1	Perm_s2	ECb_s1	ECb_s2	s.POXC_OC	BMN_ Ntot
ι	Jnit	-	mEq/100g	-	mS	5/m	F/m		mS/m		% OC	%Ntot
1	n	17	17	17	17	15	8	8	8	8	17	17
	mean	7.5	13.4	9.5	88.9	93.8	13.6	17.7	10.6	14.5	6.3	1.9
1	CV (%)	10.4	35.9	11.9	39.0	35.1	27.7	37.0	49.9	67.2	14.3	58.1
	sig.	а	а	а	а	а	а	а	а	а	а	а
	n	25	25	25	24	22	12	12	12	12	25	25
2	mean	7.3	13.7	10.1	92.7	92.9	15.4	19.4	13.2	16.9	6.5	1.9
2	CV (%)	10.8	47.1	18.0	36.4	42.9	48.1	44.5	98.3	95.5	13.8	50.7
	sig.	а	а	а	а	а	а	а	а	а	а	а
2	n	26	26	26	26	21	13	13	13	13	26	25
	mean	7.3	14.8	9.6	91.6	86.8	29.9	17.8	10.5	12.1	5.7	2.0
5	CV (%)	11.2	38.8	15.2	35.0	36.6	179.9	39.8	48.3	45.9	24.6	69.2
	sig.	а	а	а	а	а	а	а	а	а	а	а
	n	18	18	18	18	16	9	9	9	9	18	18
Л	mean	7.6	14.2	9.7	95.9	96.8	15.0	21.9	11.6	19.3	6.6	2.3
4	CV (%)	6.9	39.6	16.6	38.3	30.4	35.1	50.6	79.4	92.4	18.7	56.5
	sig.	а	а	а	а	а	а	а	а	а	а	а
	n	40	40	40	40	37	21	21	21	21	40	39
CA	mean	7.4	14.1	9.6	92.3	89.5	24.4	19.4	11.0	14.9	6.2	2.2
	CV (%)	9.7	40.0	14.1	36.3	34.7	174.0	45.1	62.9	84.2	22.8	63.5
	n	40	40	40	39	37	21	21	21	21	40	40
CONV	mean	7.4	14.1	9.7	90.8	94.9	14.6	18.8	12.1	16.0	6.3	1.8
	CV (%)	11.0	41.5	17.4	37.6	38.3	42.2	42.4	85.3	83.7	14.8	53.2
sig.		0,807	0.745	0.393	0.329	0.338	0.288	0.658	0.919	0.835	0.39	0.09

Table S7. Chemical properties 1 - Main characteristics, conductivity and permittivity, Carbon and Nitrogen.
Table S8. Chemical properties 2 - Micro and macro soil elements.

For CONV values, n=40 plots, except for K₂O measurements where n=21 since measurements were only performed in year 2. Similarly for CA values, n=40 plots, except for K₂O measurements where n=21 since measurements were only performed in year 2.

			K ₂ O		Mg		Mn			Na ₂ O			S			3	F	e	Р	Cu	N	10
Va	ariable	Tot	Av.	Av/ Tot	Tot	Tot	Av.	Av/ Tot	Tot	Av.	Av/ Tot	Tot	Av.	Av/ Tot	Tot	Av.	Av.	Av/ Tot	Av.	Av.	Av.	Av/ Tot
	Unit	mg/	′kg	%	mg/kg	mg,	/kg	%	mg	i/kg	%	mg	/kg	%	mg	/kg	mg/kg	%	mg/kg	mg/kg	mg/kg	%
	n	8	8	8	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
1	mean	6875.0	244.5	4.3	5994.1	1008.5	52.2	5.0	288.2	25.5	9.7	337.1	2.4	0.9	20.3	0.3	57.7	0.2	42.2	4.4	0.1	14.4
÷.	CV (%)	53.3	43.6	52.0	56.9	114.2	131.7	90.7	47.3	83.9	70.4	56.7	26.1	46.6	90.0	37.3	101.6	126.6	50.3	114.9	2.4	38.4
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	12	12	8	17	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
2	mean	4941.7	230.3	4.4	5512.0	618.2	37.2	5.8	248.0	23.4	9.5	337.6	4.4	1.7	17.6	0.3	70.4	0.3	60.0	2.6	0.1	15.3
2	CV (%)	60.7	81.7	48.0	65.2	66.9	92.4	65.8	70.9	79.0	67.8	73.4	118.5	106.0	114.4	37.6	69.0	76.7	56.5	60.4	24.0	49.2
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	13	13	13	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
_	mean	7853.8	348.8	5.1	7676.9	886.9	45.2	4.4	284.6	26.2	10.8	437.3	3.8	1.0	26.9	0.3	72.8	0.2	53.8	4.8	0.1	14.6
3	CV (%)	54.8	66.6	70.2	69.8	61.1	120.0	70.0	51.3	43.3	54.6	51.7	108.4	76.3	109.7	29.5	88.1	92.9	59.1	193.9	29.4	57.4
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	9	9	9	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
	mean	5500.0	258.7	5.2	5211.1	631.1	30.8	5.1	272.2	20.8	7.2	388.3	3.3	1.1	21.7	0.3	56.2	0.3	58.5	4.0	0.1	18.2
-	CV (%)	59.6	50.0	40.3	56.8	45.3	87.9	91.5	71.1	46.5	59.2	59.7	37.5	53.2	102.7	40.4	85.9	108.1	52.6	132.7	42.2	48.4
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
CA	mean	6200.0	295.8	5.2	6302.3	789.4	42.8	5.1	269.8	23.0	9.4	395.3	3.7	1.1	23.0	0.3	68.5	0.3	55.7	4.4	0.1	15.5
CA	CV (%)	58.8	67.3	56.4	73.8	64.3	110.4	79.5	62.9	47.8	60.4	54.5	91.4	66.1	111.1	35.0	83.7	102.5	56.3	178.8	33.4	52.9
С		6461.0	255.0	4.2	CODE 2	760.1	20.6	5.0	274.4	25.2	0.5	261.2	2.5	1.2	20.7	0.2	62.9	0.2	52.0	2.4	0.1	15 5
0	mean	6461.9	255.8	4.3	6095.3	769.1	39.6	5.0	274.4	25.2	9.5	361.2	3.5	1.3	20.7	0.3	62.8	0.3	52.8	3.4	0.1	15.5
N	C(1)	50.0	CAE	50.2	50.0	101.2	122 5	75.0	56.0	76.0	67.1	66.6	115.2	111 5	102.1	26.0	05.1	07.4	57.0	102.0	22.1	47.2
V	CV (%)	0.072	04.5	50.3	58.8 0.472	101.3	123.5	75.8	1	76.2	07.1	0.065	0.554	0.244	103.1	36.0	85.1 0.214	97.4	0.254	102.6	23.1	47.3
sig.		0,972	0.465	0.122	0.473	0.34	0.675	0.712	1	0.648	0.597	0.065	0.554	0.244	0.237	0.072	0.214	0.483	0.354	0.085	0.135	0.758

Ele	Element Granulometric		Carbon		Nitrogen		C/N
Granu clas	lometric s (μm)	0-50	200-2000	50-2000	200-2000	0-50	200-2000
L	Init		%C tot		%N tot	%(C/N tot
1	mean	84.5	6.8	15.5	3.9	8.9	17.7
(n-17)	CV (%)	4.3	27.5	23.5	31.2	11.4	35.4
(11-17)	sig.	а	а	а	а	а	а
2	mean	84.5	6.9	15.5	4.1	9.6	18.9
(n-25)	CV (%)	6.9	43.5	37.7	49.1	25.0	48.0
(11-23)	sig.	а	а	а	а	а	а
2	mean	83.1	6.9	16.9	4.4	9.2	15.7
(n=26)	CV (%)	6.7	43.0	32.7	44.3	17.5	31.2
	sig.	а	а	а	а	а	а
Д	mean	83.5	6.9	16.5	4.4	9.3	17.3
(n=18)	CV (%)	5.9	46.2	29.9	49.4	15.9	47.4
(11 10)	sig.	а	а	а	а	а	а
CA	mean	83.1	7.1	16.9	4.4	9.2	16.2
(n=40)	CV (%)	6.2	44.8	30.4	46.2	14.8	39.3
CONV	mean	84.9	6.7	15.1	3.9	9.2	18.3
(n=40)	CV (%)	5.9	38.3	32.9	44.7	22.6	44.8
sig.	(n=40) CV (%) sig.		0.738	0.143	0.145	0.143	0.082

Table S10. Physical soil properties.

Variable		Soil tem	perature	Soil M	oisture			
Va	riable	Temp_s1	Temp_s2	Moist_s1	Moist_s2	Cindex	Beerkan	score VESS
l	Jnit	o	C	9	%	-	mL/min	Score
	n	17	17	17	15	17	17	17
1	mean	14.5	15.3	18.9	19.7	0.9	527.6	2.7
-	CV (%)	22.1	21.2	32.2	43.0	71.4	62.8	28.5
	sig.	а	а	а	а	а	а	а
	n	25	25	25	22	25	25	25
2	mean	14.5	16.0	20.9	22.3	1.0	551.4	2.8
2	CV (%)	41.1	16.9	39.0	40.0	68.0	67.3	25.0
	sig.	а	а	а	а	а	а	а
	n	26	26	26	26	26	26	26
2	mean	14.9	16.3	19.3	22.6	0.6	507.8	2.6
5	CV (%)	41.5	16.8	23.8	41.1	78.4	88.4	29.8
	sig.	а	а	а	а	а	а	а
	n	18	16	18	16	18	18	18
4	mean	12.4	15.4	19.8	22.0	0.9	635.8	2.5
4	CV (%)	26.4	14.0	36.3	53.3	75.8	115.9	28.8
	sig.	а	а	а	а	а	а	а
	n	40	38	40	37	40	40	40
CA	mean	13.7	15.8	19.7	22.2	0.8	507.8	2.6
	CV (%)	40.3	16.0	28.9	45.0	72.0	117.3	29.2
	n	40	40	40	37	40	40	40
CONV	mean	14.5	15.9	19.6	21.4	0.9	568.9	2.8
	CV (%)	34.7	18.6	37.7	42.2	70.3	61.0	26.5
sig.		0,09	0.87	0.58	0.35	0.08	0.15	0.22

Va	iabla					Nutri	tional				Sanitary			Technolo	ogic	
Val	lable	Starch	g.TN	Prot	g.Fe	g.Mn	Polyph	Antiox	B9	DON	HT2	ZEA	FarmerSW_n	ExpSW_n	Grain_ear_n	ткw
L	Init		%		mg	/kg	mg GAE/100g	FRAP/100g	μg/100g		µg/kg		kg/h	Ι	-	g
	n	17	16	17	16	16	16	16	16	16	16	16	12	16	16	16
1	mean	61.5	1.8	10.8	27.1	25.9	73.2	166.4	2.8	19.9	0	0	76.1	79.9	36.4	36.0
1	CV (%)	1.2	10.6	11.3	17.6	20.9	10.4	16.8	30.0	400.0	0	0	3.3	4.0	15.1	8.8
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	25	24	25	24	24	24	24	24	24	24	24	19	24	24	24
2	mean	62.0	1.7	10.5	29.0	24.7	74.6	171.3	2.6	0.0	0	0	76.9	79.1	36.2	36.0
2	CV (%)	1.3	10.4	11.4	24.7	29.7	12.4	16.1	28.1	0.0	0	0	2.8	5.0	10.1	10.6
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	26	25	25	25	25	25	25	25	25	25	25	22	25	25	25
2	mean	61.5	1.8	10.7	32.4	30.3	74.4	173.8	2.8	19.4	2.5	0	75.4	78.1	36.3	33.5
3	CV (%)	2.1	18.0	15.1	44.6	42.8	10.9	16.0	37.6	303.3	500.0	0.0	3.4	4.6	11.6	13.4
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	18	17	18	17	17	17	17	17	17	17	17	16	17	17	17
л	mean	61.4	1.8	11.1	28.4	26.7	76.9	168.7	2.5	13.1	0	2.1	76.3	78.3	37.1	34.8
-	CV (%)	2.2	10.6	9.7	25.3	20.2	11.2	15.6	29.7	226.6	0	312.3	2.8	3.7	17.0	9.7
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	39	39	39	39	39	39	39	39	39	39	39	32	37	37	37
CA	mean	61.5	1.8	10.8	30.7	28.5	75.0	174.2	2.7	19.2	1.7	1.0	76.2	78.3	36.9	34.7
	CV (%)	2.0	13.3	12.7	40.5	38.6	11.8	15.8	24.3	270.0	608.3	466.3	2.9	4.5	13.8	11.9
	n	39	39	38	39	39	39	39	39	39	39	39	30	38	38	38
CONV	mean	61.6	1.8	10.6	27.7	25.5	74.0	170.7	2.8	8.4	0	0	76.2	79.1	36.2	35.2
	CV (%)	1.4	13.2	10.7	22.7	25.4	10.5	16.2	35.8	616.4	0	0	3.5	4.5	12.0	11.0
sig.		0,45	0.64	0.39	0.22	0.14	0.44	0.32	0.31	0.30	1.00	0.37	0.91	0.11	0.59	0.45

Table S12. System performances.

For the calculation of min N efficiency, one plot from cluster 3 was removed, since the farmer did not use any N mineral fertilisation in 2022 (11_CA_2022).

		Ec	conomic	Environmental		Production	
Varia	ble	SNMargin	ProductionCost	GHGtotEmiss	farmerYield_n	ExpYield_n	minN_eff _n
Un	it	€/ha	€/t	kgCO₂eq/ha	q/1	ha	%
	n	17	17	17	15	16	17
1	mean	621.5	189.6	2491	60.9	79.8	36.2
-	CV (%)	68.3	37.3	0.5	27.7	28.2	24.7
	sig.	а	а	а	а	а	а
	n	25	25	25	20	24	24
2	mean	675.2	170.4	2385	67.0	81.8	43.0
2	CV (%)	49.1	24.6	0.5	22.6	26.2	30.1
	sig.	а	а	а	а	а	а
	n	26	26	26	24	25	25
2	mean	695.2	165.7	2283	59.0	70.6	41.6
3	CV (%)	46.2	29.1	0.2	23.6	28.9	38.3
	sig.	а	а	а	а	а	а
	n	18	18	18	17	17	18
л	mean	615.1	173.0	2309	66.5	80.4	40.4
-	CV (%)	56.8	26.6	0.2	19.2	25.5	28.2
	sig.	а	а	а	а	а	а
	n	43	43	43	35	37	42
CA	mean	671.8	167.2	2301	62.8	76.8	42.2
	CV (%)	48.9	26.7	20.1	22.9	27.4	34.3
	n	43	43	43	34	38	42
CONV	mean	644.4	179.4	2418	63.0	77.8	39.1
	CV (%)	57.3	31.7	15.6	25.8	28.4	28.7
sig.		0,48	0.08	0.50	0.69	0.89	0.35

Group B: No difference CA-CONV, but differences between clusters

Table S13. Indicators showing differences in the clusters, but not in the CA vs. CONV analysis. The minimum mean values of significantly different clusters are highlighted in orange while the highest mean values are highlighted in blue. * We noted the presence of an outlier influencing the result of the comparison tests: after outlier removal, the difference was no longer significant.

Compa	irtment			Soil			Gr	ain	Performance
Sub-compartment Variable				Chemical			Nutri	tional	Economic
Vari	able	s.TFe	s.TZn	s.TCu	s.ratioCu	s.TMo*	g.Zn	Cellulose	InputExpenses
Cluster	Unit	mg/kg	mg/kg	mg/kg	%	mg/kg	mg/kg	%	€/ha
	n	17	17	17	17	17	16	17	17
1	mean	29505.9	71.6	21.7	19.2	0.8	22.6	2.5	514.3
-	cv (%)	49.6	55.0	92.1	32.8	51.2	27.4	5.2	17.3
	sig.	ab	ab	ab	ab	ab	ab	ab	а
	n	25	25	25	25	25	24	25	24
2	mean	24104.0	73.3	12.9	22.1	0.9	22.1	2.4	491.4
2	cv (%)	48.0	84.3	58.1	43.7	73.6	20.3	6.0	13.4
	sig.	а	а	а	а	ab	ab	а	а
	n	26	26	26	26	26	25	26	26
3	mean	39261.5	102.5	27.0	15.1	1.1	25.5	2.5	429.3
Ŭ	cv (%)	70.1	55.1	129.4	68.5	87.7	23.2	5.3	21.4
	sig.	b	b	b	b	а	а	b	b
	n	18	18	18	18	18	17	18	18
Л	mean	24905.6	55.8	16.3	21.5	0.7	20.0	2.5	531.2
+	cv (%)	74.0	43.1	72.8	51.7	43.3	20.5	5.4	21.5
	sig.	а	а	ab	ab	b	b	ab	а
	n	40	40	40	40	40	39	39	43
CA	mean	32409.3	78.8	21.7	18.3	0.9	22.7	2.5	473.0
	cv (%)	78.4	64.2	132.2	58.7	84.8	25.3	5.9	24.0
	n	40	40	40	40	40	39	39	43
CONV	mean	27434.9	77.5	17.6	20.2	0.8	22.9	2.5	498.0
	cv (%)	48.1	70.2	81.6	44.8	65.2	25.2	5.9	15.3
sig.		0.43	0.38	0.47	0.21	0.20	0.48	0.20	0.073

Group C: Significant differences CA-CONV, but no differences between clusters

Table S14. Indicators showing significant differences in the CA vs. CONV comparison, but not in the cluster comparisons. The minimum mean values of significantly different clusters are highlighted in orange while the highest mean values are highlighted in blue.

Compa	rtment				Biolog	Biological er						Chemical				Physical	
Su compai	b- rtment	N	Microbiology		Litter decompo- sition		Basa	I respiration		Ra	tios Av/Tot		Carbon	Nitrogen	Granulo	ometric frac	tionation
Varia	able	AbundFungi	DivFungi	F:B Ratio	Litter bags	minC (28 days)	minN (28 days)	SituResp24	SituResp48	Mg	Zn	В	s.TPOXC	BMN_tot	C_50_ 200	C_N_50 _200	C_N_50_2 000
Clu- ster	Unit	DNAr 18S	nb OTU 18S	-	g	mg C- CO₂/kg	mg/kg	Difference o	f absorbance		%		m	g/kg	%C tot	%C/	/N tot
	n	17	17	17	8	17	17	17	17	17	17	17	17	17	17	17	17
1	mean	29100.3	1173.4	3.5	21.4	218.7	11.7	1.63E-01	3.7E-01	4.6	3.0	2.6	986.2	34.8	8.7	13.9	15.4
-	cv (%)	37.5	23.9	32.8	49.3	46.9	31.2	115.7	58.9	72.6	43.3	65.3	23.8	85.2	30.3	30.1	30.1
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	25	25	25	12	25	25	25	22	25	25	25	25	25	25	25	25
2	mean	29838.2	1224.8	3.8	26.3	219.3	12.4	1.71E-01	3.5E-01	3.9	4.6	3.3	1051.4	30.9	8.6	13.0	15.4
2	cv (%)	37.7	19.1	38.3	48.6	45.3	51.5	97.5	63.2	54.1	63.6	65.6	36.5	61.3	40.7	32.0	32.7
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	26	26	26	13	25	24	26	25	26	26	26	26	25	26	26	26
2	mean	25287.8	1083.1	3.2	24.7	226.9	14.5	1.81E-01	4.2E-01	4.9	4.3	4.0	1188.6	46.3	10.0	12.1	12.7
5	cv (%)	32.0	22.6	32.0	45.3	47.0	32.6	96.6	51.2	65.3	98.1	89.9	28.8	80.9	31.6	51.0	24.4
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	18	18	18	9	18	18	18	17	18	18	18	18	18	18	18	18
л	mean	25186.3	1161.4	3.3	27.7	255.7	14.9	2.80E-01	4.8E-01	4.5	6.2	3.4	1225.7	49.4	9.6	11.5	13.3
4	cv (%)	30.6	25.6	33.2	49.2	47.9	52.1	105.3	57.7	37.3	69.5	91.1	39.9	87.8	45.6	33.9	32.7
	sig.	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а	а
	n	40	40	40	21	39	39	40	38	40	40	40	40	39	40	40	40
CA	mean	25089.1	1113.0	3.2	28.3	235.8	14.8	2.08E-01	4.4E-01	5.0	5.4	3.7	1162.3	46.3	9.8	12.2	13.1
	cv (%)	31.2	22.9	33.3	45.3	46.9	43.0	96.8	53.7	54.6	80.9	85.0	31.4	84.3	31.6	44.0	28.2
	n	40	40	40	21	40	40	40	37	40	40	40	40	40	40	40	40
CONV	mean	29588.3	1206.4	3.7	22.1	214.4	11.7	1.38E-01	3.6E-01	4.0	3.7	3.1	1032.5	32.1	8.5	13.0	15,0
	cv (%)	37.4	20.4	36.5	45.9	48.9	41.5	109.5	61.8	63.8	62.7	77.7	32.6	75.7	36.7	33.6	32.3
si	g.	0,001	0.014	0.005	0.016	0.044	0.003	0.002	0.030	5.38E-04	0.049	0.04	0.004	0.001	0.037	0.012	2.91E-04

Table S15. Grain nutritional indicators. The minimum mean values of significantly different clusters are highlighted in orange while the highest mean values are highlighted in blue.

Varia	able	Р	К	Ergothioneine
Cluster	Unit	mg/.	100g	ng/mg
	n	16	16	17
1	mean	276.4	384.1	1.9
1	cv (%)	12.0	9.8	60.2
	sig.	а	а	а
	n	24	24	24
2	mean	272.0	379.7	1.6
2	cv (%)	15.6	11.3	37.0
	sig.	а	а	а
	n	25	25	26
2	mean	296.9	410.0	2.1
3	cv (%)	18.2	18.3	38.9
	sig.	а	а	а
	n	17	17	18
_	mean	281.2	384.9	2.0
4	cv (%)	17.6	14.3	47.2
	sig.	а	а	а
	n	39	39	39
CA	mean	292.8	404.9	2.0
	cv (%)	19.0	17.0	44.2
	n	39 39		38
CONV	mean	271.8	383.4	1.8
	cv (%)	13.7	11.3	50.9
sig.		0,036	0.022	0.047

Group D: Significant differences CA-CONV, and significant differences between clusters

Table S16. Indicators showing both differences in the CA-CONV comparison and in the clusters.

The minimum mean values of significantly different clusters are highlighted in orange while the highest mean values are highlighted in blue.

Compa	rtment						Soil						Perfo	rmance
Sub-com	partment		Biologica	I				Chemica				Physical	Social	Environmental
Vari	Variable TMC MMB Phosphatase s.AM					s.AZn	ом	s.TN	N_0_50	N_50_200	N_50_ 2000	Agg	Working Time	Gas Consumption
Cluster	Unit	mgC/kg	μg DNA/g	nmol/hour/ g dry soil	m	g/kg	5	%		%N tot		Score	h/ha	L/ha
	n	17	17	17	17	17	17	17	17	17	17	17	17	17
1	mean	496.0	52.0	3.7	194.5	1.9	2.8	1.75E-01	90.0	6.2	10.0	4.0	2.4	56.5
-	cv (%)	46.6	44.4	48.6	39.1	49.6	34.2	37.1	2.3	30.6	20.2	22.1	25.4	21.5
	sig.	ab	ab	ab	ab	а	ab	ab	а	а	а	а	ab	а
	n	25	25	25	25	25	25	25	25	25	25	25	25	25
2	mean	460.2	49.0	3.5	169.8	2.8	2.9	1.69E-01	89.1	6.8	10.9	4.3	2.6	59.6
-	cv (%)	54.4	44.2	58.2	44.2	74.8	40.6	42.9	4.5	36.8	36.5	19.0	19.5	18.2
	sig.	а	а	а	а	ab	а	а	ab	а	ab	ab	а	а
	n	25	26	26	26	26	26	26	26	26	26	26	26	26
2	mean	691.2	71.2	5.1	281.7	3.8	3.8	2.42E-01	86.8	8.8	13.2	5.0	1.7	37.6
5	cv (%)	53.0	43.4	36.1	70.3	98.8	38.1	47.9	4.7	28.2	30.7	14.6	40.4	12.4
	sig.	b	b	b	b	b	b	b	b	b	b	С	С	b
	n	18	18	18	18	18	18	18	18	18	18	18	18	18
л	mean	562.8	62.6	4.2	205.3	3.0	3.3	2.03E-01	86.8	8.8	13.2	5.0	1.9	39.3
-	cv (%)	57.9	52.0	56.1	43.3	60.4	40.5	45.5	5.9	45.6	38.8	14.4	23.5	11.2
	sig.	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	bc	bc	b
	n	39	40	40	40	40	40	40	40	40	40	40	43	43
CA	mean	582.8	65.6	4.7	248.4	3.5	3.4	2.14E-01	87.0	8.6	13.0	5.0	1.8	38.6
	cv (%)	51.9	46.5	47.6	67.8	90.4	37.9	46.3	4.6	29.6	30.5	14.1	35.4	15.0
	n	40	40	40	40	40	40	40	40	40	40	40	43	43
CONV	mean	506.2	51.2	3.6	183.5	2.4	2.9	1.82E-01	89.6	6.5	10.4	4.2	2.5	57.6
	cv (%)	55.7	44.2	50.8	40.9	64.3	40.4	46.7	3.7	33.6	31.6	20.9	22.7	20.0
si	g.	4,61E-03	7.23E-04	7.60E-04	0.002	1.54E-02	3.16E-03	4.82E-03	9.53E-04	1.46E-04	9.02E-04	1.97E-05	1.21E-06	1.59E-12

Chapter 4 evidenced the positive impacts on several important parameters of Soil Health, such as increased OM contents, increased microbial carbon levels and improved soil structural stability, therefore enhancing the capacity of CA soils to act as carbon sinks and therefore contributing to climate change mitigation. CA soils appear to foster soil biological activity therefore contributing to improve soil nutrient cycling. The better soil structural stability evidenced in CA soils also promote soil fertility. In addition, CA systems are associated with a decrease in gas consumption, and input expenses for the most efficient systems, suggesting positive effects on the environment through decreased reliance on fossil fuels and better soil health.

The effects of cropping practices on grain quality were more difficult to evidence, mainly due to an important year effect on grain nutritional quality. However, we evidenced concentration increases for essential nutrients (P, K, ERGO), regardless of the year in CA vs. CONV. No loss in nutrient concentration in grains were observed in CA systems compared with CONV systems. We also observed no difference in grain yield and technological quality in CA compared with CONV, and no significantly higher concentrations of mycotoxins in CA grains compared with CONV grains, evidencing the potential of CA to promote food security and safety and therefore human and animal health.

CA systems are also associated with decreased in-field working time, suggesting additional benefits linked with human health.

However, as seen in **Chapter 3**, CA systems were associated with an increased reliance on pesticides and specifically herbicides that can question the full sustainability of these systems and the potential effects on farmers' and consumers' health, although no pesticide residues (or at a very low rate) were identified in grain (also see **Case study n°2**). The results from cluster 3 however showed limited reliance on pesticides from CA farmers, showing that CA systems have the ability to move toward most efficient systems in terms of pesticide use.

CASE STUDIES

Introduction to the three case studies

Apart from the PhD main work, three small pilot studies (hereby called "case studies") were held, each of them targeting a specific topic complementary to the main study. The idea of these case studies was to dig deeper into specific topics of interest, *i.e.* (i) the role of plant health in the practice-soil-grain continuum, (ii) the microbial ecology of soil-plant-grain interface and (iii) the possible effects of practices until a final transformed wheat product. The objective of these three case studies was to show the panel of possibilities and the variety of different topics that can be targeted in One Health studies.

To this end and from a system organisation perspective, we built different partnerships with specialised actors.

First, in the frame of One Health, it was difficult to ignore the role of plant health as a key lever of the practice-soil-production chain. However, the study of plant health needed to be carefully planned. We first tried in year 1 to include sap analysis in our sampling plan. However, the associated logistics at the beginning of the study were too complex and would not have allowed us to implement them correctly. In year 2, we decided to study wheat health through the angle of wheat resistance to pests and diseases under similar or comparable growing conditions. To do so, we established control strips on volunteer plots. No fungicides were applied in the control zones, and in most of them, no insecticides were applied. The use of herbicides was accepted. This work was made by Océane Grudé in the frame of her MSc. Thesis under my supervision.

Second, we partnered with the French National Museum of Natural History on the specific topic of the effects of cropping practices on soil-plant-grain microbial communities and set a small parallel project hereafter identified as "Agribiodiv" (for "Agricultural biodiversity"). A pilot was set in year 1 to run a first set of analyses and calibrate the study and associated costs and needs, and the "real" study was implemented in year 2 on 16 plots, mostly located in Vienne and Indre-et-Loire departments.

Last, since our work aims at evaluating the effects of cropping practices from "farm to fork" overall, we were interested in studying the last step of product transformation, including not only the nutritional quality but also the transformation step (flour and baking), which has a great impact on bread nutritional quality. To this end, we partnered with a flour mill ("Moulin Girardeau") that realised baking tests on grain wheat from 20 volunteer farmers' plots.

All the data gathered within these three case studies are available in the dataset <u>https://doi.org/10.18167/DVN1/SI026U</u>, and all protocols and methodologies are described in **Chapter 2** of this manuscript.

Table 9 shows a summary of farmers' participation in the different case studies.

Table 9. Summary of volunteer participants to case studies.

I: four farmers did not respect the requirement on "0 insecticide" on the control zone and therefore applied an insecticide in fall 2022 despite their participation in the plant health study.

Pair		Agribi	odiv	Plar	nts	
Pair	Туре	soil root leave	grain	Q1	Q2	Bread
	20	21-2022	campa	ign (Ye	ar 1)	
Λ	CA					
4	CONV					
7	CA					
/	CONV					
Q	CA					
0	CONV					
13	CA					
15	CONV					
	CA					
17	CONV					
22	CA					
22	CONV					
	20	22-2023	campa	ign (Ye	ar 2)	
1	CA					
1	CONV					
2	CA					
2	CONV					
3	CONV					

4	CA			
4	CONV			
-	CA			
5	CONV			
	CA			
6	CONV			
7	CA			
,	CONV			
Q	CA			
0	CONV			
10	CA			
10	CONV			
12	CA			
12	CONV			
12	CA			
15	CONV			
14	CA			
14	CONV			
15	CA			
15	CONV			
16	CA			
10	CONV			
17	CA			
10	CA			
10	CONV			
19	CA			

Tota	l fields	18	16*	22	22	20
22	CONV					
22	CA					
21	CONV					
21	CA					
20	CONV					
20	CA					
	CONV					

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CASE STUDY 1 : PLANT HEALTH IN CONSERVATION AGRICULTURE SYSTEMS

This case study refers Océane Grudé's MSc. thesis intitled "Effect of soil management on plant resistance to disease and pest" (In French: "Effet des modes de gestion des sols sur la résistance des végétaux aux maladies et aux ravageurs"). Océane was supervised by Jean-Pierre Sarthou and me between March and September 2023. This section, presented as a case study, displays a summary of the methods and main findings of the work. This case study came to complete the work and reinforce the "Plant" component of the study on the effects of cropping practices on the soil-production continuum.

1. Introduction

The capacity of CA systems to foster plant health with limited use of chemical inputs has been discussed for more than a decade (Palm *et al.*, 2014). Indeed, through fostered soil biology and structure, and in general improved soil health, CA systems would facilitate plant growth, nutrition and ability to resist pests and diseases (Altieri *et al.*, 2024). This might be explained by increased levels of OM in CA systems that may enhance antagonists that control soil-borne pathogens and pests (Altieri *et al.*, 2024). On the contrary, CA systems are more likely to suffer from a higher aboveground pest pressure of some generalist pests, especially slugs (Douglas and Tooker, 2012). Therefore, within our OFE network, we hypothesised that within the same plot, a non-chemically protected zone would be more likely to suffer from more pest and disease damage than a chemically protected treated zone, especially in CONV systems due to a less intrinsic ability of plants to resist pests and pathogens, due to low soil health.

To test this hypothesis, in autumn 2022 we proposed to farmers to add a non-treated strip next to the

studied zone in their selected plots, where they would not use any fungicide or insecticide. The use of herbicides was allowed. We decided to focus on the main diseases and pests found in wheat crops (Willocquet *et al.*, 2021). Fungal diseases on the one hand:

- Septoria tritici blotch, caused by ZymoSeptoria tritici
- Yellow (stripe) rust caused by *Puccinia striiformis*
- Leaf brown rust, caused by *Puccinia* triticina
- Powdery mildew, caused by *Blumeria* graminis f. sp tritici
- Eyespot, Oculimacula yallundae
- Fusarium spp.

Photo 3. Top: Slugs (Deroceras reticulatum) and their damage on wheat. Bottom: Adult leaf beetle (Oulema melanopus) and visible damage on wheat. ©Arvalis, l'institut du végétal

Pests on the other hand (Photo 3):

- Slugs Deroceras reticulatum and leaf beetles Oulema melanopus,
- Leaf miners *Agromyza* spp.

This short study aimed to address two main questions:

- 1. (Q1) Within a pair of neighbouring farmers (CA/CONV), under no fungicide or insecticide protection, does plant resistance to pests and diseases differ?
- 2. (Q2) Within the same CA or CONV plot, is there any difference between the treated and no treated zone in terms of frequency and intensity of pest and disease damage?

2. Materials and methods

2.1. Sampling design

In fall 2022, ahead of Océane's internship, the whole OFE network was asked to volunteer to leave a non-treated strip on which there would not be either fungicide or insecticide used. Our request did not concern herbicide use, since we knew from previous research field works and also discussions with our farmers that most of them (especially CA farmers) would not agree to leave a non-weeded strip and take the risk of contaminating their plots with weeds for years. Also, we accepted the presence of treated seeds, since within a pair of farmers, the same seeds were used, therefore including the same effect on crop growth. Since the objective was to compare plant health within a pair of neighbouring farmers, we asked farmers to volunteer directly by pair, to ensure that comparisons would be possible within the two systems at equal conditions. We asked volunteer farmers to leave a strip close to the main study zone that would correspond to one spray-boom width (for easiness of application) on a minimal length of 60 m. Since all farmers did not have the same sprayer, zone widths were unequal amongst the volunteer plots with a mean width of 12 to 24 meters, in addition to an approximative 3 m buffer strip left between the non-treated strip and the main (treated) studied zone (Figure 23, also

Some issues arose after the launch of the study:

- 1. Two farmers eventually did not use any fungicides on their entire plot: 1_CA and 20_CA. For these two plots, zone P and zone C overlapped. All measurements (pests, diseases and technological grain quality) were done only once on the P/C zone, since these farmers were involved in the Q1 comparison (CA vs. CONV). These farmers were not taken into account for the Q2 analysis. The yield and technological grain quality (thousand kernel weight, number of grains per ear) were assessed only once but the results were used both for the main analysis (see Chapter 4) and for the Q1 analysis. That is why in the database, data on technological wheat quality is not missing for the P and C zones, although they come from the main measurement.
- Four farmers eventually sprayed insecticides in autumn on the whole plot, including the non-treated strip: 12_CA and 12_CONV, 13_CA and 13_CONV. Since the whole pair of neighbouring farmers were concerned in that case, both pairs were kept in the Q1 and Q2 analyses.
- 3. Three farmers wanted to set a non-treated strip even if their neighbour refused to do so, hence enabling their participation in Q2 but not in Q1: 3_CONV, 17_CA, 18_CA.
- 4. Four farmers got involved in the plant analysis but did not grow the same wheat variety: Pair 11 and pair 14. Data was collected but eventually, the four farmers were excluded from Q1 analysis to avoid wheat variety biases (data not shown on the database).
- 5. Lost data: Data of pairs 11, 14 and 18_CA were lost, therefore excluding them from all plant analyses. Ears of 5_CONV were lost after sampling, therefore there is no information on *Fusarium* and eyespot for this plot in the two zones. Pair 5 was therefore not considered for the Q1 and Q2 comparisons for these two diseases.

Finally, 22 farmers were kept in the Q1 analysis, and 22 farmers were kept in Q2 analysis. Participating farmers are summarised in Table 9, and main characteristics of participating farmers are presented in Table S17.



Figure 20. Sampling design and visualisation of the two research questions. Adapted from (Grudé, 2023).

2.2. Data collection

2.2.1. Pest and disease analyses

Plant disease and pest observations were realised at wheat flowering from 8 to 13 May 2023 and at physiological maturity from 21 June to 7 July 2023. Samplings were realised both on the main study zone (treated, P) and non-treated strip (control, C). All the observations were made by Océane to avoid any observer bias.

The pest and disease measurement protocols were established with the support of Olivier Guérin from the Agricultural Chamber of the Nouvelle-Aquitaine region.

The overall view of the protocol implemented in fields is presented in **Chapter 2** (Section 3.7, Figure 9). In spring and summer, 20 wheat plants were randomly collected in each zone (P and C), 1 cm from the basis. On each of the three youngest completely developed leaves (L1, L2 and L3), pests and diseases were identified (presence/absence of disease) and the intensity of foliar or ear damage was quantified for all the monitored pests and diseases except for diseases monitored on ears (*Fusarium* and eyespot). For these two indicators, we considered that as soon as the organ was targeted by the disease, the intensity of the disease was 100%. In addition, yellow and brown rust observations were eventually merged, since on field, yellow and brown rusts were very difficult to differentiate. Slugs and leaf beetles were observed jointly, since in-field damages are hardly differentiable between the two pests.

2.2.3. Yield and grain technological quality

Grain samples for yield and grain technological quality analyses were also sampled at physiological maturity. The protocol was the same as realised in the P zone, as described in **Chapter 2** (Section 2.8), except that the 50 cm diameter ring was positioned at four positions of the diagonal of the C zone. The indicators measured were the same as in the P zone, *i.e.* the number of grains per ear

(*Grain_ear_n*), yield (*ExpYield_n*), the thousand kernel grain (*TKW*), and the specific weight (*ExpSW_n*). Each specific weight measurement was repeated three times per sample.

<u>2.3. Data analyses</u>

All the analyses were run with R studio (R Core Team, 2021). We summed the total frequency of diseases and pest attacks at the zone (P or C) scale to get one total frequency of disease and pest attacks per field and per zone. Frequency was calculated as follows:

- For Septoria tritici blotch, yellow and brown rust, powdery mildew, slugs and leaf beetles and leaf miners: $Fq(disease_pest)_zone = \sum_{n=1}^{20} fqL1 + fqL2 + fqL3$,
- and for eyespot and *Fusarium*: $Fq(disease)_zone = \sum_{n=1}^{20} fqEar$

with n=Plant_ID as described in Figure 8 (Chapter 2).

The intensity of diseases and pest damage was calculated as the mean intensity of damage caused by each pest or disease monitored, considering only the damaged organs. The mean intensity of damage was calculated at the zone scale. Therefore, there is one average value per zone and per plot.

For Q1, we ran Wilcoxon paired ranked tests to evidence differences between the C zones of each CA/CONV pair, while for Q2, Wilcoxon paired ranked tests were run to evidence differences between the C and P zones of each participating farmers' plots. For Q2, analyses were run separately on CA and CONV fields to evidence different effects of the C zone on the two different systems. Due to the low number of repetitions, we considered α =0,1 as the significant threshold.

Due to the low number of individuals participating in the study, we could not study the differences based on the cluster analysis of cropping practices (**Chapter 3 and 4**), therefore limiting the analysis to the CONV *vs.* CA comparison.

3. Results

3.1. Differences between CA and CONV systems on non-treated strips (Q1)

3.1.1. Disease and pest

On average, 53% of sampled CA leaves were affected by *Septoria*, against 66% in CONV plots of the C zone. CA and CONV plots were hardly attacked by powdery mildew, despite an important heterogeneity between plots (cv=295% and 248% in CA and CONV plots respectively). Six plots (1_CA, 10_CONV, 12_CA, 13_CA, 19_CA and 19_CONV) out of the 22 studied plots were affected by powdery mildew contaminations, at very low intensity (2.2% in CONV and 2.3% in CA). Five CA and seven CONV plots were concerned by rust attacks, although fewer leaves were attacked on CA plots. The intensity of rust damage is relatively low both in CA and CONV systems on damaged leaves (going from 0.2 to 2.6% on CONV plots and 0.2 to 3.2% on CA plots) (Figure S7). *Fusarium* was found in five CA plots (2, 6, 10, 19 and 22_CA) and six CONV plots (2, 6, 10, 13, 20, 22_CONV). Damage caused by *Fusarium* was very limited and did not differ between CA and CONV plots, with a maximum of 6 affected ears in CONV, and 5 in CA (Figure S8).

All the CA and CONV plots were concerned by the presence of slugs and leaf beetle damage, although the frequency of slug and leaf miner damage was higher on CA plots (on average half of leaves affected) than on CONV plots (one-third of leaves affected). However, the intensity of damage was similar between CA and CONV plots. Very few plots were affected by damages caused by leaf miners (three CA plots: 6, 10, 19_CA, and one CONV plot: 10_CONV only). The intensity of damage on leaves was very low, <1% both on CONV and CA plots (Figure S9). All the results presented are summarised in Table 10.

Table 10. Summary of the differences found in the frequency and intensity of diseases between CA and CONV plots in the

Ob	served damage	Frequency	Intensity (%)	
	Septoria	CA <conv< th=""><th colspan="2">No difference</th></conv<>	No difference	
	Powdery mildew	No difference	No difference	
Disease	Rust	CA <conv< td=""><td colspan="2">No difference</td></conv<>	No difference	
	Fusarium	No difference	-	
	Eyespot	No difference	-	
Post	Slugs and leaf beetles	CONV <ca< th=""><th>CONV<ca< th=""></ca<></th></ca<>	CONV <ca< th=""></ca<>	
rest	Leaf miners	No difference	No difference	

non-treated zone (C). Graphs and detailed p-values are available in Figures S7 to S9.

3.1.2. Yield and grain technological quality

There was no difference in mean yield, specific weight, thousand kernel weight (TKW) and number of grains per ear on CA *vs.* CONV non-treated plots. The mean yield was about 75 q/ha on CA plots and 77 q/ha on CONV plots, with a mean specific weight of 75 kg/hl on CA plots and 77 kg/hl on CONV plots. The mean TKW was 33 g in both CONV and CA plots. The mean number of grains per ear was 37 on CA plots against 37.8 on CONV plots (Figure S10). Results are summarised in Table 11.

Table 11. Summary of results found for the grain quality indicators on the non-treated zone (C). Graphs and detailed p-values are available in Figure S10.

Grain quality indicator	Observations
ExpYield	No difference
Specific weight	No difference
Number of grains per ear	No difference
Thousand Kernel Weight	No difference

3.2. Within-field differences between non-treated and treated strip (Q2)

3.2.1. Disease and pest

Septoria was the most recorded disease in all CA and CONV plots, and traces of Septoria were found in all the sampled plots. However, in CONV plots, C zones were more affected than P zones (p=0.003). On CA plots, there is a slight difference between P and C zones (p=0.1), although it is less pronounced than on CONV plots. Powdery mildew showed no difference in frequency of occurrence and in intensity of damage caused between zones P and C, in each of the two studied systems. It was observed both on zones C and P for 10_CONV, 12_CA and 19_CA. It was found only in the C zone for 13_CA and 19_CONV, while surprisingly it was found only in the P zones of 5 and 12_CONV. Overall, intensity of damage was very low on affected leaves in each zone of the CA and CONV plots. Rust frequency and intensity of damage were higher in C zones when analysing CONV and CA plots altogether. However, the intensity of damage was identical between the C and P zones for CA plots, while it was lower in the P zone compared with the C zone on CONV plots. Seven C zones of CONV plots were affected by rust, while five were affected on P zones. For CONV plots, when rust was found, it was always found in the two zones, except for 2_CONV and 8_CONV in which rust was only found in the C zone. For CA plots, rust was found in the two zones for three plots (5_CA, 8_CA and 22_CA), while it was only found in the C zone for 17_CA and 19_CA. Surprisingly, it was only found in the P zone for 2_CA (Figure S11).

We found less *Fusarium* in the P zones of CA plots (p=0.1), while there were no differences between the P and the C zone for CONV plots. For six CONV plots, *Fusarium* was found in the two zones of the same plot for pairs 2, 3, 6, 10, 20 and 22_CONV. For 13_CONV, *Fusarium* was only found in the C zone, while for 12_CONV, it was only found in the P zone, on 1/20 ear only. For CA plots, *Fusarium* was also found in the two zones for 2, 5, 6, 10 and 22 CA. For 19_CA, it was only found in the C zone. Eyespot was found at equal rates on CA plots (on average on 2/20 leaves for the C zone) and 2/20 leaves in the P zone. The damage was lower in the P zones for CONV plots (p=0,06) with an average of 1,6/20 leaves affected in the P zone, against 3,7 in the C zone. When present, eyespot was always found in the two zones in CONV systems, except for 2_CONV where it was only recorded in the P zone (1 ear/20) (Figure S12).

We found less slug plus leaf beetle and leaf miner frequency in the P zones of CONV plots compared with their C zones (p=0.04 for slugs and leaf beetles and p=0.1 for leaf miners) while no differences were evidenced between the P and C zones of CA plots. No difference in intensity was evidenced in CA or CONV plots between the two zones for these pests (Figure S13).

All the results are summarised in Table 12.

Table 12. Results of Wilcoxon paired-ranked tests for the comparison of frequency and intensity of damage in CONV and CA plots.

	Туре	CONV (2	L2 plots)	CA (10 plots)		
Observed damage		Frequency	Frequency Intensity (%)		Intensity (%)	
	Septoria	P <c< th=""><th>P<c< th=""><th>P<c< th=""><th>P<c< th=""></c<></th></c<></th></c<></th></c<>	P <c< th=""><th>P<c< th=""><th>P<c< th=""></c<></th></c<></th></c<>	P <c< th=""><th>P<c< th=""></c<></th></c<>	P <c< th=""></c<>	
Disease	Powdery mildew	No difference	No difference	No difference	No difference	
	Rust P <c< td=""><td>P<c< td=""><td>P<c< td=""><td>No difference</td></c<></td></c<></td></c<>		P <c< td=""><td>P<c< td=""><td>No difference</td></c<></td></c<>	P <c< td=""><td>No difference</td></c<>	No difference	
	Fusarium	No difference	-	P <c< td=""><td>-</td></c<>	-	
	Eyespot	P <c< td=""><td>-</td><td>No difference</td><td>-</td></c<>	-	No difference	-	
	Slugs and leaf	P>C	No difference	No difference	No difference	
Pest	beetles					
	Leaf miners	P>C	No difference	No difference	No difference	

Graphs and detailed p-values are available in Figures S11 to S13.

3.2.2. Yield and grain technological quality

For CONV plots, yields and TKW were higher in the P zone than in the C zone. Yield was 7% lower, and TKW was 4.3% lower in the C zone for CONV systems when compared with the P zone. We observed no difference in yield between the C and P zones in CA plots. No differences were observed in specific weight and number of grains per ear in C or P zones in each of the CONV and CA plots (Figure S14). All the results are summarised in Table 13.

Table 13. Results of Wilcoxon paired-rank tests for grain quality indicators in P vs. C zones for CONV and CA plots. Graphs and detailed p-values are available in Figure S14.

Grain variable	CONV	CA		
ExpYield	P>C	No difference		
Specific weight	No difference	No difference		
Number of grains per ear	No difference	No difference		
Thousand Kernel Weight	P>C	No difference		

3.3. Influence of crop variety, previous crop and location

In this sub-section, we considered all participating plots having a C zone, independently of Q1 or Q2. The too few repetitions of each condition prevented from running robust statistical tests. Therefore, all results are based on visual observations of graphs presented in Figure S15 and Figure S16.

It seemed that the Nemo variety was the most affected by diseases, at equal rates for the CONV and CA (Figure S15A). However, this variety was used by a sole pair of farmers (pair n°22) located in the east part of Deux-Sèvres department after oilseed flax. The effects of location and/or variety and/or previous crop were difficult to evidence. Similarly, it was visually difficult to highlight any effect of crop variety on the frequency of diseases due to the absence of repetition of different wheat varieties. On wheat varieties that were repeated in the network (KWS Ultim and the mix Absalon + Advisor), we visually observed a small tendency of increased quantity of diseases in CONV plots grown with Absalon + Advisor mix (Figure S15B). Finally, not much can be said about the possible influence of previous crop on wheat diseases due to a lack of repetition of the same previous crop across pairs of farmers. An exception can be made for winter wheat and sunflower as previous crops since three pairs of farmers had winter wheat as previous crop (pairs 1, 2 and 10), while three pairs of farmers had sunflower as previous crop (pairs 5, 6 and 8). Visually, sunflower did not lead to an increased disease frequency (mean frequency = 61 for CA, vs. mean frequency = 53.4). It appeared that when winter wheat was grown for two successive years, it was more attacked by diseases in CONV than in CA (mean frequency = 41.7 in CA and mean frequency = 82.7 in CONV) (Figure S15C). For pests and disease frequency, little visual evidence was detectable of any location, previous crop or wheat variety impact. However, we could visually confirm that CA plots were more affected by pest damage than CONV ones, as evidenced in Q1 (Figure S16).

4. Discussion

4.1. Differences between CA and CONV systems on non-treated strips (Q1)

No differences were observed between CA compared with CONV systems for powdery mildew in frequency and intensity, nor for *Fusarium*, eyespot and leaf miners' frequency and intensity of damage. The only observed differences lied in an increased frequency of *Septoria* and rust in CONV C zones compared with CA C zones. However, intensity of damage was equal in CA and in CONV on leaves affected by *Septoria* or rust. On the opposite, we observed a higher frequency of *slug plus leaf beetle damage* on CA C zones, and on damaged leaves, the intensity of damage was also higher in CA compared with CONV plots. This highlights the issue of slugs (rather than leaf beetles in fact) in CA systems (Douglas and Tooker, 2012).

However, although we observed some differences in pests and diseases, we could not identify any differences in yield or other grain technological indicators. In this regard, we received one feedback

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from a farmer participating in the study (2_CA_2023). This farmer weighed the wheat harvested from the C and P zones at the weighbridge. The farmer sampled wheat on the same surface from the P and C zones, *i.e.* 211 m x 10.2 m (= 0.22 ha), corresponding to the C zone dimension. For the C zone, he obtained a weight of 1 620 kg, *i.e.* 75.2 qx/ha. For the P zone, he obtained a weight of 1 920 kg, *i.e.* 89.21 qx/ha. In our study, the results obtained by the ring test for this farmer revealed a yield of 75 qx/ha for the C zone and 79.4 qx/ha for the P zone. The results given by the farmer should therefore be considered, although they must be put into perspective, as he was the only farmer to have measured yield in both study areas. In future seasons, it would be interesting to ask all the farmers to carry out this test to validate or not the experimental yields obtained. This was done for the P zone in the two successive years, with results of experimental yields (*ExpYield_n*) and farmers' provided yields (*farmerYield_n*) were correlated at 86% (see **Annexe 5**), therefore not excluding larger differences on specific plots.

4.2. Within field differences between non-treated and treated strips (Q2)

In both CA and CONV cropping systems, we observed a lower incidence of *Septoria* in the P zone compared with the C zone, both in terms of frequency or in intensity of damage on affected leaves. We observed no significant effect on powdery mildew, with P and C zones being affected equally in both cropping systems. Regarding rust, we observed a decrease in frequency of attacks in the P zone compared with the C zone when analysing CA and CONV altogether. For CONV plots, the intensity of attacks on damaged leaves was higher in the P zone compared with the C zone, while on CA plots, the intensity of damage was identical between the P and the C zone on affected leaves. We observed no significant difference in *Fusarium* damage between the P and the C zone for CONV plots, while on CA plots, P zones were more affected by *Fusarium* than C zones. We observed an opposite pattern for eyespot, which was found in higher rates in the C zone of CONV plots compared with the P zone, while we observed no differences in CA plots between the P and the C zone. Surprisingly, we observed an increased frequency of pest damage in the P zone compared with the C zone in CONV plots, while there were no differences in the CA plots. On the CONV plots, the intensity of the damage for affected leaves was identical between the P zone.

For CONV plots, we measured a higher yield and a higher TKW in the P zone compared with the C zone, while no difference in yield and TKW in CA was observed. This suggested that despite a decrease in pesticide use rate in CA and a possible increase in disease frequency, this has not impacted CA yields, thus creating an interesting source of savings in CA systems, in addition to a positive impact on the environment. For both CA and CONV plots, we observed no differences in grain specific weights and number of grains per ear in the P and the C zone.

4.3. Influence of crop variety, previous crop and location

Nemo variety was more affected by diseases than others, but it was used only by a pair of farmers, so there might be a doubt on a potential location or previous crop effect rather than a variety effect. Wheat variety and mix effects were difficult to evidence since there were very few repetitions of each variety in the network. However, it appeared that the Absalon + Advisor mix was interesting in terms of disease rates, compared with the monocropping of KWS Ultim, thereby confirming the interest of cultivar mixtures to limit diseases (Wolfe, 2000; Wuest, Peter and Niklaus, 2021; Zhu *et al.*, 2000). The only observations we could make on the effects of previous crops on pests and diseases concerned sunflower and wheat as previous crops. Sunflowers seemed to have no effect on the rate of disease

and pest attacks, while winter wheat may have led to more attacks in CONV plots compared with CA plots, as demonstrated by Bouatrous *et al.* (2022) and Jalli *et al.* (2021).

4.4. Agronomic relevance, limits and perspectives of the case study

The year 2023 was described as a low to moderate risk year for the pests and diseases studied in the geographical zone investigated, especially for powdery mildew, rust and eyespot (Nouvelle-Aquitaine Agricultural Chamber, 2023). However, at the time of sampling, vegetal sanitary bulletins indicated a moderated to high risk of *Septoria* and *Fusarium*, confirming our observations. It would be interesting to investigate successive years to assess the stability of these results across different climatic years.

In our study, the minimal C zone size ranged from 208 m² (10_CONV_2023) to 1.8 ha (1_CA_2023), and therefore measurements were not systematically done on the same surface areas. C zones were set proportionally to field size and had to respect buffer zones with the P zone to avoid any edge effect. However, in further studies, supplementary efforts should be made to standardise at best observation zone sizes to ensure equitable observations between fields. The choice of a 3 m buffer zone was coherent with findings from the literature on the splash dispersal of major wheat pathogens in fields (Griffiths and Ao, 1976; Karisto, Suffert and Mikaberidze, 2022). Therefore, such a choice of buffer zone width was relevant to avoid any disease transfers from zone to zone.

Although only volunteer farmers agreed to leave a non-treated strip, many of them treated anyway. Having too strict rules on herbicide use and treated crops would have reduced far too much the number of participating farmers. The network was not originally designed for this, so we had to adapt to the conditions and find a compromise between what was acceptable to allow the study.

Other interesting co-factors could influence the frequency and intensity of diseases. For example, seeding density could favour spore propagation between plants (Loyce *et al.*, 2008; Morais *et al.*, 2015), the seeding date could influence slug attacks in CA systems (Bellone *et al.*, 2023; Douglas and Tooker, 2012), or other disease and pest attacks (Loyce *et al.*, 2008).

5. Conclusion

In this case study, we investigated the frequency and intensity of five fungal diseases (*Septoria*, powdery mildew, rust, *Fusarium* and eyespot) and three pests (slugs, leaf beetles and leaf miners), on winter wheat fields. The objective was to compare the effects of soil management types (CA vs. CONV) on plant resistance to diseases and pests. We evidenced that despite a relatively low pressure in pests and diseases in 2023, *Septoria* and rust frequency were lowered in CA compared with CONV on non-treated plots, while slug plus leaf beetle damage was increased in CA compared with CONV on non-treated plots. Finally, no yield loss was revealed in non-treated compared with treated zones in CA systems while a 7% loss was observed in non-treated zones of CONV systems compared with treated zones. The use of phytosanitary products did not lead to a significant increase in yields, specifically in CA systems. We can therefore question to what extent, in such a context of low disease risk, it would be possible to reduce the use of plant protection products in order to minimise the impact on human health and the balance of ecosystems.

Supplementary materials

Table S17. Main characteristics of plots participating in the "plant health" case study.

The study was led in 2023 (Year 2). Pair = pair of neighbour farmers; Location = Administrative French department number and position in the department; Texture = Texture as found in the USDA referential; Previous crop = Crop preceding the studied winter wheat; CaCO3 = % of $CaCO_3$ in soils; pH = Soil pH measured at 0-20 cm depth; System age = "Very old" if the last ploughing was done more than 20 years before the monitored year, "Old" if the last ploughing was carried out 10 to 20 years before the monitored year, "Recent" if the last ploughing was carried out between 4 and 10 years before the monitored year and "Very recent" if the last ploughing was done in the 4 years preceding the monitored.

Pair	Туре	Location	Wheat variety	Previous crop	Texture (USDA)	CaCO3	ом	рН	System age
	СА				loam		2.41	6.04	Old
1	CONV	79 west	KWS ultim	Winter wheat	silt loam	0.1	2.02	7.21	Very recent
	СА		oregrain,		silt loam	0.1	1.42	6.35	Old
2	CONV	16 south	providence, KWS ultim	Winter wheat	loam	1.3	2.42	7.89	Very recent
3	CONV	86 south	abilene	Sunflower	clay	42.5	5.88	8.18	Very recent
	СА		ascott, cesario,		silty clay loam	37.3	2.95	8.25	Recent
5	CONV	79 south	complice, oregrain, providence	Sunflower	silty clay	56.9	3.74	8.16	Recent
	СА		absalon, complice,				2.65	6.7	Recent
6	CONV	86 south	gerry, prestance, KWS ultim	Sunflower	silt loam	0.1	1.86	6.09	Recent
	СА			-			2.4	6.88	Recent
8	CONV	86 north	absalon	Sunflower	silt loam	0.1	2.23	7.14	Very recent
	СА					29.7	5.87	8.07	Old
10	CONV	16 north	absalon, advisor	Winter wheat	silty clay	47.2	4.33	8.24	Very recent
	СА	_	amstrong, pillier,	Silage maize	_	_	2.86	6.51	Recent
12	CONV	79 west	prestance, KWS ultim	Grain maize	loam	0.1	2.47	6.46	Recent
12	СА	70		Rapeseed + legumes		0.1	3.35	6.16	Old
13	CONV	79 west	absaion, advisor	Rapeseed	Silt Ioam	0.1	2	5.55	Very recent
18	СА	49 east	absalon, sy adoration, veloski	Sunflower	loam	18.8	3.28	8.09	Old
	СА				silty clay	46.6	3.98	8.24	Recent
19	CONV	17 north	KWS ultim	Реа	clay	48.6	4.62	8.15	Very recent
20	СА	27 north	NA (farm mix of	Rapeseed + legumes	loam	1.3	2.5	7.85	Very old
20	CONV	57 1101 111	varieties)	Rapeseed clay lo		2.3	2.5	8.12	Very recent
	СА	79 east					4.07	6.78	Recent
22	CONV	79 east	nemo	Oilseed flax	silt loam	0.1	3.35	7.22	Very recent



Туре		CA (11 plots in total)		CONV (11 plots in total)			(11 plot	CA s in total)	CONV (11 plots in total)	
Disease	ncy	Mean per plot (60 leaves)	cv (%)	Mean per plot (60 leaves)	cv (%)	(%) /	Mean	cv (%)	mean	cv (%)
Septoria	reque	31.8 (n=11)	21	39,6 (n=11)	25	tensity	4.4 (n=11)	75	7.4 (n=11)	75
Powdery		12.3	172	9 (n-2)	62	<u> </u>	0.7	190	0.3	124
Rust		22.4	1/5	25	05		0.9	169	0.8	124
nust		(n=5)	87	(n=7)	53		(n=5)	150	(n=7)	189

Figure S7. Results of Q1 problematic for leave diseases.

Top: Graphs of the differences of disease frequency (left) and intensity (right) of damage between CA and CONV plots on sick leaves in the C zone. The blue bar represents the 0 line (=no difference). Bars above the blue line mean that values are higher on CA plots than on CONV plots of the same pair. Bars below the blue line mean that values are higher in CONV plots than on CA plots on the same pair. p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency and mean intensity of damage in CA and CONV plots in the C zone. n corresponds to the number of plots concerned by disease presence in zone C.

Difference of disease frequency (fqCA-fqCONV) on the non-treated zone (C)



		۱	/		
Туре		CA (10 plots in t	total*)	CONV (10 plots in to	otal*)
Disease	lency	Mean per plot (20 ears)	cv (%)	Mean per plot (20 ears)	cv (%)
Fusarium	Frequ	3.8 (n=5)	34	2,3 (n=6)	59
Eyespot		2 (n=7)	91	3,7 (n=6)	61

Figure S8. Results of Q1 problematic for ear diseases.

Top: Graphs of the differences of disease frequency of damage between CA and CONV plots in the C zone. The blue bar represents the 0 line (=no difference). Bars above the blue line mean that values are higher on CA plots than on CONV plots of the same pair. Bars below the blue line mean that values are higher in CONV plots than on CA plots on the same pair. p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency and mean intensity of damage in CA and CONV plots in the C zone. n corresponds to the number of plots concerned by disease presence in zone C.*Ear samples of 5_CONV were lost after sampling, thus excluding pair 5 from the analysis.



			CA	CONV			СА		CONV		
Туре		(11 plots in total)		(11 plots in total)			(11 plot	s in total)	(11 plots in total)		
Pests	λ	mean	cv (%)	mean	cv (%)	(%)	mean	cv (%)	mean	cv (%)	
Slugs and	ner					sity					
leaf	bə.	30.3		19.6		ens	1.5		0.9		
beetles	μ	(n=11)	32	(n=11)	55	Int	(n=11)	52	(n=11)	148	
Leaf		4.7		3			0.4		0.3		
miners		(n=3)	118	(n=1)	NA		(n=3)	134	(n=1)	NA	

Figure S9. Results of Q1 problematic for pests' damage.

Differences of pest damage frequency (left) and intensity (right) of damage between CA and CONV plots on sick leaves in the C zone. The blue bar represents the O line (=no difference). Bars above the blue line mean that values are higher on CA plots than on CONV plots of the same pair. Bars below the blue line mean that values are higher in CONV plots than on CA plots on the same pair. p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency and mean intensity of damage in CA and CONV plots in the C zone. n corresponds to the number of plots concerned by pests' damage presence in zone C.



Differences of grain technological quality (CA-CONV) on the non-treated zone (C)

Туре	Unit	(11 plot	CA s in total)	CONV (11 plots in total)		
Variable		mean	cv (%)	mean	cv (%)	
Yield	q/ha	74.7	32	78.1	21	
SW	kg/hl	75.4	6	76.9	4	
TKW	g	32.7	15	33.3	10	
Grain/ear	Number of grains in 1 ear	37.0	12	37.8	17	

Figure S10. Results of Q1 problematic on wheat grain quality and yield.

Differences of yield and technological grain quality between CA and CONV plots in the C zone. The blue bar represents the 0 line (=no difference). Bars above the blue line mean that values are higher on CA plots than on CONV plots of the same pair. Bars below the blue line mean that values are higher in CONV plots than on CA plots on the same pair. p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of yield and technological grain quality indicators on CA and CONV plots in the C zone.

Difference of disease frequency (fqP-fqC) on CONV plots



Difference of disease intensity on damaged leaves (intP-intC) on CONV plots (%)



Difference of disease frequency (fqP-fqC) on CA plots

Difference of disease intensity on damaged leaves (intP-intC) on CA plots (%)



	CA (10 plots in total)											
Zone		(C	F	Р		С		F)		
		mean	cv (%)	mean	cv (%)		mean	cv (%)	mean	cv (%)		
Septoria	cy	31.7 (n=10)	22	28.0 (n=10)	21	Y.	4.6 (n=10)	72	2.9 (n=10)	106		
Powdery mildew	eduen	16 (n=3)	152	18.5 (n=2)	73	ntensit	0.9 (n=3)	164	0.5 (n=2)	71		
Rust	Ē	21 (n=5)	97	5.5 (n=4)	130	II	0.8 (n=5)	162	0.07 (n=4)	129		
				CONV (12 plots i	n tota	I)					
Zone			C	Р		Р			C	Р)
		mean	cv (%)	mean	cv (%)		mean	cv (%)	mean	cv (%)		
Septoria	ency	38.4 (n=12)	26	29.0 (n=12)	29	٨	7 (n=12)	79	4.4 (n=12)	88		
Powdery mildew	Freque	9 (n=2)	63	4.7 (n=3)	118	ntensit	0.3 (n=2)	124	0.1 (n=3)	147		
	1					<u> </u>						

Figure S11. Results of Q2 on leave diseases.

Differences of disease frequency (left) and intensity (right) of damage between C and P zoneson sick leaves in the C zone. The blue bar represents the separation between CONV plots (two graphs on the left) and CA plots (two graphs on the right). p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency and mean intensity of damage in CA and CONV plots on the C and P zone. n corresponds to the number of plots concerned by disease presence in each zone.

Difference of disease frequency (fqP-fqC) on CONV plots



Difference of disease frequency (fqP-fqC) on CA plots



	CA (10 plots in total)										
Zo	ne	C	2	Р							
Disease		mean	cv (%)	mean	cv (%)						
Fusarium	F	3.3 (n=6)	49	2.2 (n=5)	75						
Eyespot	Frequency	1.9 (n=7)	100	2 (n=6)	55						
	CONV	(11 plots i	n total*)								
Fusarium	Fraguanay	2.3 (n=7)	55	1.7 (n=7)	73						
Eyespot	Frequency	3.7 (n=7)	55	1.6 (n=8)	65						

Figure S12. Results of Q2 on ear diseases.

Differences of Fusarium (left) and Eyespot (right) frequency and intensity of damage between C and P zoneson sick leaves in the C zone. The blue bar represents the separation between CONV plots (two graphs on the left) and CA plots (two graphs on the right). p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency of damage in CA and CONV plots on the C and P zone. n corresponds to the number of plots concerned by disease presence in each zone.*Ear samples of 5_CONV were lost after sampling thus excluding 5_CONV from the analysis.



	CA (10 plots in total)											
Zone		(2	I	Р		С		Р			
		mean	cv (%)	mean	cv (%)		mean	cv (%)	mean	cv (%)		
Slugs and leaf beetles	Fraguanay	26.7 (n=10)	48	28.1 (n=10)	53	Intensity	1.3 (n=10)	67	1.4 (n=10)	81		
Leaf miners	Frequency	4.7 (n=3)	118	6 (n=3)	88		0.4 (n=3)	134	0.6 (n=3)	123		
			(CONV (12	plots in t	otal)						
Slugs and leaf beetles	Frequency	19.9 (n=12)	51	24.6 (n=12)	47	Intensity	0.9 (n=12)	142	1.3 (n=12)	148		
Leaf miners	Frequency	6 (n=2)	71	6.5 (n=4)	77	mensity	0.7 (n=2)	84	0.6 (n=4)	75		

Figure S13. Results of Q2 on pest attacks.

Differences of pests' frequency (left) and intensity (right) of damage between C and P zoneson sick leaves in the C zone. The blue bar represents the separation between CONV plots (two graphs on the left) and CA plots (two graphs on the right). p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of the total frequency and mean intensity of damage in CA and CONV plots on the C and P zone. n corresponds to the number of plots concerned by pests' damage presence in each zone.

Difference of grain technological quality (P-C) on CONV plots

Difference of grain technological quality (P-C) on CA plots





Туре		CA (10 plots in total)				CONV (12 plots in total)			
Zone		С		Р		С		Р	
	Unit	mean	cv (%)	mean	cv (%)	mean	cv (%)	mean	cv (%)
Yield	q/ha	82.7	35	83.3	25	76.6	21	82.5	17
SW	kg/hl	76.0	7	76.7	5	77.3	4	78.2	4
ткw	g	33.7	16	34.3	12	33.5	10	35.0	8
Grain/ear	Number of grains in 1 ear	37.2	12	37.5	12	37.5	17	37.5	9

Figure S14. Results of Q2 on grain technological quality and yield.

Differences of yield and technological grain quality between C and P zoneson sick leaves in the C zone. The blue bar represents the separation between CONV plots (two graphs on the left) and CA plots (two graphs on the right). p values correspond to the results of Wilcoxon paired-rank tests. Bottom: mean, coefficient of variation (cv) of yield and grain technological quality in CA and CONV plots on the C and P zone.



Figure S15. Total diseases (Sept, Mil, Rust, Fusa, Eyespot) frequency on all participating plots (n=23) according to plot location (A), wheat variety (B) and previous crop (C).



Figure S16. Total pest frequency on all participating plots (n=23) according to plot location (A), wheat variety (B) and previous crop (C).
CASE STUDY 2: "AGRIBIODIV" PILOT PROJECT

This case study summarises the work jointly realised with Amélia Bourceret (post-doctoral researcher at the National Museum of Natural History, Paris). A scientific publication is being prepared and planned for submission in fall-winter 2024.

1. Introduction

Important research has been conducted to theoretically demonstrate the importance of soil (micro)biology for soil health and crop production, and more largely One Health (Banerjee and van der Heijden, 2023; Kendzior, Warren Raffa and Bogdanski, 2022). Although knowledge on soil biodiversity is increasing, little is still known about the taxonomic diversity of the soil biota (Bardgett and van der Putten, 2014; Wall, Bardgett and Kelly, 2010). In soils, fungi are evidenced to make up diverse functional communities composed of many distinct ecological guilds (Nguyen et al., 2016), with three main guilds being of relative importance in agricultural settings. First, saprotrophic fungi are involved in OM decomposition, carbon cycling, nutrient mobilisation, and an improved soil structure. Second, the symbiotrophic fungi support the expansion of plant roots surface area, giving plants greater access to nutrients and water in exchange for C. Third, pathotrophic fungi attack crop plants but also control populations of nematodes, insects and other animal, plant or fungal pests (Schmidt, Mitchell and Scow, 2019). Soil bacteria are also key to supplying nutrients to crops, stimulating plant growth, e.g. through the production of plant hormones, controlling or inhibiting the activity of plant pathogens, improving soil structure and also have the ability to bioremediate polluted soils through the mineralisation of organic pollutants (Hayat et al., 2010). Microbial communities also colonise plants both externally and internally and are crucial for plant growth and health (Friesen et al., 2011). Finally, wheat grain microbial composition has mostly been considered with the contamination lens (Laca et al., 2006), and little research has been made to explore the potential transfers in the plant production continuum, from soil to production (Oliveira, Zannini and Arendt, 2014).

Several studies showed the effect of soil management on microbial community assemblages, namely by comparing conventional and agroecological practices (Garnica, Rosenstein and Schön, 2020; Oehl *et al.*, 2004; Paul Chowdhury *et al.*, 2019; Schmidt *et al.*, 2019). However, many questions remain around the specific composition of microbial communities in contrasted cropping systems, and specifically in CA cropping systems where information on the soil microbial communities is very scarce (Christel, Maron and Ranjard, 2021).

This case study was developed in three main axes. First, we investigated the repartition of microbial communities across the different compartments of the soil-root-plant-grain continuum. Second, we considered the possible influence of cropping systems (CA *vs.* CONV) on the repartition of microbial communities across the different compartments of the continuum. Finally, we investigated the possible links between pesticide use and the differences observed in the repartition of microbial communities.

2. Material and methods

2.1. Study site and in-field sampling

Sixteen farmers' fields were selected from the existing OFE in North-West France. A detailed description of the global study site is proposed in **Chapters 2 and 3**. Main characteristics of farmers participating in the "Agribiodiv" experiment are available in Table S18.

In-field sampling was conducted between 12 and 16 May 2023 for soil, root and leaves, and from 27 to 29 June 2023 for grain sampling. Soil, root, leaves and grain samples were collected on each plot on the five positions A, B, C, D and E of the "W" design (cf. **Chapter 2**). All samples were frozen directly after sampling and stored at -20°C until analysis in September 2023.

On each position, a lump of soil of about 20*20 cm was collected from where we collected soil at 5-15 cm from the surface, roots, and two leaves (middle of the youngest fully developed and healthy leaves) above ground (Photo 4). Bulk soil samples were taken at each of the five positions of the W transect, while three composite soil samples were taken by sampling at three positions: 1 on the transect A-D, 1 on the transect D-C and 1 on the transect C-E of the W. Fresh soil was sieved at 2 mm before freezing.



Photo 4. Lump sampling (left) and leaves samples in Eppendorf just after sampling. ©Clara Lefèvre

2.2. Laboratory analysis

2.2.1. Soil and plant-associated microbial community

The root fraction (endosphere and rhizoplane) was obtained in the laboratory by using a protocol adapted from (Bulgarelli *et al.*, 2012). A first washing step of around 1g of root material in sterile falcon tubes with TE 1x buffer supplemented with 0.1% Triton X-100 (TE-T) using a cycle agitator (20 min, 35 rpm) and centrifugation (20 min, 4000g, 16°C) allowed to remove the rhizospheric soil from the roots. The root fraction was obtained after one more washing step in sterile water. Roots, leaves and grains were carefully dried on Whatman paper and flash-frozen in liquid nitrogen and crushed using 2 sterile metal beads of 3.2 mm and several mg of Fontainebleau sand (frequency 30, 2x30 sec, 15 sec break) (Qiagen tissue lyser (Qiagen, Germany)).

Total genomic DNA was extracted from around 100 mg of root, leaf, grain and soil, by using the DNeasy Plant Mini Kit (Qiagen, Germany). The concentration of DNA samples was measured by fluorescence (Quant-IT[™]Picogreen, Invitrogen, Oregon, USA) and samples were diluted to 3.5 ng/µl and purified by Ampure XP beads, (Agencourt, Beckman Coulter, USA) to eliminate PCR inhibitors. Different primer tag combinations and specific primer sets targeting the fungal ITS2 region (ITS86F: GTG AAT CAT CGA ATC TTT GAA; ITS4: TCC TCC GCT TAT TGA TAT GC (Turenne *et al.*, 1999; White *et al.*, 1990), and the bacterial 16S V5V7 region (799F: AAC MGG ATT AGA TAC CCK G; 1193R: ACG TCA TCC CCA CCT TCC) (Dombrowski *et al.*, 2017), were used to perform PCR amplification in triplicate. The PCR were prepared in 25 µL of reaction mixture as described in (Petrolli *et al.*, 2021). An additional three PCR negative controls per amplicon were amplified at this step. After pooling of triplicate PCR reactions, the quality of amplification was checked on agarose gels (5 µL, 2%, 100 V, 20 minutes). Fungal PCR

products were purified twice by Ampure XP beads (Agencourt, Beckman Coulter, USA). Bacterial PCR products were analysed on agarose gels (100 μ L, 1H, 70V, 2%) to separate bacterial from plant mitochondrial products. Bands corresponding to 500 bp were cut and then purified (QIAquick gel extraction kit, QIAGEN, Hilden, Germany). DNA concentration was checked (Quant-ITTMPicogreen, Invitrogen, Oregon, USA) and PCR products belonging to the same microbial kingdoms (bacteria or fungi) were pooled together in equimolar amounts of 50 ng to build one library per amplicon. The sample pools were purified twice by Ampure XP beads (Agencourt, Beckman Coulter, USA), and quantified by Qubit [®] dsDNA HS Assay Kit (Invitrogen, USA). MetaFast library preparation and sequencing were performed on an Illumina 2 × 250 MiSeq platform by Fasteris SA (Switzerland).

2.2.2. Sequencing data analysis

Data processing was performed by using a pipeline based on VSEARCH (Rognes *et al.*, 2016) and available on GitHub⁷ (Perez-Lamarque *et al.*, 2022; Petrolli *et al.*, 2021). Briefly, after merging pairedend reads (*fastq_mergepairs function*, default parameters), merged reads with more than two errors in alignment were removed. Then merged reads were demultiplexed (no error accepted in primers or tag sequences) by using cutadapt (Martin, 2011). Chimeras were removed de novo (*uchime3_denovo option* of VSEARCH) and the taxonomic assignment of the Amplicon Sequence Variants (ASV) was performed (*usearch_global option*, default parameters) by using the SILVA 138.1 and UNITE (v.8.0) databases (Kõljalg *et al.*, 2019; Quast *et al.*, 2013), for bacteria and fungi, respectively. The ASV tables were filtered from contaminants by comparing the amplification of negative controls and by using the R package DECONTAM (prevalence method) (Davis *et al.*, 2018). Only ASVs with long sequences (>200 bp), assigned to the bacterial or fungal kingdoms, presenting an acceptable abundance (\geq 10 reads), and prevalence (\geq 1 sample) were filtered for deeper analyses

2.2.3. Pesticide residues analyses

After surveying farmers' practices in years 1 and 2, an in-depth analysis of their pesticide use was made to identify the active molecules used from the commercial product names provided by farmers.

Grain pesticides analysis was done on grain harvested on the study zone by farmers in summer 2023. We excluded two participants from the analysis, since they used different wheat varieties (pair 14).

A few days after harvesting, grains were collected by farmers and stored at -20°C until analysis (see **Chapter 2**). Pesticide residues analysis was done by a certified laboratory (Phytocontrol laboratory, <u>https://www.phytocontrol.com/en/</u>) either by gaseous or liquid chromatography according to the targeted pesticides. We realised a multi-pesticides analysis targeting 650 molecules of interest in total. The list of targeted molecules, selected based on farmer use, is available in **Annexe 6** at the end of the manuscript.

2.3. Statistical analysis

Analyses were performed with R Studio (R Core Team, 2021). ASVs tables were normalised to the total number of reads per sample. Beta-diversity (between samples) was visualised with Principal Coordinate Analysis (PcOA) of the Bray-curtis distance matrix between samples using the cmdscale function of the {stats} package (R Core Project, 2019). The analysis is based on Bray-curtis matrix distance, where each point represents one sample, the more two samples are spatially close and the

⁷ <u>https://github.com/BPerezLamarque/Scripts/</u>

more they present a similar microbial community composition at the ASV level. Shared and specific ASVs between and to each condition, respectively, were visualised using Venn diagrams (presence/absence). Venn diagrams were built using the {VennDiagram} package and diversity profiles were made using the {Plyr} package (Chen, 2022; Wickham, 2011).

Root, leaf and seed results for pair n°14 were excluded (only soil was kept), since the two farmers in this pair did not grow the same wheat variety. A p-value of less than 0.05 was considered significant (with 999 permutations). Finally, the FUNGuild database (Zanne *et al.*, 2020) was used to assign each ASV to its functional guilds based on its taxonomic affiliation. Three guilds were used, *i.e.* symbiotroph, pathotroph, saproptroph. When FUNGuild results indicated two or more different guilds associated with an identical ASV, the ASV was assigned to an "unknown" trophic guild. Soil and composite soil results were pooled for FUNGuild analysis and Venn diagram visualisations.

3. Results

3.1. Microbial communities' repartition across the soil-root-plant-grain continuum

The two PCoA showed sample dissimilarities based on their common ASV. For bacteria, the PCoA explained 17% of differences on the first axis, while it explained 7.6% of differences on the second axis. For fungi, 26.1% of differences were explained by the first axis while 10.4% of differences were explained on the second axis. The first axes separated aerial and underground parts, with grain and leaf samples located on the left part of the PCoA for both fungal and bacterial communities, and soil composite and soil bulk samples located both on the right part of the PCoA (Figure 21). For fungi, root samples were clustered with soil both on the first and second axes, while for bacteria, they were clustered with soil on the first axis but not on the second, suggesting that fungal roots and soil communities were more similar than bacterial communities. The fungal community in grain seemed specific and different from the other studied compartment, since the grain samples were strongly clustered together, which was not the case for the bacterial grain community, which was spread along the first axis (Figure 21).

For both microbial kingdoms (bacteria and fungi), soil was the compartment with the highest richness and diversity and seed was the lowest (Figure 22). There was a high number of fungal ASVs in leaves compared with bacteria (Figure 22, 23).



Figure 21. Results of the PCoA, showing the effect of plant host niche and pairs of farmers on fungal and bacterial community assemblages.

(A) Bacterial communities, (B) Fungal communities.



Figure 22. Effect of host niche on microbial diversity and richness.

Shannon index represents the diversity and observed ASV correspond to richness (number of different species). Letters correspond to significant differences calculated with Kruskal Wallis (p<0.05).



Figure 23. Core and specific microbiota according to plant host niche.

Figures correspond to the number of ASVs shared between compartments and specific to leaf, root, soil and grain. 532 and 401 ASVs correspond to core fungal and core bacterial microbiota respectively. The "soil" compartment corresponds to merged soil and composite soil.

3.2. Microbial communities across cropping systems

We observed a higher bacterial diversity in CA leaves compared with CONV, and the contrary for fungi, with significantly higher diversity in CONV leaves compared with CA. For richness, the only significant results evidenced a higher richness in CA soils compared with CONV soils (Figure 24). For fungi, and bacteria, most of the ASVs were shared and corresponded to the core microbiota, except for the bacterial leaf community, where the majority of specific ASVs belonged to the CA condition (Figure 25). The percentage of unknown guild was high for each compartment, to a lesser extent for the leaf compartment. The highest proportion of unknown guild was observed in wheat grain. We observed a lower proportion of symbiotroph in roots and grain compared with leaf and soil. For each compartment a comparison of abundance of trophic guilds was performed between CA and CONV soil cropping (Kruskal Wallis test, significance < 0.05). A significant difference was observed only in soil, with higher abundance of pathotrophs in CA compared with CONV (Figure 26).



Figure 24. Effect of cropping systems on microbial diversity and richness according to host niche. Shannon index represents the diversity and observed ASV correspond to richness (number of different species). Stars correspond to significant differences calculated with Kruskal Wallis (p<0.05).



Figure 25. Core and specific microbiota according to cropping management type across compartments.



Figure 26. Effects of cropping management on fungal trophic guild according to the different plant host niches.

3.3. Digging into pesticide use on farmers' plots

We recorded forty-two active compounds in total, used by farmers. Some pesticides were only used by CONV farmers, while some others were only used by CA farmers (Figure 30, Table S19). 9 out of 42 compounds were not referenced on the international WHO reference list of Recommended Classification of Pesticides by Hazard (WHO, 2020). Of these 42 compounds, 7 were found in grain at very low concentrations close to the detection rate threshold (Table S19). 21 active herbicide compounds were used by farmers. The most used herbicides were diflufenican, followed by flufenacet and prosulfocarbs. Glyphosate was the fifth most used herbicide and was used only by CA farmers. 17 active fungicide compounds were used by farmers. The most used herbicide and was used only by CA farmers. 17 active fungicide compounds were used by farmers. The most used herbicide and was used only by CA farmers. 17 active fungicide compounds were used by farmers. The most used fungicide compounds were prothioconazole, metconazole and pyraclostrobine.

Some residues of active compounds were found in grain while they were not used by farmers in the wheat campaign 2023. It was the case for the two occurrences of tebuconazole (fungicide, 6_CA, 16_CONV at <0.01 and 0.017 mg/kg respectively), 2-methyl-4-chlorophenoxyacetic acid (MCPA, herbicide, 6_CONV). The other detected residues were used by farmers during the wheat growing campaign. It was the case for fluopyram (fungicide, 16_CONV, <0.01 mg/kg), fluxapyroxad (fungicide, 8_CA, 0.010 mg/kg) and mefentrifluconazole (fungicide, 8_CA, <0.01 mg/kg). Mefentrifluconazole was used by four farmers (7 CA, 7 CONV, 8 CA and 20 CONV). Fluopyram was applied by only the one CONV farmer in which residues were detected. Fluxapyroxad was applied by 4 farmers (1 CA and 3 CONV).

Glyphosate was used by six CA farmers (4_CA, 7_CA, 8_CA, 15_CA, 16_CA (twice), and 20_CA), but no residues of glyphosate and AMPA were found in grains in any of the Agribiodiv participating farmers.

Four active insecticide compounds were used, which were all part of the pyrethroid family. Of the 14 farmers whose grain samples were analysed, 9 applied an insecticide (4 CA farmers, 5 CONV farmers). One CONV farmer applied it twice using 2 different molecules (esfenvalerate and lambda-cyhalothrine). No insecticide residue was found in any of the analysed samples (Figure 27, Table S19).



Figure 27. Active compounds used by Agribiodiv farmers in 2023.

4. Discussion

4.1. Microbial communities in the continuum soil-root-plant-grain

Our analyses confirmed in real field conditions that the plant host niche (plant compartment) is a major driver of fungal and bacteria community composition (Fausto *et al.*, 2018; Martínez-Diz *et al.*, 2019). For both microbial kingdoms (bacteria and fungi), soil was the compartment with the highest number of ASVs and seed the lowest. This confirmed what we usually see in the literature on soils being an important reservoir of biodiversity (Banerjee and van der Heijden, 2023). However, these results question the vertical transmission of fungi and bacteria microbiota from soil to grain in wheat (Laurent-Webb *et al.*, 2024). It is well known that in soil, roots represent hot spots of interaction for microbes, due to nutrient exchange and water caption (Bonfante and Anca, 2009; Fitter and Garbaye, 1994; Martin, Perotto and Bonfante, 2000; Sahu *et al.*, 2019). The PCoA confirmed this close relationship between root and soil with similar fungal community composition, contrary to bacteria.

Finally, despite differences in wheat varieties and cropping management types, very specific fungal grain communities were observed, suggesting that grain fungal composition remains stable in winter wheat. In literature it was shown that plant genotype was a significant but relatively minor driver of plant microbiota (Wagner *et al.*, 2016; Walters *et al.*, 2018). On the contrary, leaf fungal communities appeared to be quite diversified, spread across the second axis of the PCoA. This suggested that additional factors could influence their composition (*e.g.* cropping practice, foliar inputs, wheat variety). For bacteria, grains and leaves are spread along the first axis, suggesting stronger environmental effects on bacterial composition.

4.2. Influence of cropping practices on the microbial communities

Venn diagrams showed that regardless of the compartment, most communities were shared whether the cropping system was CA or CONV, except for bacterial communities in leaves which had a strong specific CA community. This result suggested that amongst the different plant compartment characterised in this case study, leaf bacterial microbiota seemed to be the more affected by CA. Further statistical analyses could be performed to identify the effect of soil cropping on predictive functional bacterial communities by using for example PICRUSt tools (Langille *et al.*, 2013).

Regarding fungal communities, FUNGuild was an interesting tool that provided precision on the function of the fungal communities across compartments. FUNGuild showed that regardless of the cropping practice, most pathotroph fungi were situated in leaves, with an apparent relative abundance higher in CA systems than in CONV systems. This result appeared counterintuitive considering the results of **Case study n°1** which showed that on non-treated plots, fewer diseases were observed in CA compared with CONV plots. Although **Case study n°1** was not run on the exact same plots as this case study, this would suggest that despite a higher proportion of pathogens in leaves, wheat plants were able to defend themselves to remain healthy (Altieri *et al.*, 2024).

No differences were observed in the relative saprotroph and symbiotroph communities in soil, root, plant and grain. Although a higher proportion of saprotroph fungi communities could have been expected in CA soils, due to a higher quantity of residues and OM in CA soils than CONV soils (see **Chapter 3**), these results are in line with current literature evidencing no effect of no-till and cover crops on saprotrophic populations (Schmidt, Mitchell and Scow, 2019). However, literature relates a higher proportion of symbiotroph fungi in soils and roots of CA soils (Schmidt, Mitchell and Scow, 2019), likely to be explained by increased mycorrhisation under no-till and cover crops management (Tian *et al.*, 2024). Further investigations could be run to explore existing links between cropping practices and an increased rate of arbuscular mycorrhiza in wheat. The proportion of pathotrophic fungi was higher in CA soils than in CONV soils (p<0.05). This may be explained by residue retention and the associated increased humidity at the very top soil surface, likely to favour the survival of pathogens (Giller *et al.*, 2015). Last, FUNGuild results for grain showed that the great majority of ASV sequences are unknown, confirming the little knowledge around grain microbial communities' composition and functions.

4.3. Pesticide use and residues in grain

We listed all pesticide uses in CA and CONV systems (herbicides, fungicides and insecticides). Our results showed that the frequency of active herbicide compounds utilisation was highest in CA systems compared with CONV, while the contrary was observed for active fungicide compounds (CONV>CA). These results confirmed observations of **Chapter 3** where we measured on the full network a difference in TFI, with TFI herbicides higher in CA than in CONV systems, and TFI excluding herbicides higher in CONV than in CA systems. We cannot confirm any higher harmful effect of glyphosate herbicide compared with other substances on the soil-root-plant-grain continuum, mainly due to the little observed differences between CA and CONV systems, and the high number of confounding effects linked to pesticide uses in these two systems. Although glyphosate use is often pointed out in CA systems, and its use is a public health concern regarding its exposure (Xu *et al.*, 2019), evidence shows that glyphosate would have minimal impacts on soil and rhizosphere bacteria of wheat, although dying roots after glyphosate application may provide a "green-bridge" favouring some copiotrophic taxa

(Schlatter *et al.*, 2017). On the other hand, glyphosate would have more negative effects on soil fungal communities (Vázquez *et al.*, 2021), likely to explain the lower abundance fungi:bacteria ratios, and the lower abondance and diversity of fungal communities in CA compared with CONV systems, as evidenced in **Chapter 4**.

Although many active compounds were used, only a few of them were found in grains, and when found, their concentration was very low, at the edge of detection limits. Residues found in grains were mainly fungicide residues (tebuconazole, mefentrifluconazole, fluopyram and fluxapyroxad). Tebuconazole and mefentrifluconazole are part of the triazole family, while fluopyram and fluxapyroxad are part of the second-generation SDHI family. Although the use of second generation SDHI is increasing, concerns exist around their use and associated biodiversity and human health risks, since they target the respiratory chain complex II, which is a universal component of mitochondria from living organisms (Bénit et al., 2019; Duarte Hospital et al., 2023). Little evidence exists to date proving any harmful effect on human health, although recent research highlights the "absence of unacceptable cumulative dietary risk for French people exposed to SDHI", based on data from 2017-2021 (Trenteseaux et al., 2024). Recent studies showed that tebuconazole applied in the range of good agricultural practices (which was the case in our study) would not significantly disturb soil microbiology (Baćmaga et al., 2022), and had little adverse effects on human health at low concentrations (Muri et al., 2009). Little information exists on the toxicity of mefentrifluconazole to microbial communities and human health, since this molecule utilisation appears to be relatively new. One recent study has pointed out its negative effects on earthworm populations (Yao et al., 2024). One post-emergence herbicide residue was found in grain though it had not been used by the farmer during the winter wheat growing season (MCPA). MCPA is referenced to be a highly mobile herbicide, though its toxicity for humans is relatively low. Its fate on soils after application is not fully understood (Morton et al., 2020). However, at this stage it is difficult to establish any link between pesticide use, residues found in grain and their possible effects on bacterial and fungal communities. The high number of used active compounds makes it difficult to identify specific effects of their effects in real conditions. In addition, cocktail effects may occur, likely to worsen the effects on the different plant host compartments (Geissen et al., 2021; Mäder et al., 2024; Panico et al., 2022), and consumers' health (Hernández et al., 2013; Rizzati et al., 2016).

Finally, no insecticide residues were found in any grain sample, which is comforting with regard to their negative effects on health and the environment. However, all the used insecticides belonged to the pyrethroid family, which is suspected of having an important residence time in soils, with suspected negative effects on microbial communities that we were unable to assess in the frame of this study (Singh *et al.*, 2022).

4.4. Study limitations and perspectives

More research should be made to investigate on co-effects such as those of the previous crop (rapeseed with or without legume associations, sunflower), which may also affect microbial communities (D'Acunto *et al.*, 2018; Venter, Jacobs and Hawkins, 2016). The age of the CA system may also influence microbial communities (González-Chávez *et al.*, 2010; Wang *et al.*, 2017). Also, we have no information on the possible season, or wheat development effects on wheat-associated microbiota and we could assume that these parameters could also interact with the effect of soil cropping (Bourceret *et al.*, 2022; Edwards *et al.*, 2018). Our results therefore correspond to a specific situation, which may evolve as wheat grows or seasonal conditions change. Lastly, most of FUNGuild information

was "unknown", therefore the taxonomic associations remain much of a black box, leaving us with a great proportion of uncertainty in the obtained results.

Finally, we have no information regarding possible pesticide residues in the other compartments than grain (soil, roots, leaves), and therefore we are unable to conclude on the possible vertical pesticide degradation or transfers from soil to grain, increasing uncertainties on the fate of pesticides and consequences of their use on microbial communities. A recent study of pesticide residues in French soils showed that the most detected pesticide was AMPA, followed by glyphosate, fluopyram, fluxapyroxad and diflufenican, which all are pesticides used by Agribiodiv participating farmers (Froger *et al.*, 2023). We can therefore presume that these pesticides are likely to be found in the soils of our study.

5. Conclusions

This study under real conditions, carried out on 16 winter wheat fields in Northwestern France confirmed the high importance of the plant host niche regardless of the cropping system. It also showed contrasting results across farming practices, and the difficulty to establish links or precise conclusions on the effects of cropping practices on microbial communities across the different compartments of the plant continuum. However, it is to our knowledge one of the rare studies that tried to evidence and analyse in real conditions the evolution dynamics of evolution of bacterial and fungal communities along a gradient of cropping practices, including CA, and across the different compartments of the plant continuum. We therefore can only encourage the following:

- more on-field research to increase our knowledge of the effects of cropping systems and pesticide use on microbial communities, and
- more research on the activity of the different fungal and bacterial communities, to increase our level of knowledge on their ecosystemic functions in soils and the environment

This would deeply support the elucidation of today's big black box around the roles of microbes in sustaining soil, plant health and crop production.

Supplementary materials

Table S18. Main characteristics of Agribiodiv participating farmers.

The study was led in 2023 (Year 2). Pair = pair of neighbour farmers; Location = Administrative French department number and position in the department; Texture = Texture as found in the USDA referential; Previous crop = Crop preceding the studied winter wheat; CaCO3 = % of CaCO3 in soils; pH = Soil pH measured at 0-20 cm depth; System age = "Very old" if the last ploughing was done more than 20 years before the monitored year, "Old" if the last ploughing was carried out 10 to 20 years before the monitored year, "Recent" if the last ploughing was carried out between 4 and 10 years before the monitored year and "Very recent" if the last ploughing was done in the 4 years preceding the monitored.

Pair	Туре	Location	Texture	Prec crop	Wheat variety	CaCO3	Soil pH	System_age
4	CA	86 mid	Sandy clay loam	Rapeseed + legumes	absalon, complice, filon, letsgo,	0.1	7.3	Old
	CONV		Loam	Rapeseed	macaron, sacramento	0.1	5.7	Very recent
	CA			Sunflower	absalon, complice,	0.1	6.7	Recent
6	CONV	86 south	Silt loam		gerry, prestance, KWS ultim	0.1	6.1	Recent
-	CA	0C agusth		Cuefleuren		0.1	6.4	Old
7	CONV	86 South	Loam	Sunnower	montecario	0.1	6.0	Recent
	CA	0.0	C'IL I	c (1		0.1	6.9	Recent
8	CONV	86 north	Silt loam	Sunflower	absalon	0.1	7.1	Very recent
14	CA	37 north	Loam	Rapeseed + legumes	apache, prestance, providence	0.1	7.0	Very old
	CONV			Rapeseed	NA (mono variety)	0.1	7.0	Very recent
	CA	06 11		Sunflower		0.1	6.9	Recent
15	CONV	86 mid	Loam	Rapeseed	- NA	0.1	6.7	Very recent
	CA			Rapeseed +	aigle, fillon, forcali,	0.1	6.7	Old
16		86 mid Loam legumes	macaron, mondio,					
10	CONV			Rapeseed	KWS sphere, syllon, tallendor	0.1	6.7	Very recent
	CA			Rapeseed +		1.3	7.9	Very old
20		37 north	Loam	legumes	NA (mix of varieties)			
	CONV			Rapeseed		2.3	8.1	Very recent

Table S19. Active compound application and corresponding residues found in grain wheat.

Pair corresponds to the identification number of farmer, CA and CONV type correspond to Conservation Agriculture or Conventional farming. Application corresponds to whether the active compound was applied on the field or not. When an active compound appears more than once for the same farmer, it means that it was applied twice. Residue indicates if residues were found in grain wheat or not. Concentration corresponds to the residue concentration when applicable.

Pair	Туре	Pesticide type	Active compound name	Application	Residue	Concentration (mg/kg)
			metconazole	yes	no	-
		Fungicides	prothioconazole	yes	no	-
			pyraclostrobine	yes	no	-
			glyphosate	yes	no	-
			prosulfocarb	yes	no	-
	C A		flufenacet	yes	no	-
	CA	Horbicidos	diflufenican	yes	no	-
		Herbicides	prosulfocarb	yes	no	-
			halauxifen-methyl	yes	no	-
			florasulam	yes	no	-
4			cloquintocet-mexyl	yes	no	-
4		Insecticides	esfenvalerate	yes	no	Concentration (mg/kg) -
			metconazole	yes	no	-
		Fungicides	difenoconazole	yes	no	-
			azoxystrobine	yes	no	-
		Herbicides	flufenacet	yes	no	-
	CONV		picolinafen	yes	no	-
			diflufenican	yes	no	-
		Herbicides	tribenuron-methyl	yes	no	-
			fluroxypyr-meptyl	yes	no	-
			florasulam	yes	no	-
		Insecticides	esfenvalerate	yes	no	-
			fenpicoxamide	yes	no	-
		Fungicides	prothioconazole	yes	no	-
			Tebuconazole	no	yes	<0.01
			flufenacet	yes	no	-
	СА		diflufenican	yes	no	-
		Herbicides	aclonifene	yes	no	-
		Trendretere	cloquintocet-mexyl	yes	no	-
			pinoxaden	yes	no	-
6			metsulfuron-methyl	yes	no	-
			bromuconazole	yes	no	-
		Fungicides	tebuconazole	yes	no	-
		Tunglelues	fenpicoxamide	yes	no	-
	CONV		prothioconazole	yes	no	-
			flufenacet	yes	no	-
		Herbicides	diflufenican	yes	no	-
			aclonifene	yes	no	-
			МСРА	no	yes	<0.01
7	СА	Fungicides	bromuconazole	yes	no	-

Pair	Туре	Pesticide type	Active compound name	Application	Concentration (mg/kg)	
			tebuconazole	yes	no	-
			azoxystrobine	yes	no	-
			mefentrifluconazole	yes	no	-
			pyraclostrobine	yes	no	-
			prothioconazole	yes	no	-
			glyphosate dimethylamin salt	yes	no	-
			halauxifen-methyl	yes	no	-
			florasulam	yes	no	-
		Herbicides	cloquintocet-mexyl	yes	no	-
			clopyralid	yes	no	-
			florasulam	yes	no	-
			fluroxypyr	yes	no	-
		Insecticides	cypermethrine	yes	no	-
			mefentrifluconazole	yes	no	-
		Fungicides	fluxapyroxad	yes	no	-
		_	pyraclostrobine	yes	no	-
	CONV		diflufenican	yes	no	-
		Herbicides	chlorotoluron	yes	no	-
		Insecticides	cypermethrine	yes	no	-
			fluxapyroxad	yes	yes	0.01
		Fungicides	mefentrifluconazole	yes	yes	<0.01
		Herbicides	glyphosate	yes	no	-
	СА		diflufenican	yes	no	-
			flufenacet	yes	no	-
			clodinafop-propargyl	yes	no	-
8		Herbicides	cloquintocet-mexyl	yes	no	-
		Fungicides	metconazole	yes	no	-
			benzovindiflupyr	yes	no	-
			prothioconazole	yes	no	-
	CONV		diflufenican	yes	no	-
		Herbicides	flufenacet	yes	no	-
		Insecticides	tau-fluvalinate	yes	no	-
			metconazole	yes	no	-
		Fungicides	trifloxystrobine	yes	no	-
			fenpicoxamide	yes	no	-
			prothioconazole	yes	no	-
	СА	Herbicides	glyphosate	yes	no	-
4-			prosulfocarb	yes	no	-
15			diflufenican	yes	no	-
			metsulfuron-methyl	yes	no	-
		Insecticides	esfenvalerate	yes	no	-
			prothioconazole	yes	no	-
	CONV	Fungicides	fluoxastrobine	yes	no	-
			fluxapyroxad	yes	no	-
L				1	1	1

Pair	Туре	Pesticide type	Active compound name	Application	Residue	Concentration (mg/kg)
			pyraclostrobine	yes	no	-
			metconazole	yes	no	-
			sulphur	yes	no	-
			flufenacet	yes	no	-
		Herbicides	pendimethaline	yes	no	-
			prosulfocarb	yes	no	-
		Insecticides	lambda-cyhalothrine	yes	no	-
		Fungicides	trifloxystrobine yes no		no	-
			2,4-D dimethylamin salt	yes	no	-
			glyphosate	yes	no	-
			2,4-D dimethylamin salt	yes	no	-
			glyphosate	yes	no	-
	C A	Horbisidos	prosulfocarb	yes	no	-
	CA	Herbicides	flufenacet	yes	no	-
			cloquintocet-mexyl	yes	no	-
			pinoxaden	yes	no	-
			2,4-D dimethylamin salt	yes	no	-
			fluroxypyr-meptyl	yes	no	-
		Insecticides	cypermethrine	yes	no	-
16			metconazole	yes	no	-
			prothioconazole	yes	no	-
		Eurogicidos	tebuconazole	no	yes	0.016
		Fungicides	fluopyram	yes	yes	<0.01
			bixafen	yes	no	-
			azoxystrobine	yes	no	-
	CONV		prosulfocarb	yes	no	-
			flufenacet	yes	no	-
		Herbicides	diflufenican	yes	no	-
			tribenuron-methyl	yes	no	-
			metsulfuron-methyl	yes	no	-
			esfenvalerate	yes	no	-
		Insecticides	lambda-cyhalothrine	yes	no	-
			glyphosate	yes	no	-
			clodinafop-propargyl	yes	no	-
			cloquintocet-mexyl	yes	no	-
			prosulfocarb	yes	no	-
			diflufenican	yes	no	-
20	СА	Herbicides	flufenacet	yes	no	-
_			metsulfuron-methyl	yes	no	-
			diflufenican	yes	no	-
			florasulam	yes	no	-
			iodosulfuron	yes	no	-
			cloquintocet-mexyl	yes	no	-
			cloquintocet-mexyl	yes	no	-

Pair	Туре	Pesticide type	Active compound name	Application	Residue	Concentration (mg/kg)
			pinoxaden	yes	no	-
			sulphur	yes	no	-
		Fungicides	mefentrifluconazole	yes	no	-
	CONV		fluxapyroxad	yes	no	-
			pyraclostrobine	yes	no	-
			fenpicoxamide	yes	no	-
			prothioconazole	yes	no	-
			diflufenican	yes	no	-
		Horbicidos	pendimethaline	yes	no -	-
		nerbicides	prosulfocarb	yes	no	-
			metsulfuron-methyl	yes	no	-

CASE STUDY 3: FROM FARM TO BREAD!

This case study aimed to go deeper into the identification of possible links between cropping practices and the quality of human consumption products, closing the loop of this system study "from farm to fork". The analyses were run in partnership with "Moulins Girardeau" mill. In addition to the study of technical bread quality, this pilot study was also an opportunity to animate the farmers' network. Visits of the mills and bread-tasting were organised twice during the PhD project.

1. Introduction

Bread is a traditional staple food in many countries around the world. We consider that an average of 34.8 million tons of winter wheat are produced yearly in France, from which about five million tons are transformed into flour. A French adult consumes daily an average of about 100 grams of bread (Intercéréales, 2023). Roller milling is usually used to process wheat into different fractions, with the aim of separating the starchy endosperm tissue from the germ and the outer layers of the grain (including the aleurone, testa and pericarp) (Lockyer and Spiro, 2020). The bran corresponds to the germ and outer layers together. As seen in **Chapter 4**, fibre, vitamins and minerals are mostly concentrated in the bran (and particularly in the aleurone component). Therefore, flour nutrient composition varies with the extraction rate of the grain. In this regard, white flour consists of just the starchy endosperm, which comprises mainly starch and protein (about 80% and 10%, respectively), while wholemeal flour is a 100% extraction-rate flour, and brown flour contains about 85% of the grain (some bran and germ have been removed) and white flour between 75% and 80% extraction (Lockyer and Spiro, 2020). In industrial schemes, most of the wholemeal flour is produced by recombining all the edible fractions produced by roller milling.

This pilot study was launched with two objectives. The first objective was to assess the possibility of identifying the effects of cropping practices on the final product through analyses of bread technological quality. The second objective of the pilot was on the one hand to animate the farmers' network in a One Health perspective, and on the other hand to gather different stakeholders.

2. Material and methods

2.1. Farmers' selection and grain sampling

We selected five pairs of farmers in year 1 and five different pairs in year 2. For this study, we needed to select farmers whose grown wheat varieties were suitable for bread-making, therefore excluding all wheat varieties that would not comply with the "BPMF"⁸ criteria (*e.g.* stromboli, armada).

Participating farmers are indicated in Table 9. Apart from the 5 kg grain that all farmers put aside for the grain nutritional and sanitary analysis (see **Chapter 4**), we asked these farmers to keep 10 kg grain aside when harvesting the principal study zone. Grain was collected a few days after harvest and stored at ambient temperature in a dry room for a few weeks before analysis

Samples were analysed at the Girardeau Mill (<u>https://www.minoterie-girardeau.com/en/</u>) in early fall 2022 and 2023. Grains were milled on a test mill with steel grinding wheels, and the following indicators were analysed, as described in **Chapter 2**: quantity of flour ashes (*f.Ashes*), mill yield (*MillIYield*), Hagberg falling time (*f.Hagberg*), the percentage of flour hydration (*f.Hydration*). Bread-making tests were also run and several variables were measured on dough, such as the elasticity index (*f.IE*), the toughness:extensibility ratio (*f.P_L*) and the baking force (*b.W*). Bread technological

⁸ BPMF (« Blés Pour la Meunerie Française » or "Wheats for French Millers") correspond to winter wheat varieties that are suitable for Bread-making. Each year, the national association of French millers ("Association Nationale de la Meunerie Française", ANMF) releases the list of winter wheat varieties that contain the best protein rates, aptitude to milling and rheologic profiles adapted to different flour uses.

parameters were eventually considered, *i.e.* bread length (*b.Length*), volume (*b.Volume*), and the baking score (*Baking_Score*).

2.2. Stakeholders' involvement

This small project was the opportunity to organise small events with farmers. Two mill visits were organised in December 2022 and May 2024. The two visits were open to farmers and partners from the Agricultural Chambers of Deux Sèvres, Vienne and Nouvelle-Aquitaine and organised as follows: (1) visit of the mill, (2) restitution workshop on the advances and first results of the whole study and (3) bread tasting with the remaining farmers' flours (Photo 5).

2.3. Statistical analysis

Similar to the other chapters, analyses were run with R studio (R Core Team, 2021). We ran Wilcoxon paired-rank tests to compare the different results in the CA and CONV pairs. We did not use the cluster approach since the effective per cluster was too low to run robust analyses. Like **Chapter 4**, we run principal component analyses to identify potential co-factor effects, namely those of cropping practices, location, year, pair and previous crop to studied wheat. Wheat variety effects on bread quality were difficult to evidence, since we did not have enough repetitions of the same varieties or mixes to enable any robust conclusion.



Photo 5. Mill visits and bread-tasting in December 2022 (top) and May 2024 (bottom).

3. Results

Paired comparisons of CONV vs. CA showed no difference between all the indicators tested, except a significant difference in mill yield (*MillYield*) and a tendency in the baking score results (p<0.10) (*Baking_Score*) (Table 14). The first two axes of the PCA gathering all bread indicators explained 60.4% of the variability (Figure 28A). The variables that contributed the most to the first axis were the baking force (*b.W*) and the Hugberg falling number (*f.Hagberg*). It was difficult based on the PCA to evidence any *Type* or Cluster effect on grain quality indicators taken all together (Figure 28B, 28C). Based on the PCA results, we could not evidence any zone, previous crop or pair effect (Figure 28E, F, G). However, the PCA evidenced a year effect, evidencing better mill yields in 2023 compared with 2022, and higher bread volumes in 2022 compared with 2023. Similar to grain, it was difficult to evidence any wheat variety effect on bread technological quality due to the too large number of possible combinations and the lack of repetitions.

	Variable Unit	f.Hagberg	MillYield %	f.Ashes %	f.W -	f.PL -	f.IE -	f.Hydra- tation %	b.Length	b.Volume cm ³	Baking_ Score Score
СА	mean	405.4	65.0	0.44	188.8	0.73	51.8	1.6	51	1457.5	233
n=10	cv	10.5	5.2	4.3	33.0	48.8	15.1	51	9	11.5	7.0
CONV	mean	372.5	67.8	0.43	157.8	0.94	50.6	1.5	51.8	1462.5	247
n=10	cv	13.0	3.8	6.8	24.8	44.3	6.0	51.8	7.3	7.7	7.5
CA vs.											
CONV	sig.	0.065	0.019	0.591	0.153	0.12	0.492	0.72	0.552	1	0.092

Table 14. Summary of Bread-making results in CA vs. CONV paired comparison (Wilcoxon-paired rank test). sig. Significance

4. Discussion

This pilot study focused on bread technological quality indicators. It would be interesting to investigate further on the possible linkages between grain nutritional and bread nutritional quality. Specifically, we showed in **Chapter 4** that most of the effects of cropping practices on wheat grain quality were visible on P, K and ERGO concentrations which were higher in CA than in CONV cropping systems, while cellulose and Zn were higher in cluster 3 compared with cluster 4.

As highlighted by Lockyer and Spiro (2020), white bread is identified as a source of Ca, Mn, vitamin B1 and vitamin B3, while wholemeal bread is identified as a source of Mg, P, Fe, Cu, Zn, vitamin B1, vitamin B9. This suggests that although improved cropping practices have little effect on white flour nutritional values, mainly because all bran is removed, they might have an effect on wholemeal flour, and specifically on P, K, Zn and ERGO. More research is needed to generalise these findings, and investigations should be made to evidence the potential effects of milling and cooking on the resistance of these elements in bread.

5. Conclusion

Similar to grain quality, Bread-making tests evidenced an important year effect on bread technological quality. The little number of samples made it difficult to make robust statistical analyses, but overall, we evidenced very little differences between CA and CONV breads. This study was a successful means to foster dynamism within the OFE network and farmers' interest during the study.





Figure 28. PCA results for bread indicators.

(A) corresponds to the PCA graph of variables, (B) corresponds to the graph of individuals coloured according to their type of cropping system (CA vs. CONV), (C) corresponds to the graph of individuals coloured according to their cluster (1, 2, 3 or 4), (D) corresponds to the graph of individuals coloured according to monitoring year (2022 = Year 1 or 2023 = Year 2), (E) corresponds to the graph of individuals coloured according to their geographic zone, and (G) corresponds to the graph of individuals coloured according to their pair.

GENERAL DISCUSSION

1. Summary of major findings

This PhD manuscript is developed around four core chapters supplemented with three case studies used to support specific research questions. Chapter 1 aims to highlight the state-of-the-art information on the links between crop production systems and One Health, and the relevance of setting OFE to study cropping systems by adopting systemic approaches, to better qualify these complex systems. Chapter 2 proposes a detailed methodology specifically tailored to our in-field approach, detailing all protocols and specific methodologies mobilised to assess the large set of indicators used across the PhD project. In Chapter 3, we evidence heterogeneous cropping strategies within the CA and CONV groups, demonstrating the importance of considering all the components of cropping systems. In Chapter 4, we evidence the effects of these different cropping systems on the different compartments of crop production, i.e. soil, grain production and cropping systems performances. We show that although most of the indicators studied do not respond to crop management, despite the high variability observed, many soil health indicators respond positively to CA practices. The non-linear relationships observed between soil and grain variables confirm that the crop production system is a complex system that must be studied with adapted mathematical tools or even perhaps artificial intelligence. Researchers in agroecology have to admit the extraordinary complexity of phenomena in agroecosystems and the necessity, while modelling, to go beyond linear relations to successfully representing in models soil health and the effects of cropping practices on the crop production system. Chapters 3 and 4 show the high value of OFEs for the collection of large and robust datasets able to inform on-real-farm conditions. Both chapters show that it is possible to collect reliable and precise information in order to contribute to creating knowledge on cropping system effects on the environment, human and animal health.

The three case studies allow to target specific questions to investigate further specific items that fall within the scope of One Health. First the Plant Health case study (Case study n°1) demonstrates that on non-treated strips, the foliar disease frequency (Septoria and rust) was lower in CA than in CONV systems. However, it confirmed that CA systems are more likely to suffer from slug damage. Finally, no significant yield loss was observed in CA non-treated strips, which is not the case in CONV systems, where yield losses were observed. Second, the joint "Agribiodiv" project (Case study n°2), allows us to take a closer look at microbial community behaviour in CA and CONV systems. We observe the strong dependence of communities on their position in the soil-root-leaf-grain continuum. Despite a high variability and a small number of samples, we observe different patterns in CA and CONV fungal and bacterial communities in the continuum soil-root-plant-grain which would deserve to be investigated deeper to better understand the concrete causes of differences between our observations. Finally, the last case study (Case study n°3) allows to complete the circle of this "farm to fork" study, by investigating the potential observable effects of cropping practices up to the final bread product. Overall, we observe a similar pattern in the technological quality of bread to that of grain, *i.e.* an important year effect. Few significative results are observed, except for a likely higher mill yield in CONV systems than in CA systems. However, the small number of repetitions makes it difficult to perform robust statistical analyses. This whole study on bread-making was an effective way to energise the network and maintain the interest of farmers throughout the study.

This general discussion focuses on three main axes, providing first general feedback on the OFE and One Health approach. We then focus on data management and analysis aspects in the frame of

complex system studies. Finally, we conclude by providing insights on the in-field impacts of the study and the perspectives given by this three-year project.

2. Feedback on the On-Farm Experimentation (OFE)

2.1. A successful OFE with a committed community

The OFE study with farmers was successful, since all farmers who engaged in the study remained in the two consecutive years, except one pair of farmers, since they could not find an adequate field. Despite this success, challenges to OFE were observed.

On-farm research may be challenging for researchers themselves because they may have, amongst others, very narrow and focused questions, while farmers have a very large vision and knowledge of the complex agroecosystems they are working with. They are also dealing with interactions and trade-offs of the complex system in their daily working life, while researchers may be used to work on specific aspects of the agroecosystems, leading to an increase in the disconnection between farmers and researchers (Rafiq *et al.*, 2024). Working with farmers forced us to broaden our spectrum and increase our technical agronomic knowledge significantly. Although the study zone was restricted to a small region, the differences faced across farms were sometimes important, therefore increasing the complexity of our studied topic as we moved from farm to farm. It is important to find the right compromise between time steps *vs.* the complexity of research objectives and interactions. A key for researchers is therefore to identify the fundamental processes involved in the question they have, look at the interactions between them, and evidence how these can be interpreted and used to achieve quality and sustainability.

Working with a large diversity of partners and stakeholders was very enriching. However, this multistakeholder relationship was sometimes difficult to manage in the context of such a project because farmers or other partners quite often asked us to provide study results when they were not necessarily aware of the time needed to provide robust and scientifically based results. Hence, it is really important to find the right balance and good compromises between the research, which is part of the long term, and the extension, which on the contrary is part of the short term and requires immediate responses.

Thus, working with many stakeholders enriches studies indeed, and allows to get and grasp multiple perspectives, questions and objectives. However, very often, the more stakeholders are involved in a project, the more complex the project becomes since the size of the project can increase proportionally (Pagliarino *et al.*, 2020). It is therefore important to identify, right from the beginning, the specific research questions and the network size needed to address them. We believe that constituting simple networks to tackle specific questions may be challenging.

2.2. Field sampling and organisation of sampling campaigns

Several limitations shaped the way in which we proceeded with field sampling. Indeed, due to the distance between our laboratory and the farms studied, and the size of the study zone (about 200 x 200 km²), we had to carefully organise our sampling campaign calendars.

First, we considered that two weeks was the maximum possible difference between the sampling of the first farm and the sampling of the last farm on a tour, if we wanted to avoid too important climate confounding factors. Indeed, most samplings were held in spring, and the weather was likely to change greatly over this period (temperature increases, precipitation diminutions in normal years, as we move

towards summer). This meant that we had to sample the 44 plots (42 in year 2) within approximately about ten working days. In addition, a two-week period was also the maximum possible time to ensure a good conservation of several reagents used, such as the gels used for SituResp[®] soil basal respiration measurements. This meant that we had to proceed to a full sampling of four to five farms within a day, also considering the time needed to drive from farm to farm. We therefore estimated that to make it possible, we could not spend more than 1 hour and 30 minutes to 2 hours per field.

In addition, although we received support from partners from the Agricultural Chambers (most of the time, we were from 2 to 5 people per field for sampling), we had to ensure that sampling was carried out by trained people, and in most cases, we ensured that each indicator was sampled by the same experimenter over both years to avoid any experimenter bias. Experimenter bias was an important confounding factor to consider and avoid, knowing the already numerous potential existing confounding factors due to the only fact of working in real conditions, on farms each with its own particularities.



Photo 6. Full vehicle after one week of grain sampling. ©Clara Lefèvre, July 2023.

Finally, we drove from farm to farm and slept mostly at farmers' places, meaning that we had to carry all samples with us every day. When soil samples had to be stored in the fridge, we always managed to borrow a farmer's fridge or freezer and stored them in a cool box when we had transport them from one location to another. Soil samples occupied limited space in the vehicle since we did not sample a big quantity, although two weeks of samples and material was the maximum the vehicle could handle. However, for the summer session, wheat

samples were much bigger, and we reached the maximum capacity that the vehicle could handle after one week (Photo 6).

Overall, the time available and the associated workforce made it impossible to carry out the complete soil sampling in a single session.

Finally, the success of our sampling campaigns also lied in our ability to respect the set calendar. That is why we sometimes had to sample under inadequate climate conditions, therefore preventing us from carrying out all soil testing tests (*e.g.* some Beerkan tests dropped because of the rain). Also, we had planned to analyse bulk density, since we knew this was a crucial indicator to allow for OM stock calculations, but we could not, since in spring and due to drought in some regions, soil had become too hard to enable correct sampling with



Photo 7. Aspect of a soil charged in stones (22_CA_2022). ©Clara Lefèvre, April 2022.

our material. Some soils were also highly charged in stones, complicating the correct sampling of bulk density tremendously (Photo 7).

2.3. Financial aspects

This PhD project was made possible thanks to the strong involvement and support of multiple actors convinced by the need and the importance of going into the fields to collect information and knowledge. It is important to note here that our request for public financial support was refused prior to the start of this PhD study, citing objectively unfounded reasons. Therefore, this study was handled without any public funds. This can raise questions about the public sector's interest in systemic approaches and the reluctance to perform such integrative studies from the public sector. The budget allocated for this project (about 300k€) was relatively high compared with the average budget of usual agronomy PhD projects. Even if the multiple research collaborations allowed us to decrease the associated costs, it is true that such studies are increasingly expensive, mainly due to the associated logistics and the price of numerous analyses in certified laboratories. However, the quantity of information gathered through this network overpasses what is usually done in agronomic studies, therefore decreasing the costs related to single indicator measurements.

3. Data management and analyses in complex systems

We observed an important number of non-linear relations between variables, especially between grain and soil elements indicators, but also inside compartments, such as relations between soil parameters indicators (**Chapter 4**). These non-linear relations hampered the efficient application of known models, from the simple correlation or principal component analyses to the more systemic ones such as path analyses (*e.g.* structural equations models, partial least square path analyses (PLS/PM) etc.), to explain possible links and interaction between different variables of the system. In addition, these models, such as PLS/PM do not handle non-independent variables, which is an issue when modelling compartments of the crop production system, since very numerous retroactions do exist in the real life, creating mathematical dependencies between variables. For example, a compacted soil structure could lead to increased soil tillage, while increased soil tillage could lead to increased soil structural issues.

We tried to set up PLS/PMs to evidence specific effects of cropping practices on soil and grain quality components, but model adjustments would have required to abandon a large number of variables, which we considered as important to consider in the frame of a systemic study. In the case of complex systems modelling, linear models show several limits. Although they are relatively simple to implement, they tend to underfit the reality and under-considerate the role of specific variables only due to mathematical reasons, while they do have an important role in the system reality (San Miguel *et al.*, 2012).

Other modelling solutions should be investigated to allow a better modelling of complex systems, which would allow the inclusion of retroactions and the identification of non-linear relations between variables. However, to date and at the best of our knowledge, such systemic modelling tools do not exist.

A feasible alternative could therefore be to focus on a single response variable and try to estimate it from other explanatory variables using additive (*e.g.* Generalised additive (mixed) models such as spline or third-degree polynomial, Hastie and Tibshirani (1990)) or non-parametric (*e.g.* Random Forest

(Breiman, 2001)) models that allow for non-linear relations between variables. However, other limits arise when dealing with such models, since their application imply much more parameters to be estimated than simple linear models, and therefore require a higher number of observations to remain robust. Specifically, non-parametric models such as Random Forest seem to be an interesting solution to enable the explanation of a response variable through others. It is relatively simpler to implement compared with spline models, since it allows to automatically identify interactions between variables. However Random Forest are more costly in degrees of freedom than additive models.

The use of explanatory variables can also be questioned, depending on the type of variable used. For example, using simple indicators as explained variables, such as soil OM content, or protein concentration in grain, and trying to explain them according to other variables seems to be a fair approach. However, when dealing with more complex indicators such as soil health indexes, the question of the methodology used and the representativeness of such an index to represent the complex soil system can always be questioned (cf. Janzen, Janzen and Gregorich, 2021; Powlson, 2020; Rinot *et al.*, 2019; also see **Chapter 1**).

The question of existing thresholds is also an issue in mathematical analyses. Indeed, specific thresholds exist, adapted to each situation and soil type (for soil health indicators), and of uses (for nutritional indicators). One might wish to establish thresholds in the values that a soil health index or a nutritional quality index would take. However, this would be without taking into account the infinite diversity of production situations, such as whether the soils are carbonated or not, and the uses of foodstuffs, thus defining an equally enormous variability of specific thresholds and making the task almost impossible. We believe that the virtue of such indicators lies above all in their use in comparative, synchronic or diachronic modes.

Lastly, we observed an important intra-plot variability of soil health indicators (*e.g.* Beerkan). This intrinsic variability of soil conditions will always put researchers in front of the challenge of finding the best compromise between the extent of the sampling to be carried out to guarantee its representativeness, and the human, financial and material resources to achieve it. New numerical technologies will likely provide assistance in addressing these challenges in the future, usefully complementing the necessary field and research experience.

In a nutshell, and as very well explained by San Miguel *et al.* (2012), "an important source of difficulties stems from the fundamental problem of the level of detail and complexity needed for the understanding of the structure, function, and response of a complex system of interest". Indeed, One Health and systemic studies (our study brings together the two) are often associated with an important quantity of collected data, to holistically cover all the system compartments. These data may be in interaction with each other in linear or non-linear forms. The importance of data management and analyses in making the best mathematical choice in the analyses, and the ability of system researchers to manage large databases should not be underestimated in such studies. That is why we would recommend always including statisticians or data analysts fluent in data management of complex systems in One Health projects. There are at the moment no fully satisfying approaches to model the complex agronomic systems, and each approach comes with its pros and cons. The most important issue is choosing the best compromise to get as close as possible to reality, without leaving aside important factors for the wrong reasons. The key is to find the best compromise between model simplicity and the necessary capture of complex emergent behaviours in multi-scale systems (San Miguel, 2023).

4. Methodological developments and associated questions

4.1. Choice of indicators

An important part of the work of this PhD project also involved testing and using a large set of indicators, some of which were intended to measure the same elements (*e.g.* POXC, C mineralisation). Entering the frame of systemic science and looking for holisticity in the representativeness of the compartments studied was challenging. Finding the right compromise between covering most elements of the system while accepting it is not feasible to measure them all may lead to criticism on a lack of holisticity. On the other hand, measuring as many elements as possible may be criticised as an attempt to look for too many things, impossible to address in the data analysis or modelling step.

In our case, we are aware of some lack, especially regarding soil fauna. Berlese were our only means to enable a quick and simple monitoring of soil fauna, but we failed to establish performant devices. We did not perform any earthworm counting or nematode analyses, or other known soil fauna tests. Actually, we tried to include earthworm counting within VESS analyses, but we did it only on a small part of the total sampling. Therefore, we did not include the results in the analyses. We also decided not to apply the two indicators of nutrient cycling, i.e. ion exchange membranes and mineral N extraction of the Biofunctool® kit, because of the complexity of their implementation and the extremely fluctuating nature of N levels over time, largely due to the influence of fertiliser inputs by farmers (that we retrieved thanks to the surveys). Also, although we tried at best to repeat all protocols on the two successive years, some were performed only for one year (e.g. lamina baits replaced by Litter bags, K in soils, texture with no decarbonation). Regarding lamina baits, at the time of our study, it was advised to buy pre-filled lamina baits in a specific German laboratory (Terra Protecta). This laboratory also owned the "official" formula for the substrate (cellulose-based), of right consistency to fill the plastic strips' holes. The cost of one filled lamina was approximately 5€. The protocol indicated that 8 laminas per sampled point were needed, *i.e.* 24 lamina per plot. Although lamina baits are presented as an economic indicator (Griffiths et al., 2016), on the whole design, with 1 056 laminas needed, the total budget rose to slightly more than 5 000€. This represented a very high and unbearable cost. In year 1, we managed to borrow pre-filled laminas from a partner laboratory (Eco & Sols in Montpellier), while we had the rest of the strips stamped by a 3D printer, with extra substrate provided by a local farmers' extension service (ALPAD 40 - https://www.alpad40.fr). This allowed us to maintain the application of lamina baits in year 1, at reasonable costs, but with associated quite heavy logistics. In addition, we encountered issues on very rocky soils, where it was sometimes very difficult or even impossible to insert the lamina baits into the soils (especially in the 79-north zone, Photo 7).

Plant health analyses were also performed only over one year. It would have been very complicated to launch them directly in year 1, since plots were selected just before seeding, and we did not know the farmers well enough at that time to identify those who may accept *vs.* those who may refuse to implement a control zone.

Concerning wheat nutritional quality, we did not investigate gluten aspects, which are linked to the quality of grain proteins, the importance of which for human health was evidenced (Shewry, 2019), and likely to be influenced by cropping practices (Calzarano *et al.*, 2018; Galieni *et al.*, 2016).

Overall, we believe that in-field indicators were useful and interesting to implement at least once with farmers, in a pedagogical and awareness-raising objective. They showed to be a relevant complement

to laboratory analyses. The set of analysed data was the result of a compromise between research of holisticity, at bearable costs and associated logistics.

4.2. Carbonate soils vs. Non carbonate soils

We were confronted in the OFE with a non-negligible proportion of carbonate soils, especially in the north of Charente and South of Vienne. These soils have a particular functioning due to their high CaCO₃ concentration leading to OC stabilisation despite their high OC concentration, hence to reduced respiration rates (Rowley, Grand and Verrecchia, 2018) amongst others. Further research would be needed to investigate the specific responses of crop management in carbonate soils and understand how they might differ from non-carbonate soils.

4.3. Simple indicator of subsurface soil structure

In hindsight, we observed that most of the soils we studied, in CONV as in CA, were compacted to very compacted on the lower part of the A horizon and especially below (subsurface). This hinders root prospecting and therefore reduces the water potential available to crops, especially in summer. In the future, it would be interesting to develop a rapid indicator based on manual sampling of soil cores over 50 to 70 cm (using a manual corer equipped with a pressure gauge) and including a simple test for measuring pH, active limestone and empirical measurement of the texture on the surface and subsurface. This would make it possible to identify the possible presence of a sealed horizon in the subsurface and to identify pedological factors promoting or slowing its appearance in addition to soil management factors.

5. In-field impacts of the study and way forwards

To be maintained, the OFE must be agile and flexible in accepting new farmers or manage the possibility for farmers to quit the experiment. Indeed, although the aim of the study was not to make CONV farmers shifting their practices, four CONV farmers expressed during the different interviews and discussions that they were willing to shift practices or had already started the shift in practices while starting the study. These farmers were well identified by the clustering (**Chapter 3**) since they appeared in cluster 3 (with many other CA farmers) in the second year of the study. Also, one CONV and one CA farmer retired, and one CA farmer stopped his activity, making three farmers being unable to pursue the study further.

We hope that our study will support an acceleration of the transition as well as help "demystify" CA systems. Indeed, one rather important barrier to farmers' transition to soil conservation systems, lies in the fact that farmers have a limited perception of the role and potential of ecological processes at work in agroecological, regenerative or CA settings (Cartailler *et al.*, 2024). This demonstrates again the vital importance of explaining the existence of these processes on farmers' or their neighbours' land (such as what we did in this PhD work). Making them understand how these processes can be improved and, above all, how this translates economically, at least in terms of savings on costs, will be key to fostering transition.
The study could therefore be pursued but arrangements would be needed which could be:

- 1. Investigate further on the large and unique database created, since there might be more to discover regarding specific questions or parameters based on the database. The database was published to offer possibilities to other researchers to benefit from this collective effort of onfarm data acquisition.
- 2. Refocus the research questions on specific questions that target farmers' in-field problems that need to be solved or studied by research. From our previous discussions with farmers, some of the identified questions could target weeding operations, the diminution of herbicide use in CA systems and a better accounting for residual N after cover crop and legume insertion in the rotation, allowing for diminution of N mineral fertilisation. These issues are directly related to management but others, indirectly important also for farmers' activity, could emerge such as the problem of subsurface soil compaction.
- 3. Shift to more farmer-driven OFE to really tackle farmers' questions and support them on trial designs and analysis of results. In this way, establish significant economic support for farmers hosting participatory research devices on their farm, in relation to research and/or extension organisations.
- 4. Dig in the social and economical aspects of CA and CONV farming to identify the causes, levers and barriers to initiate cropping practices shifts, especially at the transition stage.
- 5. Extend the network to new CA/CONV couples to test the methodology and potentially identify new CA or CONV profiles specific to other production areas or pedoclimatic conditions.

These three last proposals will be implemented by the OFE *Pour Une Agriculture du Vivant* through different projects that were recently approved and are now entering their implementation phase. Amongst them, the "In-field research grant" (in French: "Bourse de recherche à la ferme")⁹, funded by the Mirova Foundation¹⁰ started in 2024. It will enable the creation and testing of a simple and appropriate support scheme (scientific, technical and financial) for farmers to accelerate the emergence and scaling-up of innovative agricultural practices. The project has been designed around four main actions:

- Set up a network of pioneer agroecological farms in France, working closely with all existing French farmer networks (such as "Groupes 30 000" as already mentioned). The objective is to identify 200 farms across all types of production,
- 2. Identify and map the scientific and technical partners capable of enhancing the robustness of farmers' OFE, to facilitate contacts between farmers, researchers and relevant technicians,
- 3. Based on it, create the "In-field research grant" scheme (operation, partners, funds, governance, etc.) to finance farmers' OFE, with a first call for projects launched in 2025 to finance 3 farmers' projects,
- 4. Valorise the knowledge produced (contributions to technical days, feedback, publication of scientific articles, etc.) amongst farmers, technicians, agronomists and scientists, in order to accelerate the transition.

 ⁹ <u>https://agricultureduvivant.org/leviers-daction/projet-bourse-recherche-action-a-la-ferme-brf/</u> (in French)
 ¹⁰ https://mirova-foundation.org/en/

GENERAL CONCLUSION

This work was undertaken to contribute to fill a gap in data and existing knowledge on CA systems and practices, and the potential effects on soil and their performance on crop production, both quantitatively and qualitatively. Without this being expressly set as an initial objective, the work also filled a gap in our knowledge of CONV systems, allowing to categorise them with a high level of precision.

In addition to providing conclusions on the potential beneficial effects of CA systems on soil health, the study allowed the analysis of a large set of indicators, evidencing links or disputable results for similar indicators. CA was evidenced as a good alternative to conventional cropping systems (*i.e.* with soil tillage or even ploughing) with beneficial effects on One Health. However, a difference should be made between CA systems with increased efficiency and limited reliance on chemical inputs, and CA systems which remain very dependent on chemical inputs (fertilisers and biocide chemicals). Efforts should certainly be made to foster the transition of farmers to more efficient systems.

This study was also relevant regarding its multi-stakeholder and multi-disciplinary approach. It showed the multiple potential of a unique OFE network to address global questions, but also more specific questions as evidenced in the case studies.

If agronomic research has largely relied on experimental plots with single-factor analyses and put forwards a top-down organisation for the dissemination of knowledge, agroecological research must today rely heavily on on-farm experiments, and organise a much more horizontal dissemination of knowledge. Some talk about "bottom-up" knowledge dissemination, but this expression maintains the vision of socio-professional categories "at the top", *i.e.* the researchers and other institutions, while other socio-professional categories would be "at the bottom", *i.e.* the farmers...

Simply let's admit that the two main dimensions of a territory are indeed horizontal!

ANNEXES

Annexe 1: Indicators studied in the PhD project

<u>1. General indicators</u>

Abbreviation	Sub Compartment	Description	Unit	Laboratory/Institute	Date of sampling	Orientation
SD_1	Date	Sampling date for the first spring campaign	dmy	INP ENSAT Toulouse	NA	NA
SD_2	Date	Sampling date for the second spring campaign	dmy	INP ENSAT Toulouse	NA	NA
SD_3	Date	Sampling date for the third summer campaign	dmy	INP ENSAT Toulouse	NA	NA
SD_Aurea	Date	Sampling date	dmy	INP ENSAT Toulouse	NA	NA
date_seeding	Date	Wheat seeding date in 2022 or 2023	dmy	INP ENSAT Toulouse	NA	NA
date_harvest	Date	Wheat harvesting date in 2022 or 2023	dmy	INP ENSAT Toulouse	NA	NA
d.samp1	Date	Number of days between wheat seeding in 2021 or 2022 and the first spring sampling in 2022 or 2023	days	INP ENSAT Toulouse	NA	NA
d.samp2	Date	Number of days between wheat seeding in 2021 or 2022 and the second spring sampling in 2022 or 2023	days	INP ENSAT Toulouse	NA	NA
s.samp_ aur	Date	Number of days between wheat seeding in 2021 or 2022 and the laboratory sampling in 2022 or 2023	days	INP ENSAT Toulouse	NA	NA
d.samp3	Date	Number of days between wheat seeding in 2021 or 2022 and the summer sampling in 2022 or 2023	days	INP ENSAT Toulouse	NA	NA
d.seed	Date	Number of days between 1/01/2021 or 01/01/2022 and wheat seeding date in 2021 or 2022	days	INP ENSAT Toulouse	NA	NA
d.harv	Date	Number of days between 1/01/2022 or 2023 and wheat harvest date in 2021 or 2022	days	INP ENSAT Toulouse	NA	NA
d.growth	Date	Number of days of wheat growth in 2022 or 2023	days	INP ENSAT Toulouse	NA	NA
Farmtype	Farm	Type of farm: 1 = Breeder or animals' presence on farm (some farmers establish exchanges with other farmers so they can graze cover crops), 0 = cereal grower with no animals on farm	No_unit	INP ENSAT Toulouse	survey	NA
FarmSize	Farm	Useful Agricultural Area	ha	Farmers	survey	NA
FieldSize	Field	Total size of the studied field. Measurements were held on a 1 ha square of each field	ha	INP ENSAT Toulouse	survey	Optimum
Pair	ld	Farmer associated pair. 22 pairs of farmers participated in the experiment in 2021-2022 and 21 in 2022-2023	No_unit	INP ENSAT Toulouse	NA	NA
Туре	ld	Type of management practice: CA = Conservation agriculture, CONV= Conventional agriculture	No_unit	INP ENSAT Toulouse	NA	NA
Year	Id	Year of sampling	No_unit	INP ENSAT Toulouse	NA	NA

Abbreviation	Sub Compartment	Description	Unit	Laboratory/Institute	Date of sampling	Orientation
Zone	Id	Zone = P corresponds to the main zone of the study where farmers' practices	No_unit	INP ENSAT Toulouse	NA	NA
		were observed as normal. Zone = C corresponds to a control zone that was				
		set up only with a few farmers and only for the 2023 harvest year, for which				
		no fungicides were applied, and in some cases no insecticides				

2. Local conditions

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
Abbreviation	Compartment	Description	Onic	Laboratory/institute	sampling	onentation
GPS_X	Location	Longitude in WGS84	0	NA	NA	NA
GPS_Y	Location	Latitude in WGS84	0	NA	NA	NA
Location	Location	Position of the field within the département in mainland France.	No_unit	NA	NA	NA
		Departments are described by their administrative number				
Hedge	SNH	Presence (1) or absence (0) of hedge at the edge of the plot studied in year n	No_unit	INP ENSAT Toulouse	NA	More the
						better
SNH	SNH	Score from 0 to 3 defined as follows:	No_unit	INP ENSAT Toulouse	NA	More the
		- 3 points if forest (as defined by the Corine Land Cover 2021) and/or				better
		watercourse less than 200 m from the study area				
		- 2 points if forest AND watercourse more than 200 m from the study				
		area but less than 1km from the study area				
		- 1 point if forest OR watercourse more than 200 m from the study				
		area but less than 1 km from the study area				
		- 0 if neither forest nor watercourse within 1 km of the study area				
Texture_USDA	Soil_texture	Soil texture classified in the USDA classification	No_unit	INP ENSAT Toulouse	NA	NA
Clay	Soil_texture	Soil clay on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
		plot studied in 2022 or 2023, measured after decarbonation and deduction of			Aurea	
		soil OM				
Silt	Soil_texture	Soil loam on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
		plot studied in 2022 or 2023, measured after decarbonation and deduction of			Aurea	
		soil OM				

	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
Appreviation	Compartment	Description	Onit	Laboratory/institute	sampling	
Sand	Soil_texture	Soil sand on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
		plot studied in 2022 or 2023, measured after decarbonation and deduction of			Aurea	
		soil OM				
Clay_no_	Soil_texture	Soil clay on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
decarb		plot studied in 2023, measured without decarbonation and deduction of soil			Aurea	
		ОМ				
Silt_no_	Soil_texture	Soil loam on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
decarb		plot studied in 2022 or 2023, measured without decarbonation and			Aurea	
		deduction of soil OM				
Sand_no_	Soil_texture	Soil sand on a composite soil sample representative of the study area of the	%	Aurea	SD_	Optimum
decarb		plot studied in 2023, measured without decarbonation and deduction of soil			Aurea	
		ОМ				
DM	Soil_texture	Soil dry matter at 105°C, on a composite soil sample representative of the	%	Aurea	SD_	Optimum
		area of the plot studied in 2022 or 2023			Aurea	
Coarse_	Soil_texture	% coarse matter (>2 mm) on a composite soil sample representative of the	%	Aurea	SD_	Less the
elements		study area of the plot studied in 2022			Aurea	better
CaCO3	Soil_texture	Soil CaCO ₃ on a composite soil sample representative of the area of the plot	%	Aurea	SD_	Optimum
		under study in 2022 or 2023			Aurea	

3. Cropping practices

Ale la service di autore	Sub	Description	l lucit	laharataru/lastituta	Date of	Orientation
Appreviation	Compartment	Description	Unit	Laboratory/institute	sampling	Orientation
nbCC_rot	Cover	Number intercrops seeded between 2017-2022 or 2018-2023. Value ranging	No_unit	Farmers	survey	More the
		from 0 (no cover planted) to 5 (cover planted at each intercrop). The				better
		presence of rapeseed regrowth in the intercropping period, in the case of no-				
		tillage, is counted as a plant cover				
OppCC_rot	Cover	Number of opportunities to implant a cover crop in the rotation, to be	No_unit	Farmers	survey	More the
		considered as an opportunity, there must be:				better
		1) A period \geq 8 weeks between the harvesting of one crop and the seeding of				
		the next				
		2) There is no opportunity if the soil is permanently covered by a perennial				
		crop (<i>e.g.</i> alfalfa) or by a meadow, or by a catch crop				
EffCC_rot	Cover	Ratio between the number of opportunities to plant a cover crop and the	No_unit	INP ENSAT Toulouse	survey	More the
		number of cover crops planted in the rotation. The ratio ranges from 0 (of all				better
		the opportunities to plant a cover crop, none were taken) to 1 (of all the				
		opportunities to plant a cover crop, all were taken)				
RestitRes_n	Cover	1 if the residues of the previous crop have been restored, and 0 if the	No_unit	Farmers	survey	More the
		residues of the previous crop have been exported				better
RestitRes_rot	Cover	Number of times main crop residues were returned over the 5 years	nb	Farmers	survey	More the
		preceding the campaign under study as well as the campaign under study				better
minN_n	Crop_nutrition	Mineral nitrogen units used to grow winter wheat in 2022 or 2023	kg/ha/yr	Farmers	survey	Less the
						better
minN_eff_n	Crop_nutrition	Nitrogen efficiency: Ratio between the yield given by the farmer and the	%	INP ENSAT Toulouse	survey	More the
		quantity of mineral nitrogen supplied to the system				better
minP2O5_n	Crop_nutrition	P_2O_5 inputs by farmer in harvest year 2022 or 2023	kg/ha/yr	Farmers	survey	Less the
						better
minK2O_n	Crop_nutrition	K_2O inputs by farmer in harvest year 2022 or 2023	kg/ha/yr	INP ENSAT Toulouse	survey	Less the
						better
minSO3_n	Crop_nutrition	SO_3 inputs by farmer in harvest year 2022 or 2023	kg/ha/yr	Farmers	survey	Less the
						better
minN_rot	Crop_nutrition	Average mineral N inputs by farmer in harvest year 2022 or 2023 and the 5	kg/ha/yr	Farmers	survey	Less the
		years preceding these harvest years				better

Abbroviation	Sub	Description	Linit	Laboratory/Instituto	Date of	Orientation
ADDIEVIATION	Compartment	Description	Onit	Laboratory/institute	sampling	Onentation
minP2O5_rot	Crop_nutrition	Average P_2O_5 inputs by the farmer in harvest year 2022 or 2023 and the 5	kg/ha/yr	Farmers	survey	Less the
		years preceding these harvest years				better
minK2O_rot	Crop_nutrition	Average K_2O inputs by the farmer in harvest year 2022 or 2023 and the 5	kg/ha/yr	INP ENSAT Toulouse	survey	Less the
		years preceding these harvest years				better
minSO3_rot	Crop_nutrition	Average SO $_3$ inputs by the farmer in the 2022 or 2023 harvest year and the 5	kg/ha/yr	Farmers	survey	Less the
		years preceding these harvest years				better
C_entries_rot	Crop_nutrition	Annual inputs of organic carbon on the plot, via organic amendments, roots	tC/ha/yr	INP ENSAT Toulouse	survey	More the
		and aerial parts returned, either by crops or by cover crops in studied year				better
		and the 5 preceding years, <i>i.e.</i> 2017-2022 or 2018-2023				
nbLeg_rot	Crop_nutrition	Number of times a legume was planted (crop or cover) between 2017-2022	No_unit	Farmers	survey	More the
		or between 2018-2023				better
orgN_rot	Crop_nutrition	Annual organic nitrogen inputs on the plot, via organic amendments over the	kg/ha/yr	INP ENSAT Toulouse	survey	More the
		5 years preceding the study year, <i>i.e.</i> 2017-2022 or 2018-2023				better
nbOrginput_	Crop_nutrition	Number of applications of organic fertiliser over the 5 years preceding the	nb	Farmers	survey	More the
rot		study campaign and the study campaign.				better
orgN_n	Crop_nutrition	Quantity of organic nitrogen provided by organic fertilisation over the 2021-	kg N/ha	Systerre®	survey	More the
		2022 or 2022-2023 campaign				better
orgP2O5_n	Crop_nutrition	Quantity of organic phosphorus provided by organic fertilisation over the	kg	Systerre®	survey	More the
		2021-2022 or 2022-2024 campaign	P₂O₅/ha			better
orgK2O_n	Crop_nutrition	Quantity of organic potash provided by organic fertilisation over the 2021-	kg	Systerre®	survey	More the
		2022 or 2022-2025 campaign	K₂O/ha			better
CropDiv_rot	Diversification	Number of different crops grown in the 5 years prior to the study year (cover	No_unit	INP ENSAT Toulouse	survey	More the
		crops or main crop)				better
Time_return_	Diversification	Length of time winter wheat has been back in the rotation. This number may	year	Farmers	survey	More the
wheat		be higher than 5 if no winter wheat has been grown since 2017. It is then				better
		noted as 6				
Prec_crop_n	Diversification	Previous crop harvested in 2021 or 2022	No_unit	Farmers	survey	NA
RI_farm	Index	Score of Regeneration Index. Score /100 that provides information on the	Score	Pour une Agriculture	survey	More the
		farm agronomic performance		du Vivant		better
RI_field	Index	Regeneration index adapted to a field scale. Works at a 5-year rotation scale	Score	INP ENSAT Toulouse	survey	More the
						better

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment		•		sampling	
TFIh_n	Pesticide_	sum of the ratios of applied herbicide dose over recommended dose	No_unit	Farmers	survey	Less the
	use					better
TFleh_n	Pesticide_	sum of the ratios of applied treatment dose (except herbicide) over	No_unit	Farmers	survey	Less the
	use	recommended dose. Seed treatment excluded				better
nbHerbi_n	Pesticide_	Number of herbicide applications between the destruction of the previous	No_unit	Farmers	survey	Less the
	use	crop (or intercrop) and wheat harvest in 2022 or 2023				better
nbFungi_n	Pesticide_	Number of fungicide applications between the destruction of the previous	No_unit	Farmers	survey	Less the
	use	crop (or intercrop) and wheat harvest in 2022 or 2023				better
nbIns_n	Pesticide_	Number of insecticide applications between the destruction of the previous	No_unit	Farmers	survey	Less the
	use	crop (or intercrop) and wheat harvest in 2022 or 2023				better
nbMoll_n	Pesticide_	Number of molluscicides in the 2022 and 2023 study years	No_unit	Farmers	survey	Less the
	use					better
nbSDHI_n	Pesticide_	Number of SDHIs used in the 2022 and 2023 study years	No_unit	Farmers	survey	Less the
	use					better
Tillage_	Tillage	Score out of 18 representing the sum of <i>Tillage_n</i> scores over the last 5 years	Score	Farmers	survey	More the
intensity_rot		prior to the study year (<i>i.e.</i> 2017-2022, or 2018-2023)				better
lastPlough	Tillage	Number of years since the last ploughing on the plot. For plots studied in	year	Farmers	survey	More the
		2022, the reference year is 2021 (2021 = 0, corresponding to autumn				better
		ploughing before BT implantation), and for plots studied in 2023, the				
		reference year used is 2022 (2022=0, corresponding to autumn ploughing				
		before BT implantation)				
System_age	Tillage	Categorical variable defining the age of the system in place as follows:	No_unit	INP ENSAT Toulouse	survey	NA
		 "Very old" if the last ploughing was done more than 20 years before 				
		the monitored year,				
		 "Very recent" if the last ploughing was done in the 4 years preceding 				
		the monitored,				
		 "Recent" if the last ploughing was carried out between 4 and 10 				
		years before the monitored year,				
		 "Old" if the last ploughing was carried out 10 to 20 years before the 				
		monitored year				
Tillage_n	Tillage	Type of tillage for seeding in 2022 or 2023:	Score	Farmers	survey	More the
		- 0 = Ploughing;				better

Abbroviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
ADDIEVIATION	Compartment	Description	Onit		sampling	
		- 1 =Heavy tillage;				
		- 2 = Light tillage;				
		- 3= Direct seeding.				
		We consider that light tillage = less 10 cm deep and strictly less than 3				
		machinery operations. Heavy tillage = at least one operation is > 10 cm depth				
		or more than 3 machinery operations. Decompaction is not considered as a				
		tillage operation				
nbOperations	Tillage	Total number of machine operations during the crop year	nb	INP ENSAT Toulouse	survey	Less the
_n						better
nbVar	Variety	Number of cultivated wheat varieties in 2022 or 2023	No_unit	Farmers	survey	More the
						better
Var_mix	Variety	0 if monovariety, 1 if mix of varieties in field in 2022 or 2023	No_unit	Farmers	survey	More the
						better
Wheatvar	Variety	Name of cultivated varieties in 2022 or 2023, when available	No_unit	Farmers	survey	NA

<u>3. Soil health</u>

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment			···· // ·····	sampling	
ТМС	Biological	Total Microbial Carbon on a composite soil sample representative of the	mgC/kg	Aurea	SD_	More the
		study area of the plot studied in 2022 or 2023. Microbial carbon is the	dry soil		Aurea	better
		carbon contained in living soil microorganisms. It is also known as				
		microbial biomass or living organic matter				
TMC_OC	Biological	Total Microbial Carbon expressed as a percentage of the total organic	% OC	Aurea	SD_	More the
		carbon concentration on a composite soil sample representative of the			Aurea	better
		study area of the plot studied in 2022 or 2023				
s.POXC_OC	Biological	Concentration of KMnO ₄ carbon in a composite soil sample representative	% OC	Aurea	SD_	Optimum
		of the study area of the plot studied in 2022 or 2023, expressed as a			Aurea	
		proportion of OC. Carbon oxidised with potassium permanganate, also				
		known as labile carbon or active carbon, represents the least stable				
		fraction of C. It is rapidly degradable (a few days to a few months) and				
		constitutes a source of oxidised carbon: an energy fraction for living				
		organisms in the soil				
s.TPOXC	Biological	Concentration of KMnO ₄ carbon in a composite soil sample representative	mg/kg	Aurea	SD_	Optimum
		of the study area of the plot studied in 2022 or 2023	dry soil		Aurea	
BMN_Ntot	Biological	Biologically Mineralisable Nitrogen on a composite soil sample	% of	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023,	total		Aurea	
		expressed as a percentage of total N. Biologically mineralisable nitrogen is	nitrogen			
		used to estimate the potential for mineralisation of organic nitrogen. It is				
		an alternative method to the 28-day nitrogen mineralisation potential at				
		28°C.				
BMN_tot	Biological	Biologically mineralisable nitrogen on a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023	dry soil		Aurea	
MMB	Biological	Molecular Microbial Biomass on a composite soil sample representative	μg	Aurea	SD_	Optimum
		of the study area of the plot studied in 2022 or 2023. Molecular microbial	DNA/g		Aurea	
		biomass is used to measure the abundance of microorganisms in the soil,	soil			
		based on total microbial DNA				
AbundBact	Biological	Bacterial abundance on a composite soil sample representative of the	DNAr	Aurea	SD_	Optimum
		area of the plot studied in 2022 or 2023	16S		Aurea	

Abbroviation	Sub	Description	l lasit	Laboratory/Institute	Date of	Orientation
Appreviation	Compartment	Description	Unit	Laboratory/institute	sampling	Orientation
AbundFungi	Biological	Fungal abundance on a composite soil sample representative of the area	DNAr	Aurea	SD_	Optimum
		of the plot studied in 2022 or 2023	18S		Aurea	
FBRatio	Biological	Fungi/Bacteria abundance ratio on a composite soil sample representative	No_unit	Aurea	SD_	Optimum
		of the study area of the plot studied in 2022 or 2023. The Fungi/Bacteria			Aurea	
		ratio is the ratio of the abundance of fungi and bacteria in the soil				
		(quantities of 18S and 16S rDNA measured by qPCR)				
DivBact	Biological	Bacterial taxonomic diversity (nb OTU 16S) on a composite soil sample	nb OTU	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023.	16S		Aurea	
		Bacterial diversity corresponds to the number of bacterial taxa				
DivFungi	Biological	Fungal taxonomic diversity (nb OTU 18S) on a composite soil sample	nb OTU	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023.	18S		Aurea	
		Mushroom diversity corresponds to the number of mushroom taxa				
minC_3d	Biological	Respiration after 3 days of incubation. Soil microbial respiration is the	mg C-	Aurea	SD_	Optimum
		result of the mineralisation of organic substances. During this process, the	CO ₂ /kg		Aurea	
		substances are oxidised into CO_2 and H_2O , while the microorganisms	dry soil			
		consume O ₂ . Respiration is therefore a measure of the overall activity of				
		soil microorganisms				
minC_7d	Biological	Respiration after 7 days of incubation. Soil microbial respiration is the	mg C-	Aurea	SD_	Optimum
		result of the mineralisation of organic substances. During this process, the	CO ₂ /kg		Aurea	
		substances are oxidised into CO ₂ and H ₂ O, while the microorganisms	dry soil			
		consume O ₂ . Respiration is therefore a measure of the overall activity of				
		soil microorganisms				
minC_14d	Biological	Respiration after 14 days of incubation. Soil microbial respiration is the	mg C-	Aurea	SD_	Optimum
		result of the mineralisation of organic substances. During this process, the	CO ₂ /kg		Aurea	
		substances are oxidised into CO ₂ and H ₂ O, while the microorganisms	dry soil			
		consume O ₂ . Respiration is therefore a measure of the overall activity of				
		soil microorganisms				
minC_21d	Biological	Respiration after 21 days of incubation. Soil microbial respiration is the	mg C-	Aurea	SD_	Optimum
		result of the mineralisation of organic substances. During this process, the	CO ₂ /kg		Aurea	
		substances are oxidised into CO ₂ and H ₂ O, while the microorganisms	dry soil			
		consume O2. Respiration is therefore a measure of the overall activity of				
		soil microorganisms. It is highly sensitive to cultivation practices.				

Abbroviation	Sub	Description	Unit	Laboratory/Institute	Date of	Oriontation
Appreviation	Compartment	Description	Unit	Laboratory/institute	sampling	Unentation
minC_28d	Biological	Respiration after 28 days of incubation. Soil microbial respiration is the	mg C-	Aurea	SD_	Optimum
		result of the mineralisation of organic substances. During this process, the	CO ₂ /kg		Aurea	
		substances are oxidised into CO_2 and H_2O , while the microorganisms	dry soil			
		consume O ₂ . Respiration is therefore a measure of the overall activity of				
		soil microorganisms. It is highly sensitive to cultivation practices.				
minC_3d_OC	Biological	Respiration after 3 days of incubation expressed as a percentage of OC	% OC	Aurea	SD_	Optimum
					Aurea	
minC_7d_OC	Biological	Respiration after 7 days of incubation expressed as a percentage of OC	% OC	Aurea	SD_	Optimum
					Aurea	
minC_14d_OC	Biological	Respiration after 14 days of incubation expressed as a percentage of OC	% OC	Aurea	SD_	Optimum
					Aurea	
minC_21d_OC	Biological	Respiration after 21 days of incubation expressed as a percentage of OC	% OC	Aurea	SD_	Optimum
					Aurea	
minC_28d_OC	Biological	Respiration after 28 days of incubation expressed as a percentage of OC	% OC	Aurea	SD_	Optimum
					Aurea	
minN_7d_Ntot	Biological	Nitrogen mineralisation after 7 days of incubation expressed as a	% N	Aurea	SD_	Optimum
		percentage of total soil N			Aurea	
minN_14d_Ntot	Biological	Nitrogen mineralisation after 14 days of incubation expressed as a	% N	Aurea	SD_	Optimum
		percentage of total soil N			Aurea	
minN_21d_Ntot	Biological	Nitrogen mineralisation after 21 days of incubation expressed as a	% N	Aurea	SD_	Optimum
		percentage of total soil N			Aurea	
minN_28d_Ntot	Biological	Nitrogen mineralisation after 28 days of incubation expressed as a	% N	Aurea	SD_	Optimum
		percentage of total soil N			Aurea	
minN_7d	Biological	Nitrogen mineralisation after 7 days of incubation. Gross mineralisation of	mg/kg	Aurea	SD_	Optimum
		nitrogen is the change from organic to mineral form, in two stages: 1)			Aurea	
		Ammonification where organic nitrogen is converted to ammonium (NH_4^+)				
		by heterotrophic microorganisms, and 2) Nitrification where under non-				
		limiting conditions, NH_4^+ is then oxidised to nitrite (NO_2^-) and then nitrate				
		(NO ₃ ⁻) by autotrophic bacteria				
minN_14d	Biological	Nitrogen mineralisation after 14 days of incubation. Gross mineralisation	mg/kg	Aurea	SD_	Optimum
		of nitrogen is the change from organic to mineral form, in two stages: 1)			Aurea	

Abbreviation	Sub	Description	Unit	nit Laboratory/Institute	Date of	Orientation
Abbreviation	Compartment	Description	Onit	Laboratory/institute	sampling	Onentation
		Ammonification where organic nitrogen is converted to ammonium (NH_4^+)				
		by heterotrophic microorganisms, and 2) Nitrification where under non-				
		limiting conditions, NH_4^+ is then oxidised to nitrite (NO_2^-) and then nitrate				
		(NO ₃ ⁻) by autotrophic bacteria				
minN_21d	Biological	Nitrogen mineralisation after 21 days of incubation. Gross mineralisation	mg/kg	Aurea	SD_	Optimum
		of nitrogen is the change from organic to mineral form, in two stages: 1)			Aurea	
		Ammonification where organic nitrogen is converted to ammonium (NH_4^+)				
		by heterotrophic microorganisms, and 2) Nitrification where under non-				
		limiting conditions, NH_4^+ is then oxidised to nitrite (NO_2^-) and then nitrate				
		(NO ₃ ⁻) by autotrophic bacteria				
minN_28d	Biological	Nitrogen mineralisation after 28 days of incubation. Gross mineralisation	mg/kg	Aurea	SD_	Optimum
		of nitrogen is the change from organic to mineral form, in two stages: 1)			Aurea	
		Ammonification where organic nitrogen is converted to ammonium (NH_4^+)				
		by heterotrophic microorganisms, and 2) Nitrification where under non-				
		limiting conditions, NH_4^+ is then oxidised to nitrite (NO_2^-) and then nitrate				
		(NO ₃ ⁻) by autotrophic bacteria				
NAG	Biological	Activity of the enzyme N-Acetylglucosaminidase (NAG), which is involved	nmol/	CNRS Toulouse	SD_2	More the
		at the end of chitin degradation, degraded into compounds that can be	hour/g			better
		used directly by micro-organisms. NAG reflects the activity of	dry soil			
		decomposers, as well as the release of organic nitrogen and carbon				
Beta_Glu	Biological	B-glucosidase activity. B-glucosidase is involved in the final stage in the	nmol/	CNRS Toulouse	SD_2	More the
		decomposition of cellulose and hemicellulose (major components of OM),	hour/g			better
		releasing glucose units, which serve as a readily available energy source	dry soil			
		for microorganisms. This enzyme is produced by bacteria, saprophytic				
		fungi and root exudates. It plays an important role in the carbon cycle				
Phosphatase	Biological	Activity of the phosphatase enzyme, whose role is to release phosphate	nmol/	CNRS Toulouse	SD_2	More the
		that can be used directly in plants	hour/g			better
			dry soil			
Lamina	Biological	Bait laminas are a bio-indicator that assess the rate of degradation of a	Score	INP ENSAT Toulouse	SD_2;	More the
		cellulose substrate by the soil mesofauna (it can also reflect the activity of			SD_3	better
		small macrofauna)				

Abbroviation	Sub	Description	11		Date of	Orientation
Appreviation	Compartment	Description	Unit	Laboratory/institute	sampling	Orientation
Litter_bag	Biological	Degradation of litter bags after 3 months of incubation	g	INP ENSAT Toulouse	SD_1;	More the
					SD_3	better
ECp_s1	Chemical	Pore water conductivity, represents the ion content of the water available	mS/m	INP ENSAT Toulouse	SD_1	Optimum
		to plants, measured at 0-10 cm during the first sampling in spring				
Perm_s1	Chemical	Soil permittivity measured at 0-10 cm depth at the first sampling in spring	F/m	INP ENSAT Toulouse	SD_1	Optimum
ECb_s1	Chemical	Soil bulk electrical conductivity measured at 0-10 cm depth at the first	mS/m	INP ENSAT Toulouse	SD_1	Optimum
		sampling in spring				
ECp_s2	Chemical	Pore water conductivity, represents the ion content of the water available	mS/m	INP ENSAT Toulouse	SD_2	Optimum
		to plants, measured at 0-10 cm during the second sampling in spring				
Perm_s2	Chemical	Soil permittivity measured at 0-10 cm depth at the second sampling in	F/m	INP ENSAT Toulouse	SD_2	Optimum
		spring				
ECb_s2	Chemical	Soil bulk electrical conductivity measured at 0-10 cm depth at the second	mS/m	INP ENSAT Toulouse	SD_2	Optimum
		sampling in spring				
Paramag_LF	Chemical	Paramagnetism in Low frequency (LF = 465 Hz)	m³/kg	CIRAD Montpellier	SD_2	More the
						better
Paramag_Xld	Chemical	Difference (LF-HF)/LF% (LF = Low frequency, HF = High Frequency).	%	CIRAD Montpellier	SD_2	More the
		 XLd% < 2%: Most paramagnetism linked to big particles (>30 nm) 				better
		due to parent material or heavy metal pollution;				
		 2% < XLd% < 5% paramagnetism linked to biogenic particles <30 				
		nm;				
		 Ld% > 5% paramagnetism linked to fine particles of biogenic 				
		origin.				
		This parameter is mostly accurate when LF > 1E-7				
рН	Chemical	Soil water pH, on a composite soil sample representative of the area of	No_unit	Aurea	SD_	Optimum
		the plot under study in 2022 or 2023			Aurea	
s.AP	Chemical	Phosphorus concentration P_2O_5 Olsen on a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023	soil		Aurea	
CEC	Chemical	Metson CEC on a composite soil sample representative of the study area	mEq/100	Aurea	SD_	Optimum
		of the plot studied in 2022 or 2023	g soil		Aurea	
s.AK2O	Chemical	Concentration of exchangeable K_2O in a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2023	soil		Aurea	

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment	Description		Laboratory/institute	sampling	onentation
s.AMg	Chemical	Concentration of exchangeable MgO in a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the area of the plot studied in 2022 or 2023	soil		Aurea	
s.ANa2O	Chemical	Concentration of exchangeable Na ₂ O on a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 or 2023.	soil		Aurea	
		Where the value was below the laboratory detection limit (10 mg/kg), the				
		concentration was set at 10 mg/kg				
s.AZn	Chemical	Concentration of zinc EDTA in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 and 2023. If the EDTA zinc	soil		Aurea	
		concentration is below the laboratory detection limit (1 mg/kg), then				
		value 1 is applied				
s.AMn	Chemical	Manganese EDTA concentration on a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 and 2023.	soil		Aurea	
		Where the value is below the detection limit (4 mg/kg), the value 4 is				
		applied				
s.ACu	Chemical	EDTA copper concentration in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 and 2023	soil		Aurea	
s.AFe	Chemical	Concentration of EDTA iron in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area surveyed in 2022 and 2023. Where the value was below	soil		Aurea	
		the laboratory detection limit (10 mg/kg), the concentration was set at 10				
		mg/kg				
s.AB	Chemical	Boron concentration extracted with boiling water on a composite soil	mg/kg	Aurea	SD_	Optimum
		sample representative of the study area of the plot studied in 2022 and	soil		Aurea	
		2023				
s.ASO4	Chemical	Concentration of exchangeable sulphur in a composite soil sample	% raw	Aurea	SD_	Optimum
		representative of the area of the plot studied in 2022 or 2023	soil		Aurea	
s.AMo	Chemical	Concentration of DTPA molybdenum in a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot studied in 2022 and 2023.	soil		Aurea	
		When the raw values were noted as Mb bioavailable < 0.10, then we				
		adapted the notation as Mb bioavailable = 0.10. Where no value was				
		provided, the laboratory indicated that this was because no trace had				
		been detected. In this case we assigned 0 to this missing value				

Abbreviation	Sub Compartment	Description	Unit	Laboratory/Institute	Date of sampling	Orientation
s.TMn	Chemical	Total manganese concentration in a composite soil sample representative	mg/kg	Aurea	SD_	Optimum
		of the study area of the plot studied in 2022 and 2023	soil		Aurea	
s.TCu	Chemical	Total copper concentration in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 or 2023	soil		Aurea	
s.TZn	Chemical	Total Zinc concentration in a composite soil sample representative of the	mg/kg	Aurea	SD_	Optimum
		study area of the plot studied in 2022 or 2023	soil		Aurea	
s.TMo	Chemical	Total molybdenum concentration on a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the study area of the plot surveyed in 2022 and 2023.	soil		Aurea	
		Where the value was noted as < 0.5 mg/kg, then the value 0.5 mg/kg is				
		assigned				
s.TB	Chemical	Total boron concentration on a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 and 2023. When the raw values	soil		Aurea	
		were noted as Br total < 2.5 mg/kg, we adapted the notation as				
		B total = 2.5 mg/kg				
s.TNa2O	Chemical	Concentration of total Na2O on a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 or 2023	soil		Aurea	
s.TS	Chemical	Total sulphur concentration in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 or 2023	soil		Aurea	
s.TK2O	Chemical	Concentration of total potassium in a composite soil sample	mg/kg	Aurea	SD_	Optimum
		representative of the area of the plot studied in 2022 or 2024.	soil		Aurea	
s.TMg	Chemical	Concentration of total MgO in a composite soil sample representative of	mg/kg	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 or 2023	soil		Aurea	
s.TFe	Chemical	Total iron concentration in a composite soil sample representative of the	mg/kg	Aurea	SD_	Optimum
		study area in 2022 or 2023	soil		Aurea	
s.ratioMg	Chemical	Ratio s.AMg:s.TMg	%	Aurea	SD_	Optimum
					Aurea	
s.ratioK2O	Chemical	Ratio s.AK2O:s.TK2O	%	Aurea	SD_	Optimum
					Aurea	
s.ratioNa	Chemical	Ratio s.ANa2O:s.TNa2O	%	Aurea	SD_	Optimum
					Aurea	
s.ratioZn	Chemical	Ratio s.AZn:s.TZn	%	Aurea	SD_	Optimum

Abbroviation	Sub	Description	Unit	Laboratory/Instituto	Date of	Oriontation
ADDIEVIATION	Compartment	Description	Onit	Laboratory/institute	sampling	Onentation
					Aurea	
s.ratioMn	Chemical	Ratio s.AMn:s.TMn	%	Aurea	SD_	Optimum
					Aurea	
s.ratioFe	Chemical	Ratio s.AFe/s.TFe	%	Aurea	SD_	Optimum
					Aurea	
s.ratioB	Chemical	Ratio s.AB:s.TB	%	Aurea	SD_	Optimum
					Aurea	
s.ratioS	Chemical	Ratio s.ASO4 :s.TS	%	Aurea	SD_Aure	Optimum
					а	
s.ratioMo	Chemical	Ratio s.AMg:s.TMg	%	Aurea	SD_Aure	Optimum
					а	
s.ratioCu	Chemical	Ratio s.ACu:s.TCu	%	Aurea	SD_	Optimum
					Aurea	
OM	Chemical	Concentration of soil OM by dry combustion on a composite soil sample	%	Aurea	SD_	More the
		representative of the study area of the plot studied in 2022 or 2023			Aurea	better
OC	Chemical	Soil OC concentration in a composite soil sample representative of the	%	Aurea	SD_	More the
		study area of the plot studied in 2022 or 2023			Aurea	better
s.TN	Chemical	Dumas total N concentration on a composite soil sample representative of	%	Aurea	SD_	Optimum
		the study area of the plot studied in 2022 or 2023			Aurea	
C_N	Chemical	C/N of a composite soil sample representative of the study area of the	No_unit	Aurea	SD_	Optimum
		plot studied in 2022 or 2023			Aurea	
C_0_50	Chemical	Granulometric fractionation of C in the 0-50 μm fraction on a composite	%C tot	Aurea	SD_	Optimum
		soil sample representative of the study area of the plot studied in 2022 or			Aurea	
		2023. This separation makes it possible to define OM fractions with				
		different roles in the soil: the stable fraction (0-50 μm) represents the				
		finest and most evolved organic matter (> 50 years). It plays a role in the				
		long-term structuring and stabilisation of soils, as well as in their cation				
		exchange capacity (chemical reservoir)				
C_50_200	Chemical	Granulometric fractionation of C in the 50-200 μm fraction on a	%C tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023. This separation makes it possible to define OM fractions				

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment			,	sampling	
		with different roles in the soil: the labile fraction (50-200 μ m) groups				
		together intermediate, transitory organic matter in the process of				
		decomposition. It represents the reserve of energy and nutrients available				
		over the medium term (10 to 20 years) for living soil organisms and crops				
C_200_2000	Chemical	Granulometric fractionation of C in the 200-2000 μm fraction on a	%C tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023. This separation makes it possible to define OM fractions				
		with different roles in the soil: the fresh fraction (200-2000 $\mu m)$ groups				
		together the coarsest and youngest organic matter (2 to 5 years old), such				
		as recent crop residues and plant cover or fragments of organic products				
		added (manure, etc.) that have not yet decomposed. As well as acting as a				
		reserve of elements and energy over the next 2-5 years, it also provides				
		physical protection for the soil (limiting soil compaction)				
C_50_2000	Chemical	Granulometric fractionation of C in the 50-2000 μm fraction on a	%C tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023.				
N_0_50	Chemical	Granulometric fractionation of N in the 0-50 μm fraction on a composite	%N tot	Aurea	SD_	Optimum
		soil sample representative of the study area of the plot studied in 2022 or			Aurea	
		2023				
N_50_200	Chemical	Granulometric fractionation of N in the 50-200 μm fraction on a	%N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				
N_200_2000	Chemical	Granulometric fractionation of N in the 200-2000 μm fraction on a	%N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				
N_50_2000	Chemical	Granulometric fractionation of N in the 50-2000 μm fraction on a	%N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				
C_N_0_50	Chemical	Granulometric fractionation of C/N in the 0-50 μm fraction on a	%C/N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment				sampling	
C_N_50_200	Chemical	Granulometric fractionation of C/N in the 50-200 μ m fraction on a	%C/N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				
C_N_200_2000	Chemical	Granulometric fractionation of C/N in the 200-2000 μm fraction on a	%C/N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot studied			Aurea	
		in 2022 or 2023				
C_N_50_2000	Chemical	Granulometric fractionation of C/N in the 50-2000 μm fraction on a	%C/N tot	Aurea	SD_	Optimum
		composite soil sample representative of the study area of the plot in 2022			Aurea	
		or 2023				
SituResp24	Chemical	Basal soil respiration measured using the Biofunctool® protocol at 3	Absorba	INP ENSAT Toulouse	SD_2	More the
		positions in the study area of the study plot in 2022 or 2023 after 24 h	nce			better
SituResp48	Chemical	Basal soil respiration measured using the Biofunctool® protocol at 3	Absorba	INP ENSAT Toulouse	SD_2	More the
		positions in the study area of the study plot in 2022 or 2023 after 48 h	nce			better
Moist_s1	Physical	Volumetric soil moisture measured at 0-10 cm depth at the first sampling	%	INP ENSAT Toulouse	SD_1	Optimum
		in spring				
Temp_s1	Physical	Soil temperature measured at 0-10 cm depth at the first sampling in	°C	INP ENSAT Toulouse	SD_1	Optimum
		spring				
Moist_s2	Physical	Volumetric soil moisture measured at 0-10 cm depth at the first sampling	%	INP ENSAT Toulouse	SD_2	Optimum
		in spring				
Temp_s2	Physical	Soil temperature measured at 0-10 cm depth at the second sampling in	°C	INP ENSAT Toulouse	SD_2	Optimum
		spring				
Cindex	Physical	Crusting Index	No_unit	INP ENSAT Toulouse	SD_	Less the
					Aurea	better
Agg	Physical	Stability of aggregates. Good aggregate stability means that the soil	Score	INP ENSAT Toulouse	SD_1	More the
		particles are resistant to water or wind erosion. The principle of the				better
		protocol is based on the attribution of a single score linked to the stability				
		of aggregates in water				
Beerkan	Physical	Speed of infiltration of water into the soil: A fixed volume of water 1 cm	mL/min	INP ENSAT Toulouse	SD_1	Optimum
		high (310 mL for a 20 cm diameter cylinder) is poured onto the surface of				
		the soil. The infiltration time in the soil is measured for each volume of				

Abbreviation	Sub	Description	Unit	l aboratory/Institute	Date of	Orientation
Abbreviation	Compartment	Description	onic	Euboratory/institute	sampling	
		water. It is then possible to calculate the infiltration rate of the water in ml				
		per minute using the infiltration curve for water in its steady state				
VESS	Physical	The VESS (Visual Evaluation of Soil Structure) method is based on a visual	Score	INP ENSAT Toulouse	SD_1	Optimum
		description of the structure of the different layers of a square block of soil				
		structure of the different layers of a square block of soil measuring				
		20 cm x 20 cm in surface area and 25 cm deep depth. The description is				
		guided by a description sheet which characterises various structural				
		parameters of each soil layer. The final score is calculated by averaging				
		each score assigned to each layer, between 1 (very friable soil with no				
		structure) and 5 (very compact soil). An adaptation to the original				
		protocol was made since assigned scores were attributed from 0 (very				
		friable with no structure) to 6 (very compact soil). VESS scores were				
		measured in 2022 and 2023 with 3 repetitions on each plot				
scoreVESS	Physical	Adaptation of VESS to reflect the "More the better" orientation	Score	INP ENSAT Toulouse	SD_1	More the
						better

<u>4. Plant health</u>

Abbreviation	Sub Compartment	Description	Unit	Laboratory/Institute	Date of sampling	Orientation
fqSept	Disease	Total number of organs (L1, L2, L3) affected by Septoria per 20	No_unit	INP ENSAT Toulouse	SD_2	Less the
		plants assessed per plot and per zone				better
fqMil	Disease	Total number of organs (L1, L2, L3) affected by powdery	No_unit	INP ENSAT Toulouse	SD_2	Less the
		mildew per 20 plants assessed per plot and per zone				better
fqRust	Disease	Total number of organs (L1, L2, L3) affected by rust on 20	No_unit	INP ENSAT Toulouse	SD_2	Less the
		plants assessed per plot and per zone				better
fqFusa	Disease	Total number of ears affected by fusariosis on 20 plants	No_unit	INP ENSAT Toulouse	SD_3	Less the
		assessed per plot and per zone				better
fqEyespot	Disease	Total number of ears affected by foot rot on 20 plants assessed	No_unit	INP ENSAT Toulouse	SD_3	Less the
		per plot and per zone				better

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
-	Compartment				sampling	-
fqTotDis	Disease	Total number of organs (L1, L2, L3, ears) affected by diseases	No_unit	INP ENSAT Toulouse	SD_3	Less the
		on the 20 plants assessed per plot and per zone				better
intSept	Disease	Average intensity of Septoria damage on affected leaves per	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		individual and per zone	sick leaves			better
intMil	Disease	Average intensity of powdery mildew damage on affected	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		leaves per individual and per zone	sick leaves			better
intRust	Disease	Average intensity of rust damage on affected leaves per	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		individual and per zone	sick leaves			better
intAvDis	Disease	Average intensity of disease on affected leaves per individual	% average intensity on	INP ENSAT Toulouse	SD_3	Less the
		and per zone	sick leaves			better
Height	Growth	Average height of wheat at maturity on the plot studied in	cm	INP ENSAT Toulouse	SD_3	Optimum
		2022 or 2023				
fqslugs_lbeetles	Pest	Total number of organs (L1, L2, L3) affected by slug and locust	No_unit	INP ENSAT Toulouse	SD_2	Less the
		damage on the 20 plants assessed per plot and per zone				better
fqlminers	Pest	Total number of organs (L1, L2, L3) affected by leaf miners on	No_unit	INP ENSAT Toulouse	SD_2	Less the
		20 plants assessed per plot and per zone				better
fqPests	Pest	Total number of diseases found on 20 plants assessed by	No_unit	INP ENSAT Toulouse	SD_2	Less the
		organ, plot and zone				better
intslugs_lbeetles	Pest	Average intensity of locust and slug damage on affected leaves	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		per individual and per zone	sick leaves			better
intlminers	Pest	Average intensity of leaf miners attacks on leaves per individual	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		and per zone	sick leaves			better
intPests	Pest	Average intensity of insect damage on affected leaves per	% average intensity on	INP ENSAT Toulouse	SD_2	Less the
		individual and per zone	sick leaves			better

<u>5. Grain quality</u>

Abbreviation	Sub	Description	Unit	Laboratory/Institute	Date of	Orientation
	Compartment		onic	Euboratory/institute	sampling	onentation
Polyph	Nutritional	grain polyphenols, measured on a composite sample representative of	mg GAE/100g	Valorex, James	date_	More the
		the portion of the plot studied in 2022	fresh	Hutton Institute	harvest	better
Antiox	Nutritional	grain antioxidants, measured on a composite sample representative of	FRAP/100g	Valorex, James	date_	More the
		the portion of the plot studied in 2022 or 2023	fresh	Hutton Institute	harvest	better
g.TN	Nutritional	Total nitrogen in grain, measured on a composite sample representative	%	Phytocontrol	date_	More the
		of the portion of the plot studied in 2022 or 2023			harvest	better
Prot	Nutritional	Grain protein, measured on a composite sample representative of the	%	Valorex	date_	More the
		portion of the plot studied in 2022 or 2023			harvest	better
g.Fe	Nutritional	Grain Fe, measured on a composite sample representative of the	mg/kg	Phytocontrol	date_	Optimum
		portion of the plot studied in 2022 or 2023			harvest	
g.Mn	Nutritional	Grain manganese, measured on a composite sample representative of	mg/kg	Phytocontrol	date_	Optimum
		the portion of the plot studied in 2022 or 2023			harvest	
g.P	Nutritional	Grain phosphorus, measured on a composite sample representative of	mg/100g	Phytocontrol	date_	Optimum
		the portion of the plot studied in 2022 or 2023			harvest	
g.K	Nutritional	Grain potassium, measured on a composite sample representative of	mg/100g	Phytocontrol	date_	Optimum
		the portion of the plot studied in 2022 or 2023			harvest	
g.Zn	Nutritional	Grain zinc, measured on a composite sample representative of the	mg/kg	Phytocontrol	date_	Optimum
		portion of the plot studied in 2022 or 2023			harvest	
B9	Nutritional	Vitamin B9 in grain, measured on a composite sample representative of	µg/100g	Phytocontrol	date_	More the
		the portion of the plot studied in 2022 or 2023			harvest	better
Starch	Nutritional	Grain starch, measured on a composite sample representative of the	%	Valorex	date_	Optimum
		portion of the plot studied in 2022 or 2023			harvest	
Cellulose	Nutritional	Grain cellulose, measured on a composite sample representative of the	%	Valorex	date_	Optimum
		portion of the plot studied in 2022 or 2023			harvest	
Ergo	Nutritional	Ergothionein in grain, measured on a composite sample representative	ng/mg	University of	date_	More the
		of the portion of the plot studied in 2022 or 2023		Pennsylvania	harvest	better
DON	Sanitary	Grain Deoxinivalenol (DON), measured on a composite sample	μg/kg	Phytocontrol	date_	Less the
		representative of the portion of the plot studied in 2022 or 2023			harvest	better
HT2	Sanitary	HT2 of the grain, measured on a composite sample representative of the	µg/kg	Phytocontrol	date_	Less the
		portion of the plot studied in 2022 or 2023			harvest	better

Abbreviation	Sub	Description	Unit	laboratory/Institute	Date of	Orientation
	Compartment	Description	onic		sampling	
ZEA	Sanitary	Zearalenone (ZEA) in grain, measured on a composite sample	µg/kg	Phytocontrol	date_	Less the
		representative of the portion of the plot studied in 2022 or 2023			harvest	better
FarmerSW_n	Technologic	Average specific weight of wheat harvested in 2022 or 2023, supplied by	kg/hl	Farmers	date_	Optimum
		the farmer			harvest	
ExpSW_n	Technologic	Specific weight measured at the laboratory on 3 sub-samples of the	kg/hl	INP ENSAT Toulouse	SD_3	Optimum
		studied zone and averaged				
Grain_ear_n	Technologic	Average number of grains per ear on wheat harvested in 2022 or 2023	No_unit	INP ENSAT Toulouse	SD_3	Optimum
ТКШ	Technologic	Thousand Kernel Weight: weight in grams of 1 000 seeds	g	INP ENSAT Toulouse	SD_3	Optimum

6. Bread quality

Abbreviation	Sub	Description	Unit	Laboratory/	Date of	Orientation
Abbreviation	Compartment	Description	Onit	Institute	sampling	Orientation
f.Hagberg	Technologic	Hagberg falling number on a composite sample of wheat in 2022 or	S	Flour mill	date_harvest	Optimum
		2023		"Girardeau"		
MillYield	Technologic	Mill yield, which corresponds to the ratio between the weight of flour	%	Flour mill	date_harvest	More the
		and the weight of milled grain		"Girardeau"		better
f.Ashes	Technologic	Flour weight after incineration of 5g at 900°C for 1:30 hours. Determines	%	Flour mill	date_harvest	Optimum
		flour type		"Girardeau"		
b.W	Technologic	The baking force characterises the viscoelastic capacity of the dough	No_	Flour mill	date_harvest	Optimum
		according to two parameters: elasticity and extensibility. Elasticity	unit	"Girardeau"		
		expresses the dough ability to stretch and then return to its original				
		shape after stress. Extensibility expresses its capacity to stretch without				
		tearing. More than a force, it represents the work necessary for the				
		deformation of the dough				
f.PL	Technologic	toughness/extensibility ratio	No_unit	Flour mill	date_harvest	Optimum
				"Girardeau"		
f.IE	Technologic	PO/P ratio (PO: pressure reached for dough length of 4 cm, P: maximum	No_unit	Flour mill	date_harvest	Optimum
		pressure recorded before dough starts to swell). Gives an indication of		"Girardeau"		
		the elasticity of the dough. Low elasticity indices indicate an extensible				
		dough, while high values indicate a dough with a tendency to be tough				

Abbroviation	Sub	Description	Unit	Laboratory/	Date of	Orientation
ADDIEVIATION	Compartment	Description	Onit	Institute	sampling	
f.Hydration	Technologic	Proportion of water in dough for bread fabrication	%	Flour mill	date_harvest	Optimum
				"Girardeau"		
b.Length	Technologic	Length of bread	cm	Flour mill	date_harvest	Optimum
				"Girardeau"		
b.Volume	Technologic	Bread volume in cm ³	cm ³	Flour mill	date_harvest	Optimum
				"Girardeau"		
Baking_Score	Technologic	The French bread baking test is used to assess the bread-making ability	Score	Flour mill	date_harvest	More the
		of a wheat or wheat blend, or even a flour		"Girardeau"		better

<u>7. Performances</u>

Abbroviation	Sub	Description		Laboratory/	Date of	Orientation
Abbreviation	Compartment			Institute	sampling	
farmerYield_n	Economic	yield of winter wheat in 2022 or 2023 given by farmer	q/ha	Farmers	date_	More the
					harvest	better
ExpYield_n	Economic	Wheat yield in 2022 or 2023 on the study plot, values measured at 3 points on each	q/ha	INP ENSAT	SD_3	More the
		plot using the ring method. Value then averaged to obtain a value per plot		Toulouse		better
SNMargin	Economic	Indicator of system profitability	€/ha	Systerre®	survey	More the
						better
InputExpenses	Economic	Costs related to the purchase of inputs: fertilisers, seeds, plant protection products,	€/ha	Systerre®	survey	Less the
		other inputs, etc.				better
Production	Economic	Indicator of a crop competitiveness. It considers all costs involved in producing a	€/t	Systerre®	survey	Less the
Cost		crop				better
Gas	Environmental	Quantity of fuel used by equipment during operations on the plot. Additional	L/ha	Systerre®	survey	Less the
Consumption		consumption (maintenance, etc.) is not included				better
GHGtotEmiss	Environmental	Greenhouse gas (GHG) emissions correspond to the cumulative emissions of CO ₂ ,	kgCO2eq/	Systerre®	survey	Less the
		CH_4 and N_2O weighted by coefficients specific to each gas, based on their global	ha			better
		warming power (GWP9, IPCC method 2007). Indirect emissions (upstream of the				
		farm, to produce inputs, etc.) and direct emissions (in situ) are taken into account				

Abbreviation	Sub Compartment	Description		Laboratory/ Institute	Date of sampling	Orientation
WorkingTime	Social	Working time calculated by the Systerre [®] tool in hours per hectare. This corresponds	h/ha	Systerre®	survey	Less the
		to the time spent pulling on the plots; it does not therefore include time spent on				better
		administrative management, crop observation, etc.				



Annexe 2: Cropping practices' survey provided to farmers in year 2 (the same was provided in Year 1 on the 2017-2022 period).

Cropping practices 2018-2023

In order to study the links between farming practices and the results obtained in terms of soil, plant and grain quality, we need to know the precise cultivation history of the plots made available over a 5-year period. This document will therefore enable us to compile information on tillage, fertilisation and the use of plant protection products, which will form the basis of our analyses for this second year of the study.

It is also possible to print out directly the cropping itineraries as described in your monitoring software (Geofolia, Terciel, MesParcelles...) if you have access to it.

Ideally, please submit the completed documents (years 2018-2022) (or send them to clara.lefevre@agricultureduvivant.org) preferably before 1 June 2023.

The '2023 harvest year' section will have to be returned at the end of the harvest. Please return the information before 20 July 2023.

2018 Campaign

Principal crop :

1) Intercrop or catch crop :

- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../....../
 - Cover composition :
 - Destruction date of cover:/...../...../....../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :
- Was a catch crop seeded before the main crop? Yes/No
 - Seeding date of catch crop:/..../...../
 - Catch crop composition :



- Yield :

2) <u>Principal crop (Fill in the below tables):</u>

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

<u>Notes :</u>

- a. If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- b. For seeding: Please also indicate row spacing (cm) and seeding density (grains/ m^2).

Date	Type of soil tillage and tillage depth

<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity



2.3) Phytosanitary products (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide, herbicide)	Commercial name + dose		

Harvest date :.... Residue returned: YES – NO Yield (qx/ha) :....



2019 Campaign

Principal crop:

- 1) Intercrop or catch crop:
- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../...../....../
 - Cover composition :
 - Destruction date of cover:/...../...../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :
- Was a catch crop seeded before the main crop? Yes/No
 - Seeding date of catch crop:/..../...../
 - Catch crop composition :
 - Harvest date :/...../...../....../
 - Yield :

2) Principal crop (Fill in the below tables):

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

<u>Notes :</u>

- c. If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- d. For seeding: Please also indicate row spacing (cm) and seeding density (grains/m²).

Date	Type of soil tillage and tillage depth		



<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity

2.3) Phytosanitary products (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide,	Commercial name + dose
	herbicide)	

Name/Surname: « One Health » study – Winter Wheat - Year 2 – April 2023



Harvest date :.... Residue returned: YES – NO Yield (qx/ha) :....



2020 Campaign

Principal crop :

1) Intercrop or catch crop :

- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../...../
 - Cover composition :
 - Destruction date of cover:/...../....../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :
- Was a catch crop seeded before the main crop? Yes/No
 - Seeding date of catch crop:/..../...../
 - Catch crop composition :
 - Harvest date :/..../...../...../
 - Yield :

2) Principal crop (Fill in the below tables):

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

Notes :

- e. If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- f. For seeding: Please also indicate row spacing (cm) and seeding density (grains/m²).

Date	Type of soil tillage and tillage depth	





<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity	

2.3) Phytosanitary products (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide, herbicide)	Commercial name + dose

Name/Surname: « One Health » study – Winter Wheat - Year 2 – April 2023



Harvest date :.... Residue returned: YES – NO Yield (qx/ha) :....


2021 Campaign

Principal crop :

1) Intercrop or catch crop :

- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../...../
 - Cover composition :
 - Destruction date of cover:/...../....../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :

• Was a catch crop seeded before the main crop? Yes/No

- Seeding date of catch crop:/..../...../
- Catch crop composition :
- Harvest date :/..../...../...../
- Yield :

2) Principal crop (Fill in the below tables):

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

<u>Notes :</u>

- g. If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- h. For seeding: Please also indicate row spacing (cm) and seeding density (grains/m²).

Date	Type of soil tillage and tillage depth	





<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity	

2.3) Phytosanitary products (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide, herbicide)	Commercial name + dose



Harvest date :.... Residue returned: YES – NO Yield (qx/ha) :....

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<u>2022 Campaign</u>

Principal crop :

1) Intercrop or catch crop :

- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../...../
 - Cover composition :
 - Destruction date of cover:/...../...../....../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :
- Was a catch crop seeded before the main crop? Yes/No
 - Seeding date of catch crop:/..../...../
 - Catch crop composition :
 - Harvest date :/..../...../...../
 - Yield :

2) Principal crop (Fill in the below tables):

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

Notes :

- *i.* If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- *j.* For seeding: Please also indicate row spacing (cm) and seeding density (grains/m²).

Date	Type of soil tillage and tillage depth	





<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity	

2.3) Phytosanitary products (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide, herbicide)	Commercial name + dose



Harvest date :.... Residue returned: YES – NO Yield (qx/ha) :....

2023 Campaign

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Principal crop : Winter wheat

Wheat variety(ies) used:.....

Name of seed treatment:

- 1) Intercrop or catch crop :
- Was a cover crop seeded before the main crop? Yes / No
- If yes :
 - Seeding date of cover:/...../...../....../
 - Cover composition :
 - Destruction date of cover:/...../...../
 - Mean of destruction (ploughing, herbicide application, grazing...) :
 - Estimated returned biomass (in t/ha) by cover crop (please refer to the image on the document last page) :

• Was a catch crop seeded before the main crop? Yes/No

- Seeding date of catch crop:/..../...../
- Catch crop composition :
- Harvest date :/...../...../....../
- Yield :

2) <u>Principal crop (Fill in the below tables):</u>

<u>2.1) Soil tillage</u> since the last principal crop (or cover crop)

Notes :

- *k.* If a catch crop was grown before the main crop, include in this table the tillage carried out since the catch crop was seeded (including seeding).
- I. For seeding: Please also indicate row spacing (cm) and seeding density (grains/m²).

Date	Type of soil tillage and tillage depth



<u>2.2) Fertilisation</u> (Mineral + Organic + Biostimulants + Oligoelements) since last harvest or cover destruction

Note: If a catch crop was grown before the main crop, include the fertilisation since the catch crop was planted in this table.

Date	Type of input + quantity

<u>2.3) Phytosanitary products</u> (Herbicides, fongicides, Herbi, Fongis, Regulators/biocontrols, slug control, seed treatments) (since last harvest)

<u>Note:</u> If a catch crop was grown before the main crop, include the plant protection products grown since the catch crop was planted in this table.

Date	Type (fongicide, herbicide)	Commercial name + dose



Harvest date :..... Residue returned: YES – NO Yield (qx/ha) :.....



Specific Weigth: Humidity (%): Proteins level (%):.....

IF the plot was irrigated, how much water did it receive? How often?

.....

RAINFALL:

If possible, could you estimate the amount of rain that fell during the wheat growing months from last October until harvest?

Month	Precipitations (mm)	Month	Précipitations (mm)	Month	Precipitations (mm)
October 22		February		May 23	
		23			
November 22		March 23		June 23	
December 22		April 23		July 23	
January 23					

How did you record the rainfall in the table above? (Tick the appropriate box) :

O Manual rain gauge

\bigcirc Personal weather station near the plot. Distance to plot:	
--	--

Other, please specify:

Were there any weather events during the wheat growing season this year (very wet or very dry winter, heatwave, hail, etc.), if so, over what periods?



OTHER COMMENTS:

Make a note here of any other specific comments you feel are important (*e.g.* hail damage to the plot, disease problems, rodent damage, % of estimated loss due to damage, etc....):

Annexe 3: Towards the adaptation of RI farm to the field scale

The RI used for the initial farmer's selection was measured at the farm scale on the 2019-2020 campaign, while our study was performed at the field scale on the 2021-2022 and 2022-2023 campaigns. The adaption of the Regeneration Index at the farm scale (*RI_farm*) to the field scale (*RI_field*) had two principal objectives:

- quantitatively assess the correlation between the farm and field cropping management performance indexes
- quantitatively classify cropping systems according to their agronomic performance at the field scale,
- test the feasibility and relevance of backing a quantitative index to the identified groups of farmers (the four identified clusters, **Chapter 3**).

The shift from farm to field requested the adaptation of some of the *RI_farm* questions to fit the field scale (Table 15). Indeed, *RI_farm* was developed to be computed with yearly information. To correctly assess management at the field scale in accounting for possible legacy effects of past practices, we chose to work with year and the past five years' information. In some cases, adapting indicators from the farm to the field scale was not possible or very redundant. Therefore, some questions were dropped in *RI_field*. Table 15 shows all the adaptations from *RI_farm* to *RI_field* with associated scores. Finally, since the final *RI_field* score was not equal to 100 but 96, we adjusted the score to get a score over 100 as follows:

$$RI_field = (EffCC_{rot} + Tillage_{intensity_{rot}} + C_{entries_{rot}} + nbLeg_{rot} + Norga_{rot} + TFI_{h_n} + TFI_{h_n} + CropDiv_{rot} + scoreSNH) * \frac{100}{90}$$

Like *RI_farm*, the objective of *RI_field* was to quantitatively inform the cropping system performance at the six-year rotation scale (five years past management + monitored campaign) on a given field.

As a result, *RI_field* scale evidenced least contrasted couples such as pair n°2, 6 and 9 (Figure 32). After discussing with farmers, CONV famers of pair 6 and 9 were transitioning farmers with high interest in CA systems (also see discussion of **Chapter 3**).

RI_field and *RI_farm* were correlated at 76%. However, when looking at the correlation between *RI_field* and *RI_farm* according to the type of cropping system, the R² decreased (Figure 33).

In this case, the threshold values to establish RI field scores for some important indicators (*e.g.* TFI scores, C entries scores) were dependent on the minimum and maximum value found within our network. IR field was therefore an interesting tool to classify farmers between them, although the associated scores cannot be interpreted such as RI farm scores. Since RI farm and RI field were well correlated, we continued the work with RI farm in the rest of the analyses.



Figure 29. (left) RI at the field scale according to the type of cropping system (CA in red, CONV in blue), (right) Differences of RI between pairs of farmers (Score CA- Score CONV) for each monitored year.



Figure 30. RI field and RI farm scores.

RI farm scores are identical across the two years. Blue and red lines show the two regression lines and the grey shadow corresponds to the standard error.

Table 15. Adaptations of the Regeneration Index from farm to field scale.

Compartment corresponds to the different compartments of cropping practices considered in the Regeneration Index at farm and field scale. Indicators in italics correspond to calculated indicators that were used in the main analysis of practices (see **Chapter 3**). *The OM:clay ratio was removed from score calculation since the ratio was problematic on very calcareous soils (also see **Annexe 4**). **The indicator was removed from the calculation since no adaptation was possible from farm to field scale and the question was redundant from farm to field.

	RI at the farm scale		RI at the field scale		
Axis	Indicator	Associated rating	Corresponding indicator at the field scale (also see Chapter 2)	Associated rating	
	Average annual rate of soil cover at the farm scale	0 point if yearly soil	Efficiency of cover cropping at the rotation scale	0 point if 0% efficiency	
		cover < 60%	(EffCC_rot)	18 points if 100% efficiency	
Soil Cover		18 points if yearly		Linear relation in between	
18 points		soil cover > 90%			
		Linear relation in			
		between			
	Farmer's tillage practices as a percentage of cultivated area:	A: 18 points	Farmer tillage practice at the rotation scale	0 point if ploughing is repeated	
	A: > 30% direct seeding, < 30% heavy tillage/ploughing and	B: 12 points	(Tillage_intensity_rot)	each of the six years	
	the rest light tillage	C: 6 points	For each of the six years:	18 points if direct seeding each of	
Soil tillage	B: < 30% direct seeding, < 30% heavy tillage/ploughing and	D: 0 point	0 if ploughing	the six years	
18 points	the rest light tillage OR > 50% direct seeding and > 30%		1 if light tillage		
10 points	heavy tillage/ploughing		2 if heavy tillage		
	C: < 30% direct seeding, > 30% heavy tillage/ploughing and		3 is direct seeding		
	the rest light tillage				
	D: 100% in heavy tillage or ploughed				
	On-farm average OM:clay ratio:	A: 5 points			
	A: ≥ 20	B: 3.3 points			
	B: >20 and ≥ 17	C: 1.7 points	Indicator removed	*	
	C: < 17 and ≥ 12	D: 0 point			
Carbon	D: <12				
18 noints	Annual organic carbon inputs (organic amendments roots,	0 point if < 2.5	Average yearly organic carbon inputs (organic	0 point if ≤ 0.6 tC/ha/yr	
10 points	aerial parts returned to soil) in tC/ha/year	tC/ha	amendments roots, aerial parts returned to soil) in	18 points if ≥ 5.9 tC/ha/yr	
		13 points if > 6	tC/ha/year (C_entries_rot)	Linear relation in between	
		tC/ha			
		Linear relation in			
		between			

	RI at the farm scale		RI at the field scale			
Axis	Indicator	Associated rating	Corresponding indicator at the field scale (also see Chapter 2)	Associated rating		
Nitrogen fertilisation 6 points	Farm surface area cultivated with legumes (crop or cover) or organic nitrogen	0 point if <30% of the total farm area 6 points if >70% of the total farm area Linear relation in between	Number of legumes grown in six years (crops + cover crops) (<i>nbLeg_rot</i>) Average organic nitrogen inputs (kgN/ha/year) from organic fertilisation (orgN rot)	0 point if 0 3 points if ≥ 10 Linear relation in between 0 point if 0 kgN/ha/year 3 points if ≥ 150 kgN/ha/year Linear relation in between		
	TFI of herbicides compared with the average TFI of herbicides in the same region (based on public data)	0 point if > 0% 3.75 points if ≤ - 50%	TFI of herbicides on monitored campaign (<i>TFIh_n</i>)	0 point if ≥ 5.14 7.5 points if 0		
Phytosanitary	TFI of fungicides, insecticides and molluscicides compared with the average TFI of fungicides, insecticides and molluscicides in the same region (based on public data)	0 if > 0% 3.75 if ≤ - 50%	TFI of fungicides, insecticides and molluscicides on monitored campaign (<i>TFI_eh_n</i>)	0 point if ≥ 4.92 7.5 points if 0		
management 15 points	 Farmer's strategy for reducing TFI: A: Cropping system redesign (<i>e.g.</i> choice of resistant or low-susceptible varieties, intra-plot varietal mixes, etc) B: Substitution of chemical means by alternatives (<i>e.g.</i> biocontrol products, biological control, essential oils) C: Efficient use of plant protection products (low-volume, precision tools, etc) D: No specific reduction strategy implemented 	A: 7.5 points B: 5 points C: 2.5 points D: 0 point	Indicator removed**			
Biodiversity 15 points	Percentage of on-farm Ecological Interest Areas as declared to the Common Agricultural Policy declarations	0 point if <5% 7.5 points if > 10%	Presence of a hedge at the edge of the plot studied in year n (Hedge) Presence of semi-natural habitats around the plot (SNH): 3 points if forest and/or watercourse less than 200m from the study area 2 points if forest AND watercourse more than 200 m from the study area but less than 1 km from the study area 1 point if forest OR watercourse more than 200 m from the study area but less than 1 km from the study area	0 point if absence of hedge 3.75 points if presence of hedge 3.75 points if SNH = 3 2.5 points if SNH = 2 1.25 points if SNH = 1 0 point if SNH = 0		

	RI at the farm scale		RI at the field scale			
Axis	Indicator	Associated rating	Corresponding indicator at the field scale (also see Chapter 2)	Associated rating		
			0 if neither forest nor watercourse within 1km of			
			the study area			
	Number of species grown on farm and over the cropping	A: 7.5 points	Number of crop species grown over the rotation	0 point if 2 different crops		
	campaign (crops + cover crops)	B: 5 points	(crops + cover crops)	7.5 if 18 crops		
	A: ≥ 13	C: 2.5 points	(cropDiv_rot)			
	$B: \ge 9 \text{ and } \le 12$	D: 0 point				
	C: ≥ 5 and ≤ 8					
	D: ≤ 4					
	On-farm agroforestry project (hedgerow planting, intra-	A: 5 points				
	parcel planting, etc.):	B: 3.3 points				
Agroforestry	A: An in-field agroforestry project is implemented	C: 1.7 points	Indicator removed**			
5 points	B: Hedgerows are managed and/or a hedgerow planting	D: 0 point				
	program is planned					
	C: No agroforestry project on the farm					
	Farmer's participation in agroecology training or experience-	Yes: 5 points				
Knowledge	sharing networks	No: 0 point				
acquisition Yes: Yes, the farmer takes part in agroecology training or			Indicator removed	**		
and sharing	nd sharing experience-sharing networks					
5 points	No: No, the farmer does not take part in agroecology					
	training or experience-sharing networks					

Annexe 4: Investigating the OM:clay ratio

In 2021, soil textures were analysed after decarbonation. However, many carbonate soils were referenced in the study site (10 out of 44 plots with CaCO₃ concentrations >20% in year 1). Thus we decided in year 2 to analyse soil textures with and without decarbonation. These analyses could not be run for year 1 soils, because we had no remaining soil, and our calendar did not allow us to go sampling again on year 1 plots after year 1 campaign.

These different analyses influenced individual soil textural class, as defined in the USDA referential, specifically for soils with high CaCO₃ concentration (Table 16). 14 plots with low CaCO₃ concentration also changed textural class, when shifting from a granulometry calculated with to without decarbonation. This happened likely because their texture was already on the edge of a textural class with granulometry measured with decarbonation (*e.g.* 6_CA). Since these analyses were run in year 2 only, we focused on textures measured after decarbonated textures" in the CA vs. CONV and cluster comparison and obtained the same results as the "No decarbonation textures" (*i.e.* they were found in group A, thus showed no difference between the simple CA vs. CONV dichotomy and the clusters analysis) (Table 17).

In parallel, we calculated the OM:clay ratios, known as an indicator of soil structure stability (Johannes *et al.*, 2017), for textures measured according to the two methodologies. As expected, this led to different results when calculating the OM:clay ratio. As shown in Figure 34, values of OM:clay were always overestimated when using textures after decarbonation compared with results based on textures with no decarbonation.

As explained in the main manuscript, we could not include the OM:clay ratio in our main analysis, since the exclusion of soils with OM > 3.5% (as recommended in Mäkipää *et al.* (2024)) and clay content comprised between 12 and 40% (P. Boivin, pers. comm., 2023) led to the exclusion of too many plots from the analysis. However, as shown in Figure 34B, when comparing the OM:clay ratio with *vs.* without decarbonation of soils that match the criteria of OM<3.5% and clay comprised between 12 and 40%, we still had an overestimation of the OM:clay ratio by using the textures with decarbonation compared with textures without decarbonation (regression line coefficient = 0.89). Also, we observed that below 12% clay or above 3.5% OM, ratios gave values that were still in the "normal limits" (*i.e.* between 12 and 23%). Keeping these soils in our analysis could have led to a risk of misinterpretation or error.

Overall, the comparison between OM:clay ratios measured with or without decarbonation does not appear like a limitation to the use of the OM:clay as a recognised indicator with a unique threshold such as what is intended at the European level (European Environment Agency, 2023), provided that texture measurement methods are always clearly documented. In our study, the main limitation to using these ratios lied in the high number of soils which were out of the domain of use (below 12% clay and above 3.5% OM), including carbonate soils. Nevertheless, we acknowledge the need for more research on the topic to evaluate specific ratios of OM:clay or adapted indicators associated with these carbonate soils, allowing to simply characterise their structural stability.

Table 16. Orange-coloured cells correspond to different textures between after decarbonation granulometries and withoutdecarbonation granulometries.These tests were only realised in 2023.

	CaC	O₃ (%)	Soil texture with granulometry measured after decarbonation			Soil texture with granulometry measured without decarbonation			
Pair	СА	CONV	СА	CONV	Differences in textural classes	СА	CONV	Differences in textural classes	
1	0.1	0.1	Loam	Silt Loam	1	Loam	Silt Loam	1	
2	0.1	1.3	Silt Loam	Loam	1	Silt Loam	Sandy clay loam	2	
3	35.9	42.5	Silty Clay	Clay	1	Clay Loam	Silt Loam	1	
4	0.1	0.1	Sandy Clay Loam	Loam	1	Sandy Clay Loam	Loam	1	
5	37.3	56.9	Silty Clay Loam	Silty Clay	1	Silt Loam	Silty Clay Loam	1	
6	0.1	0.1	Silt Loam	Silt Loam	0	Loam	Silt Loam	1	
7	0.1	0.1	Loam	Loam	0	Loam	Loam	0	
8	0.1	0.1	Silt Loam	Silt Loam	0	Silt Loam	Silt Loam	0	
10	29.7	47.2	Silty Clay	Silty Clay	0	Clay Loam	Clay Loam	0	
11	16.2	27.8	Silty Clay	Silty Clay	0	Clay	Clay Loam	1	
12	0.1	0.1	Loa m	Loam	0	Loam	Loam	0	
13	0.1	0.1	Silt Loam	Silt Loam	0	Silt Loam	Silt Loam	0	
14	0.1	0.1	Loam	Loam	0	Loam	Loam	0	
15	0.1	0.1	Loam	Loam	0	Clay Loam	Loam	1	
16	0.1	0.1	Loam	Sandy Loam	1	Loam	Sandy loam	1	
17	8.7	0.1	Clay Loam	Clay Loam	0	Clay Loam	Clay Loam	0	
18	18.8	4.2	Loam	Loam	0	Loam	Sandy Clay Loam	1	
19	46.6	48.6	Silty Clay	Clay	1	Silt Loam	Silt Loam	0	
20	1.3	2.3	Loam	Clay Loam	1	Loam	Clay Loam	1	
21	0.1	0.1	Silt Loam	Silt Loam	0	Silt Loam	Silt Loam	0	
22	0.1	0.1	Silt Loam	Silt Loam	0	Silt Loam	Loam	1	



Figure 31. OM:Clay ratios with clay measured with no decarbonation according to OM:Clay ratio with decarbonation on 2023 samples.

(A) shows results faceted according to their CaCO3 concentration while (B) shows results faceted according to their OM concentrations. The yellow rectangle represents the interval [12%:23%] in the OM:clay ratio, where values <12% show low structural stability and values >23% show excellent structural stability. The left graph shows results of OM:clay ratios where soils have a CaCO₃ concentration lower than 40%, and the right graph shows results when soils have CaCO₃ concentration > 40%.

Table 17. Repartition of soil textures with no decarbonation between clusters.

These analyses were only performed on 2023 soil sample only. Clay, silt and sand values are given in percentage (%).

Variable	(n	1 (n=8 plots)		2 (n=12 plots)		3 (n=13 plots)		4 (n=9 plots)		CA (n=21 plots)		CONV (n=21 plots)		CA <i>vs.</i> CONV			
	mean	CV (%)	sig.	mean	CV (%)	sig.	mean	CV (%)	sig.	mean	CV (%)	sig.	mean	CV (%)	mean	CV (%)	sig
Clay_no_decarb	22.7	30	а	20.3	35	а	23.3	34	а	21.0	39	а	22.4	36	21.3	32	0.52
Silt_no_decarb	43.8	32	а	43.3	28	а	44.8	25	а	44.3	39	а	43.2	30	45.0	30	0.41
Sand_no_decarb	30.8	41	а	33.8	40	а	28.3	34	а	31.6	42	а	31.2	35	30.8	43	0.93

Annexe 5: Comparison of in-field vs. laboratory indicators

1. Comparison of Biofunctool[®] POXC protocol and certified laboratory POXC protocol

With regards to soil POXC measurements, our initial plan in year 1 was to perform them only based on the Biofunctool[®] proposed protocol. We had the opportunity to perform a double measurement by partnering with the certified laboratory Aurea Agrosciences (<u>https://aurea.eu/en/</u>). Aurea therefore proposed to measure POXC values in sampled soils, with an objective to compare results from Aurea's and Biofunctool[®] measurements. This allowed us to compare results between in-field and laboratory measured indicators. As described in **Chapter 2**, Aurea sampling was done during the "SD_Aurea" period while Biofunctool[®] POXC sampling was done slightly before, at the indicated date "SD_2" (from 3 to 28 days before Aurea depending on the plot). The main similarities and differences in soil sampling and laboratory protocols are shown in Table 17.

Aurea sampling was made at 0-20 cm depth, with samples taken all along the W transect. The soil was pooled to get a composite soil sample per plot. In the case of Biofunctool analyses, POXC concentration measurements were performed at the research centre laboratory in Toulouse. Measurements started in September 2022 but were interrupted due to values fluctuating greatly during a measurement day. Various tests were carried out afterwards (results not shown in this manuscript), to understand the source of variation, which eventually was mainly explained by differing reactions depending on the reagents' temperature (KMnO₄ solution, osmosis water). Measurements were repeated a second time in February 2023 with controlled temperatures of the reagents, and with new lab micropipettes, shifting from manual measurement pipettes to precision micropipettes. The KMnO4 solution used in February 2023 was the same as in September 2022 but appeared stable when the protocol was set up, as shown by the calibration curve made when the measurements started in February 2023 (Figure 35). No white deposit (film) was observed on the surface of the solution, indicating that the solution could be used. The mean variation between September 2022 and February 2023 measurements was also low, except for pair 15 and 18_CONV which showed a greater variability. Overall, the mean difference was 11.8 mg/kg between the two measurement dates (Figure 36).

Results of February measurements showed a 0.7 correlation value between the certified laboratory measurements, and the Biofunctool[®] measurements (Figure 37). However, POXC values using the Auréa protocol appeared similar within a pair of farmers, whereas for most pairs, POXC values were higher in CONV than in CA using the Biofunctool[®] protocol (Table 18). POXC concentrations also appeared to be generally higher with the Auréa protocol (Table 18), averaging 1 200 mg/kg dry soil (1 315 and 1 165 mg/kg soil respectively for CA and CONV) compared with an average of less than 1 000 mg/kg dry soil (848 and 985 mg/kg respectively for CA and CONV) with the Biofunctool[®] protocol. The intra-plot variability captured by measuring Biofunctool values does not seem to explain such differences.

Several possible explanations can be proposed to explain the observed differences:

- 1. <u>Different sampling dates:</u> POXC concentrations could have been higher in April (Biofunctool[®]) than in May (Aurea sampling), because of the spring 2022 drought that could have led to labile carbon being consumed in spring,
- Soils stored for 10 months before measurement (Biofunctool[®]): Could have led to a decrease in POXC concentrations in February when compared with September measurements. This result is not confirmed by our measurements.

- 3. <u>Sampling depth (0-10 cm (Biofunctool[®]) vs. 0-20 cm (Auréa))</u>: We expected an overall higher POXC concentration on the first 10 cm of soils, leading to higher average Biofunctool-POXC concentrations, which was not confirmed by our measurements.
- 4. **Different initial KMnO4 concentrations and agitation time:** Expected to lead to higher POXC concentrations in Aurea measurements, though stronger oxidation which is confirmed by our measurements.

These uncertainties added to the time spent in the laboratory trying to understand the source of the differences led us to decide to interrupt Biofunctool[®]-POXC measurements in year 2 and to continue measurements of POXC indicator with the certified laboratory. Biofunctool[®]-POXC measurements were not considered in the main analysis showed in main chapters of this manuscript.

	Biofunctool©	Certified laboratory
Sampling	-	
Time of sampling	25/04/2022 to 06/05/2022, analysis	03/05/2022 to 21/05/2022
	made in 02/2023	
Depth of sampling	0-10 cm	0-20 cm
Sampling mode	3 intraplot repetitions at positions	Sampling at 9 positions along the W
	A,B,C of the W pattern. No pooling.	pattern and pooled to obtain a
		composite sample.
Samples	Dried and sieved at 2 mm. Stored in	Dried and sieved at 2 mm
preparation	a dry a place at room temperature	
	before analysis	
Laboratory analysis		
KMnO4 solution	Prepared in September 2022, stored	Changed daily, 0.5M
	at 4°C, 0.5M	
Time of solution	10 min	1 hour
agitation		

Table 18. Main similarities and differences in POXC analysis between in-field analysis and the certified laboratory analysis.



Figure 32. Calibration curve of POXC KMnO4 solution made on 07/02/2023. The x-axis shows absorbance values at 550 nm, y-axis values show KMnO4 concentrations in mol/L.

Table 19. Descriptive statistics of certified laboratory-POXC measurements and Biofunctool-measurements. CA-CONV corresponds to the mean pair-by-pair difference. n=21 plots per cropping management system. sd: standard deviation.

	CA		COI	VV	CA-CONV		
Total POXC measurement method	Mean	sd	Mean	sd	Mean	sd	
	(n=22)		(n=22)				
Certified laboratory (Auréa)	1315	490.3	1165.1	447.8	150.1	515.2	
In-house laboratory (Biofunctool)	847.6	162.1	985.4	447.8	-137.8	189.3	



Figure 33. Differences between Biofunctool® POXC values measured in September 2022 and February 2023 on the same samples and the same KMnO4 solution on 12 soil samples.

y-axis shows the difference in POXC concentrations in mg/kg dry soil.



Figure 34. Pearson correlations between POXC-certified laboratory measured values and POXC-Biofunctool measured values.

<u>2. Comparison between Biofunctool® SituResp protocol and certified laboratory</u> protocol of C mineralisation

Spearman correlations between *minC_3d minC_7d minC_14d minC_21d* and *minC_28d* were very high, therefore, only *minC_28d* was considered for the rest of the main analysis (Figure 35). Similarly, *SituResp24* or *SituResp48* were correlated at 89%. Thus, only *SituResp24* was considered in the rest of the main analysis (Figure 35). Surprisingly, there was no correlation between *SituResp24*, *SituResp48* and *Cmin_28d*, and especially between *SituResp* after 24 hours (*SituResp24*) and carbon mineralisation after 3 days (*minC_3d*) (Figure 35, Figure 36).



Figure 35. Spearman correlation between the different soil basal respiration measurements on year 1 and year 2 samples. All variables were measured for n=86 plots. SituResp48 was measured on n=81 plots. Cells were left empty when correlations were not significant.



Figure 36. SituResp24 in function of Carbon mineralisation after 3 days (minC_3d) on year 1 and year 2 plots in CONV and CA systems.

Although the incubation methods slightly differ between the Aurea methodology and SituResp from Biofunctool, both methods were designed to measure the soil microbial activity in several timeframes (Thoumazeau *et al.*, 2017). We therefore expected to find a positive correlation between SituResp values, and the carbon mineralisation values measured by Aurea. Little documentation exists in the literature, apart from Thoumazeau's article presenting the indicator (Thoumazeau *et al.*, 2017), and few in-field applications, like in Cambodia (Koun *et al.*, 2024; Pheap *et al.*, 2019) or in Thailand (Heepngoen *et al.*, 2021). Our SituResp results are within the range of values found in tropical conditions. Mean SituResp values approximated 0.74 in East Cambodia in maize systems, and averaged 0.3 under soybean cultivation in central Cambodia (Pheap *et al.*, 2019), while they reached up to 1.20 in Thailand under rubber tree plantations (Heepngoen *et al.*, 2021). Overall, these three studies show an important variation in the SituResp values.

To our knowledge, it is the first time that SituResp[®] is compared with carbon mineralisation analyses. More research is be needed to further investigate on the links between the two indicators and interpretation of the differing results between both indicators.

<u>3. Farmers provided data vs. experimentally measured data for specific weight and yield</u>

We noted a very small spearman correlation of 0.30 between SW provided by farmers (*FarmerSW_n*, n=62 plots with available data) and the experimentally measured SW (*ExpSW_n*, n=74 plots analysed) (Figure 37). We noted a 0.86 correlation between the yield provided by farmers (*farmerYield_n*) and the experimentally measured yield (*ExpYield_n*) (Figure 37).

We observed good correlations between values of indicators provided by farmers and those measured experimentally, especially for Yield, despite different measurement methods. This is an interesting result also showing that farmers' information could be trusted. This is likely to be the result of a healthy relationship and mutual trust between researchers and each of the farmers.



Figure 37. Spearman correlations between farmers provided and laboratory measurements of yield and specific weights. Cells were left empty when correlations were not significant.

LISTE MULTIRESIDUS GC-MS/MS 250 PESTICIDES Organo chlorés – Pyréthrinoides – Organo phosphorés – Organo azotés						
2-Phenylphenol* ^{(1)(m)}	Cypermethrine $(\alpha+\beta+\theta+7)^{*(2)(3)}$	Fenchlorphos* ^{(1)(m)}	Malathion(+Malaoxon)	Prothoate		
3,4-Dichloroaniline	Cyproconazole ^{*(1)}	Fenhexamide* ⁽¹⁾	Mepanipyrim* ⁽¹⁾	Pyrazophos		
4,4-Dichlorobenzophenone	Cvprodinil ^{*(1)}	Fenitrothion* ^{(2) (3)}	Mepronil ^{*(1)}	Pvridaben* ⁽⁴⁾		
Acétochlore* ⁽⁴⁾	p.p'-DDT* ⁽¹⁾⁽²⁾⁽³⁾	Fenobucarbe	Metalaxyl dont Metalaxyl-M* ⁽²⁾	Pyridalyl		
Acibenzolar-S-methyl ^(m)	o,p'-DDT	Fenpropathrine* ⁽⁴⁾	Metazachlor	Pyridaphenthion* ⁽²⁾		
Aclonifen* ⁽²⁾	p,p'-DDE* ⁽¹⁾⁽²⁾⁽³⁾	Fenpropimorphe	Methacrifos	Pyrifenox		
Acrinathrine	p,p'-TDE (DDD)	Fenvalerate(Σisomères)* ⁽⁴⁾	Methidathion ^{*(2)}	Pyrimethanil* ⁽¹⁾		
Alachlore* ⁽³⁾⁽⁴⁾	DDT(Σ des isomères)	Fipronil	Methoxychlore	Pyriproxyfen* ⁽¹⁾		
Amisulbrom	Deltamethrine ^{* (2) (3)}	Fipronil sulfone	Metolachlore dont S- Metolachlore * ⁽³⁾⁽⁴⁾	Quinalphos* ⁽³⁾		
Ametryn	Demeton-S-methyl	Fipronil(+sulfone)	Mirex* ⁽¹⁾	Quinomethionate		
Atrazine	Dialifos	Fipronil desulfinil	Myclobutanil* ^{(1) (2)}	Quinoxyfen		
Benalaxyl dont Benalaxyl-M* ⁽¹⁾	Dichlobenil* ⁽³⁾	Fluazifop p butyl ^(m)	Nitrofène	Quintozene		
Bendiocarb	Dichlofenthion* ⁽¹⁾	Fluchloraline	Nitrothal isopropyle	Pentachloroaniline (PCA)		
Benfluraline* ⁽⁴⁾	Dichlofluanide	Flucythrinate	Oxadiazon* ^{(1) (3)}	Quintozene(+PCA)		
Benoxacor	Dichlorvos	Fludioxonil* ⁽¹⁾⁽²⁾	Oxadixyl* ⁽¹⁾	Quizalofop-ethyl		
Bifenox	Diclofop-methyl* ^{(1) (m)}	Flufenacet ^(m)	Oxyfluorfene* (2) (3)	S421		
Bifenthrine (Σ des isomères)* ⁽¹⁾⁽²⁾	Dicofol(Σ des isomères)	Fluopicolide* ⁽⁴⁾	Parathion-ethyl* ⁽²⁾	Sebuthylazine		
Biphenyl	Dicrotophos	Flurochloridone	Parathion-methyl* (1) (2) (3) (m)	Sectburneton		
Bitertanol(Σ des isomères)* ⁽¹⁾	Dieldrin* ⁽²⁾⁽³⁾	Fluroxypyr-methylheptyl	PCB 028* ⁽¹⁾	Sulfotep		
Bromocyclen	Aldrin	Flusilazole* ⁽¹⁾	PCB 052* ⁽¹⁾	Sulprofos		
Bromophos-ethyl	Dieldrin(+Aldrin)	Flutolanil	PCB 101* ⁽¹⁾	Tebuconazole* ⁽¹⁾⁽²⁾		
Bromophos-methyl	Diethofencarb	Flutriafol	PCB 118* ⁽¹⁾	Tebufenpyrad* ^{(1) (3)}		
Bromopropylate ^{*(1)(2)(3)}	Difenoconazole* (1) (3)	Fluvalinate(Tau)* ⁽²⁾⁽³⁾	PCB 138* ⁽¹⁾	Tebupirimphos		
Butachlor	Diflufenican* ⁽²⁾⁽⁴⁾	Folpet	PCB 153* ⁽¹⁾	Tecnazene		
Butraline	Dimetachlor	Phtalimide	PCB 180* ⁽¹⁾	Tefluthrine* ⁽²⁾⁽⁴⁾		
Captafol	Dinitramine	Folpet(+Phtalimide)	Penconazole(∑ des isomères)* ⁽¹⁾	Terbacil		
Captan	Diphenylamine* ⁽¹⁾	Fonofos* ⁽¹⁾	Pendimethaline	Terbufos* ⁽⁴⁾		
Tetrahydrophtalimide (THPI)	Disulfoton ^(m)	Formothion	Pentachloroanisole* ⁽¹⁾	Terbuthylazine* ⁽⁴⁾		
Captan(+THPI)	Ditalimphos	Furalaxyl	Permethrine(cis+trans)* ⁽³⁾⁽⁴⁾	Terbutryne		
Carbaryl	Edifenphos	Haloxyfop-2-ethoxyethyl ^(m)	Perthane* ⁽¹⁾	Tetrachlorvinphos		
Carbophenothion	Endosulfan $\alpha^{*(2)(3)}$	Haloxyfop methyl(R+S) ^(m)	Phenothrine	Tetradifon* ⁽³⁾		
Carfentrazone-ethyl* ^{(1)(m)}	Endosulfan B* ⁽²⁾⁽³⁾	HCB* ⁽¹⁾⁽²⁾	Phenthoate	Tetramethrine* ⁽²⁾		
Chlorbenside	Endosulfan sulfate* ⁽²⁾	HCH α ^{*(1)(3)}	Phosalone * ⁽¹⁾⁽²⁾	Tetrasul		
Chlordane (cis+trans)* (3)	Endosulfan(α + β +sulfate)* ⁽²⁾	HCH β* ⁽¹⁾⁽³⁾	Piperonyl butoxide*(4)	Tolclofos-methyl*(1)		
Chlorfenapyr	Endrin* ⁽³⁾	HCH gamma (lindane)	Pirimicarb* ⁽¹⁾	Tolylfluanid ^(m)		
Chlorfenson	Endrin Ketone	Heptachlore	Pirimiphos-ethyl	Tralomethrine		
Chlorfenvinphos*(1)(2)	EPN	Heptachlore epoxyde cis	Pirimiphos-methyl ^{*(1)(2)(3)}	Transfluthrine		
Chlorobenzilate ^{*(1) (3)}	Ethalfluraline	Heptachlore epoxyde trans	Plifenate	Triadimefon* ⁽¹⁾		
Chlorothalonil	Ethiofencarb	Heptachlore(+epoxyde)	Pretilachlore	Triadimenol* ⁽¹⁾		
Chlorpropham ^{*(1)(2)}	Ethion* ⁽³⁾	Heptenophos	Procymidone ^{*(1)(2) (3)}	Triallate ^{*(4)}		
Chlorpyrifos* (1) (2) (3)	Ethofumesate* ^{(m)(4)}	Hexazinone* ⁽⁴⁾	Profenophos* (3)	Triamiphos		
Chlorpyrifos-methyl*(1)(2) (3)	Ethoprophos* ⁽¹⁾	Iodofenphos	Prometryn	Triazophos		
Chlorthal dimethyl* ⁽¹⁾	Ethoxyquin	Iprodione	Propachlore ^(m)	Trichloronat		
Chlorthiophos	Etofenprox * ⁽⁴⁾	Isobenzan	Propazine	Trifluraline* ⁽³⁾		
Chlozolinate	Etridiazole	Isodrine	Propetamphos	Valifenalate		
Clomazone ^{*(4)}	Etrimphos	Isofenphos-ethyl	Prophame	Vinclozoline ^{*(1)(2)(3)}		
Coumaphos ^{*(2)}	Famoxadone	Isofenphos-methyl* ⁽¹⁾	Propiconazole ^{*(1)(2)}	Zoxamide ^{*(4)}		
Cyfluthrine(β+y)* ⁽²⁾	Famphur	Isoxadifen ethyl	Propyzamide ^{*(1)(2)}			
Cyhalofop-butyl	Fenamiphos ^(m)	Leptophos	Proquinazid ^{*(1)}			
Lambda-Cyhalothrine (λ + γ + Σ isomères)* ^{(1) (2)}	Fenarimol ^{*(1)}	Malathion* ⁽¹⁾⁽²⁾⁽³⁾	Prosulfocarbe* ⁽²⁾			
Seules (1) MOC3/25 : 1 (2) MOC3/26 : Déte (3) MOC3/76 : Dét (4) MOC3/55 : 1 La limit	certaines prestations sont couvertes pa Détermination de la teneur en résidus de prmination de la teneur en résidus de p Détermination de la teneur en résidus de Les autres pesticides sont analys e de quantification est de 0,01 mg/kg sa (m): dosé(s sans son(ses))	ar l'accréditation. Elles sont identifié de pesticides dans les produits non n esticides dans les produits gras d'or pesticides dans les produits de la ru de pesticides dans les produits non n sés par les méthodes MOC3/05 ou MC our pour le Fipronil(+sulfone) et le pi analyte(s) associés dans le règlemen	es par le symbole * suivi de : gras d'origine végétale par GC-MS(n); igine végétale et animale par GC-MS(n). che y compris les abeilles par GC-MS(n). gras d'origine végétale par GC-MS(n). DC3/06 Version 0. péronyh butxide : 0,005mg/kg. tt 396/2005.	1		

Annexe 6. List of analysed molecules in the "Agribiodiv" pilot

Figure 38. List of traced active compounds based on gaseous chromatography.

Traced compounds are organochlorine, pyrethroids, organophosphates and organo-nitrogen compounds. Document provided by the Phytocontrol[®] laboratory (<u>https://www.phytocontrol.com/en/</u>), molecules' names written in French.

LISTE MULTIRESIDUS LC-MS/MS 400 PESTICIDES						
Triazo	les – Triazines – Urées – Benzimida	zoles – Carbamates – Strok	pilurine – Auxiniques – Divers			
2,4-D(acide libre) ^(m)	Cyantraniliprole ^{*(1)}	Flonicamide(+TFNA+TFNG)	Metamitron* ⁽¹⁾	Pyraclofos ^{*(1)}		
3,4,5-trimethacarb	Cyazofamide* ⁽¹⁾	Florasulam ^{*(1)}	Metazachlor ESA (479M08)	Pyraclostrobine ^{*(1)(2)(3)}		
6-Benzyladenine* ⁽¹⁾ Avermectine B1a	Cybutryne	Florpyrauxifen-benzyl	Metazachlor OXA (479M04) Metazachlor 479M16	Pyraflufen-ethyl ^{*(1)(m)} Pyrethrine I		
Avermectine B1b	Cycluron* ⁽¹⁾	Fluazinam* ⁽¹⁾	Metazachlor (somme)	Pyrethrine II		
8,9-Z-AvermectinB1a	Cyflufenamid* ⁽¹⁾⁽²⁾	Fluazuron	Metconazole(∑ des isomères)* ⁽¹⁾⁽²⁾	Cinerine I		
Acephate* ⁽¹⁾	Cyprosulfamide ^{*(1)}	Flufenacet ESA	Methabenzthiazuron* ⁽¹⁾	Jasmoline I		
Acequinocyl	Cyromazine	Flufenacet FOE 5043	Methiocarb	Jasmoline II		
Acetamipride *(1,2,15)	Daminozide ^(III)	Flufenacet OA	Methiocarbe-sulfone Methiocarbe-sulfoxide	Pyréthrines (Somme)		
Aldicarb-sulfone	Demeton-S* ⁽¹⁾	Flufenoxuron* ⁽¹⁾	Methiocarbe-sulfone+sulfoxide)	Pyridate		
Aldicarb-sulfoxide	Demeton-S-methylsulfone* ⁽¹⁾	Flufenzine	Methomyl* ⁽¹⁾	Pyridate(+pyridafol) ^(m)		
Aldicarb(+sulfone+sulfoxide)	Oxydemethon-methyl* ⁽¹⁾	Fluindapyr	Méthoprotryne	Pyrimidifen* ⁽¹⁾		
Ametoctradine* ⁽¹⁾⁽²⁾	Oxydemeton-methyl(+Demeton-S-methyl sulfone)* ⁽¹⁾	Flumetralin	Methoxyfenozide* ⁽¹⁾⁽²⁾	Pyriofenone* ⁽¹⁾		
Amidosulfuron* ⁽¹⁾	Denatonium benzoate (=bitrex)	Fluometuron* ⁽¹⁾	Metobromuron* ^{(1)(m)}	Pyroquilon* ⁽¹⁾		
Amitraze	Desmediphame	Fluopyram* ⁽¹⁾	Metolcarb* ⁽¹⁾	Pyroxsulam* ⁽¹⁾		
N-(2,4-Dimethylphenyl)formamide	Diafenthiuron	Flupyradifurone ^{*(1)}	Metosuam ¹	Quinoclamine		
N-2,4-Dimethylphenyl-Np-methylformamidine HCl	Diallate	Flupyrsulfuron methyl* ⁽¹⁾	Metrafenone* ⁽¹⁾⁽³⁾	Quizalofop(somme) ^(m)		
Amitraze(+Amitraze metabolites) Amitrole	Diazinon Dichlororon(acide libre) ^(m)	Fluquinconazole* (*) Fluridone	Metribuzine Metsulfuron-methyl* ⁽¹⁾	Quizalotop dont quizalotop-P Propaguizafon* ⁽¹⁾⁽²⁾		
Asulam	Dichlorophen	Fluroxypyr(acide libre) ^(m)	Mevinphos* ⁽¹⁾	Quizalofop-p-tefuryl		
Atrazine-deisopropyl	Diclobutrazol	Flurprimidol	Milbernectin A3	Resmethrine		
Azaconazole* ⁽¹⁾	Difenacoum	Flutianil	Milbernectin A3 + A4	Rotenone* ⁽¹⁾		
Azadirachtin A	Difenamide* ⁽¹⁾⁽²⁾	Fluxapyroxad* ⁽¹⁾	MNBA	Sedaxane* ⁽¹⁾		
Azadirachtin B	Difethialone	Fomesafen	Molinate	Siduron		
Azamethinhos	Dimeturon	Forchlorfenuron* ⁽¹⁾	Monocrotophos* ⁽¹⁾	Simazine* ⁽¹⁾		
Azimsulfuron* ⁽¹⁾	Dimepiperate	Formetanate(hydrochlorure de)	Monolinuron* ⁽¹⁾	Simetryn		
Azinphos-ethyl* ⁽¹⁾	Dimethenamid(Σ des isomères)* ⁽¹⁾	Fosthiazate ^{*(1)}	Monuron* ⁽¹⁾	Spinetoram XDE-175-J* ⁽¹⁾		
Azinphos-methyl*(1) Azoxystrohine*(1)(2)	Dimethoate* ⁽¹⁾⁽³⁾	Fuberidazole* ⁽¹⁾	NAD(1-naphthyl acetamide)* ^{(1)(m)} Naled	Spinetoram XDE-175-L* ⁽¹⁾		
Beflubutamide* ⁽¹⁾	Dimetionolphe(2 des isomeres)	Furmecyclox	Napropamide* ⁽¹⁾	Spinosyne A* ⁽¹⁾		
Benodanil	Dimoxystrobine	Halauxifen-methyl*(1)(m)	Neburon* ⁽¹⁾	Spinosyne D* ⁽¹⁾		
Bensulfuron-methyl***/ Bensulide	Diniconazole(z isomeres) Dinocap(Σ isomères) ^(m)	Halfenprox ^{*(*)} Halosulfuron-methyl ^{*(1)}	Nicosulfuron*19	Spinosad(A+D)* ⁽¹⁾ Spirodiclofen* ⁽¹⁾⁽²⁾		
Bentazone	Meptyldinocap-phenol (2,4-DNOP) ^(m)	Haloxyfop(acide libre) ^(m)	Nitralin	Spiromesifen* ⁽¹⁾		
Bentazone 6-OH	Dinoseb* ^{(1)(m)}	Hexaconazole	Norflurazon* ⁽¹⁾	Spirotetramat(somme)* ⁽¹⁾		
Bentazone(+6-OH+8-OH) ^(m)	Dinoterb* ⁽¹⁾	Hexythiazox* ⁽¹⁾	Nuarimol	Spirotetramat-enol* ⁽¹⁾		
Benthiavalicarb-isopropyl*(1)(2)	Dipropetryn	Hydramethylnon* ⁽¹⁾	Ofurace ^{*(1)}	Spiroxamine* ⁽¹⁾		
Benzobicyclon Benzovindifluovr	Disulfoton-sulfone*(*) Disulfoton-sulfoxide*(1)	Imazalil* ⁽¹⁾ Imazamethabenz (acide libre)	Omethoate ^{*(1)} Orthosulfamuron ^{*(1)}	Sulcotrione Sulfosulfuron* ⁽¹⁾		
Bifenazate	Disulfoton-sulfone(+sulfoxide) ^(m)	Imazamethabenz methyl	Oryzalin	Sulfoxaflor		
Bifenazate diazene	Dithianon	Imazamox* ⁽¹⁾	Oxamyl* ⁽¹⁾	TCMTB* ⁽¹⁾		
Bispyribac-sodium ^(m)	Diuron* ⁽¹⁾	Imazaquin	Oxasulturon	Tebutam ^{*(1)}		
Bixafen* ⁽¹⁾	DMST* ^{(1)(m)}	Imazosulfuron ^{*(1)}	Oxfendazole	Tebuthiuron* ⁽¹⁾		
Boscalid* ⁽¹⁾⁽²⁾	DNOC Dodemorphe* ⁽¹⁾	Imibenconazole Imidaclopride* ⁽¹⁾⁽²⁾⁽³⁾	Oxycarboxine Paclobutratol/S des isomères (* ⁽¹⁾⁽³⁾	Teflubenzuron* ⁽¹⁾		
Bromfenvinphos-ethyl	Dodine* ⁽¹⁾	Indaziflam	Paraoxon-ethyl* ^{(1)(m)}	Temephos		
Bromoxynil	Emamectine B1a* ⁽¹⁾⁽²⁾	Indoxacarb(∑énantiomères)* ⁽¹⁾	Pebulate	Tepraloxydim* ^{(1)(m)}		
Bromuconazole* (*) Bunirimate* ⁽¹⁾	Emamectine benzoate B1b*** Epoxiconazole* ⁽¹⁾	Inpyrituxam Iodosulfuron-methyl* ⁽¹⁾	Pencycuron* ⁽¹⁾	Terbumeton desethyl* ⁽¹⁾		
Buprofezin* ⁽¹⁾	EPTC	loxynil* ⁽¹⁾	Penoxsulame* ⁽¹⁾	Tetraconazole*(1)(2)		
Butamifos Butavucarbovim	Ethametsulfuron-methyl*(1)(3)	Ipconazole Iprobenfos	Penthiopyrad* ⁽¹⁾ Pethoxamide	Thiabendazole ^{*(1)} Thiaclopride ^{*(1)(3)}		
Butoxycarboxim sulfoxide	Ethiofencarb-sulfone	Iprovalicarbe* ⁽¹⁾⁽²⁾	Phenmediphame* ⁽¹⁾	Thiadone		
Buturon* ⁽¹⁾	Ethiofencarb-sulfoxide	Isazofos* ⁽¹⁾	Phorate	Thiamethoxam* ⁽¹⁾		
Butylate Cadusafos* ⁽¹⁾	Ethiprole*10	Isocarbophos*(*) Isofetamid	Phorate-oxon* ^{1*7} Phorate-oxon-sulfone	Thiencarbazone-methyl*(1) Thifensulfuron-methyl*(1)		
Carbendazim(+Benomyl)* ⁽¹⁾	Ethoxysulfuron	Isoprocarb* ⁽¹⁾	Phorate sulfone ^{*(1)}	Thiobencarb* ^{(1)(m)}		
Carbetamide (∑carbetamide et isomere)* ⁽¹⁾	Etoxazole* ⁽¹⁾	Isopropaline	Phorate(+Oxon+Sulfone)	Thiocyclam		
Carbofuran Carbofuran-3-hydroxy	Fenamidone*(*/*) Fenaminhos-sulfone*(1)	Isoprothiolane*(*)	Phosmet Phosmamidon* ⁽¹⁾	Thiodicarb***/ Thiometon		
Carbofuran(somme)	Fenamiphos-sulfoxide* ⁽¹⁾	Isopyrazam* ⁽¹⁾	Phoxim ^{*(1)}	Thionazin* ⁽¹⁾		
Carboxine(somme)	Fenamiphos-sulfone(+sulfoxide)*(1)(m)	Isouron	Picaridin	Thiophanate-methyl* ⁽¹⁾		
Carboxine-sulfoxide	Fenchlorphos-oxon* ^{(1)(m)}	Isoxaflutole ^{*(1)}	Picoxystrobine* ⁽¹⁾	Tolpyralate		
Oxycarboxine	Fenoxaprop-ethyl* ⁽¹⁾	RPA 202248	Pinoxadene* ⁽¹⁾	Tralkoxydim(∑ isomères)		
Chlorantraniliprole*(*) Chlorbromuron	Fenoxycarbe***/ Fenpicionil	Isoxatlutole(+RPA 202248) Isoxathion* ⁽¹⁾⁽²⁾⁽³⁾	Prallethrin	Triasulturon Triazamate		
Chlorfluazuron	Fenpicoxamide	Karanjin	Primisulfuron-méthyl	Tribenuron-methyl		
Chloridazon* ⁽¹⁾	Fenpropidine* ⁽¹⁾	Kresoxim-methyl* ⁽¹⁾	Prochloraz	Trichlorfon		
Chloridazon desphenyl Chloridazon (+desphenyl)	Fenpyrazamine*17 Fenpyroximate* ⁽¹⁾⁽²⁾	Lenacil***/	BTS 44595 BTS 44596	Triciopyr Tricyclazole* ⁽¹⁾		
Chlorpyrifos methyl desmethyl ^(m)	Fensulfothion* ⁽¹⁾	Lufenurone* ⁽¹⁾	Prochloraz(somme)	Tridemorphe		
Chlorotoluron* ⁽¹⁾	Fensulfothion-oxon=sulfone=s ⁽¹⁾	Mandestrobine	Promecarb* ⁽¹⁾	Trifloxystrobine*(1)(2) Triflumuron*(1)(2)		
Chlorsulfuron* ⁽¹⁾	Fensulfothion-sulfone* ⁽¹⁾	Matrine	Propamocarbe* ⁽¹⁾	Triflusulfuron (IN-M7222)		
Chromafenozide* ⁽¹⁾	Fenthion* ⁽¹⁾	Oxymatrine	Propanil	Triflusulfuron-methyl* ⁽¹⁾		
Cinidon-ethyl*'' Cinmethylin	Fenthion-sulfone*() Fenthion-sulfoxide*()	MCPA(acide libre)*** MCPB(acide libre)	Propaphos* ^(*) Propargite	Trinexapac-ethyl		
Cinosulfuron* ⁽¹⁾	Fenthion-oxon	MCPA(somme) ^(m)	Propoxur* ⁽¹⁾	Triticonazole*(1)		
Clethodim	Fenthion-oxon-sulfone Fenthion-oxon-sulfovide	Mecarbam* ⁽¹⁾ Mefenacet	Propoxycarbazone	Tritosulfuron* ⁽¹⁾		
Sethoxydim	Fenthion(+metabolites)	Mefentrifluconazole	Propoxycarbazone(+2-OH)	Vamidothion* ⁽¹⁾		
Clethodim(+Sulfoxide)+Sethoxydim ^(m)	Fenuron* ⁽¹⁾	Mephosfolan	Prosulfuron	Warfarin* ⁽¹⁾		
Clodinafop propargyl Clofentezine* ⁽¹⁾	Flazasulfuron Flonicamide	Mesosulfuron-methyl*(1) Mesotrione	Prothioconazole desthio*(1) Pydiflumetofen			
Clothianidine ^{*(1)}	TFNA	Metaflumizone* ⁽¹⁾	Pymetrozine			
Cyanazine* ⁽¹⁾	TFNG	Metaldehyde	Pyracarbolid			
Seules certaines prestations sont couvertes par l'accréditation. Elles sont identifiées par le symbole * et concernent les matrices citées dans la MOC ci-dessous : (1) MOC3/407: Détermination de la teneur en pesticides par LC-MS-MS dans les produits non gras d'origine végétale : méthode interne (2) MOC3/417: Détermination de la teneur en pesticides par LC-MS/MS dans les plantes aromatiques et médicinales, méthode interne (3) MOC3/417: Détermination de la teneur en pesticides par LC-MS/MS dans les épices : méthode interne (3) MOC3/417: Détermination de la teneur en pesticides par LC-MS/MS dans les épices : méthode interne La limite de quantification est de 0,01 mg/kg sur fuits, légumes, céréales et boissons. L'emanectine BLa : 0,002 mg/kg sur fuits, légumes, céréales et boissons.						

Le fluometuron, le propoxur et le phosmet : 0,005 mg/kg sur fruits, légumes, cérèales et boissons. L'avernectine B1a, l'avernectine B1 bet le 8,9-2-AvernectinB1B : 0,006 mg/kg sur fruits, légumes, céréales et boissons. (m) : dosé(s) sans son(ses) analyte(s) associés dans le règlement 396/2005.

Figure 39. List of traced active compounds based on liquid chromatography.

Traced compounds are organochlorine, pyrethroids, organophosphates and organo-nitrogen compounds. Document provided by the Phytocontrol[®] laboratory (<u>https://www.phytocontrol.com/en/</u>), molecules' names written in French

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Titre : Déterminants agroécologiques liant pratiques de gestion des sols, santé des sols, santé des plantes et qualités nutritionnelle et sanitaire des produits : le cas du blé tendre d'hiver pour illustrer l'approche "One Health"

Mots clés : Agriculture de conservation, Agroécologie, Gestion durable des sols, Recherche à la ferme, Blé tendre d'hiver

Résumé : L'ampleur de la transition agroécologique à effectuer transcrit la nécessité de tous les acteurs, du champ, à l'assiette, à s'associer et travailler ensemble. Le concept d'« Une seule santé » (« One Health »), se base sur le principe qu'il existe une connexion entre la santé des humains, des animaux, des plantes et de l'environnement. Ce travail de thèse visait à démontrer que cette théorie appliquée à la production de blé mais en incluant les pratiques culturales qui en sont au point de départ, n'est pas qu'un concept théorique, mais que ces liens entre les pratiques, la santé des sols, celle des plantes et les qualités nutritionnelle et sanitaire des produits récoltés, sont bien vérifiables sur la base de données de terrain.

Pour ce faire, nous avons mis en place en 2021 un réseau d'étude à la ferme, composé de 86 parcelles agricoles cultivées en blé tendre d'hiver. La moitié de ces parcelles étaient cultivées selon les principes de l'Agriculture de Conservation des Sols (ACS), et l'autre moitié de manière « conventionnelle » (CONV). Les parcelles d'un agriculteur en ACS et d'un agriculteur en CONV ont été sélectionnées de manière à ce qu'elles puissent se comparer en binôme.

En complément d'un diagnostic agroécologique (à l'aide de l'Indice de Régénération), nous avons procédé à une analyse fine et systémique des pratiques culturales appliquées à chaque parcelle étudiée, en prenant en compte les cinq campagnes précédant celle du blé étudié, ainsi que cette dernière. Un panel d'indicateurs de santé du sol, de santé de la plante, de qualité nutritionnelle et sanitaire de la récolte et de performances socio-économiques et environnementaux, a ensuite été testé sur chaque parcelle. Des investigations supplémentaires ont aussi été menées pour étudier des aspects spécifiques de composition des communautés microbiennes sur le continuum sol-plante-grain, en collaboration avec le Muséum d'Histoire Naturelle de Paris. Afin de clore la boucle de la « fourche à la fourchette », nos recherches sont allées jusqu'à la fabrication de pain, afin de potentiellement mettre en évidence les effets de ces pratiques agricoles contrastées sur le produit de consommation final.

Notre étude a permis de mettre en évidence des niveaux de performance agroécologique plus ou moins élevés au sein même des groupes ACS et CONV, menant à des effets variables sur la santé des sols. De manière générale, nous démontrons pour ce réseau les effets positifs des systèmes ACS comparés aux systèmes CONV sur les principales fonctions qui contribuent à la santé des sols. Les effets sur la qualité du grain ont été plus délicats à mettre en évidence, du fait d'un important effet de l'année d'étude. Toutefois, nous avons pu observer les effets positifs de l'ACS sur les qualités nutritionnelles du grain (concentration plus élevée en P, K et ergothionéine). Enfin, nous montrons, dans nos conditions d'étude, que l'ACS se pratique sans perte de rendement tout en diminuant la consommation de gasoil et la charge de travail pour l'agriculteur. Nous montrons aussi qu'il est possible chez les ACS les plus performants, de limiter la dépendance aux engrais minéraux et aux pesticides de synthèse par rapport à leurs homologues CONV, ce qui est encourageant pour le développement de cette pratique d'un point de vue environnemental et de santé publique. Tous ces résultats suggèrent des effets positifs de la pratique de l'ACS sur l'environnement et sur la santé des consommateurs (humains ou animaux).

Cette étude est le résultat d'un travail collaboratif à plusieurs échelles : avec les agriculteurs tout d'abord, qui ont pris part à la création du réseau d'étude, puis avec les multiples partenaires techniques, scientifiques et industriels, qui ont permis le déroulement de cette étude tout en horizontalité. Ce travail a finalement permis d'investiguer des aspects méthodologiques et analytiques de la santé des sols, des plantes et de la qualité nutritionnelle du blé en conditions réelles.

Title: Agroecological drivers connecting soil management practices, soil health, plant health and nutritional and sanitary qualities of grain yield: the case of winter wheat to illustrate the "One Health" approach

Key words: Conservation agriculture, Agroecology, Sustainable soil management, On-farm research, Winter wheat

Abstract: The magnitude of the task reflects the need for all stakeholders, from farm to fork, to join forces and work together. The concept of "One Health", is based on the principle that there is a connection between the health of humans, animals, plants and the environment. This thesis aimed at demonstrating that this theory applied to wheat production but including the cultural practices that are at the starting point, is not just a theoretical concept. Indeed, these links between practices, soil health, plant health and the nutritional and health qualities of the harvested products, are verifiable on the basis of field data.

To do this, we set up in 2021 an on-farm study network (OFE in the rest of the document: On-Farm Experiment), located mainly in the northwestern quarter of France and composed of 86 agricultural plots cultivated with winter wheat. Half of these plots were cultivated according to the principles of Conservation Agriculture (CA), and the other half in a "conventional" manner (CONV). The plots of a farmer in CA and a farmer in CONV were selected so that they could be compared in pairs.

After setting up this network of plots, we monitored each of them over a production campaign. In addition to an agroecological diagnosis (using the Regeneration Index), we carried out a detailed and systemic analysis of the cultivation practices applied to each plot studied, taking into account the five campaigns preceding that of the wheat studied, as well as the latter. A panel of indicators of soil health, plant health, nutritional and sanitary quality of the crop and socio-economic and environmental performances were then tested on each plot. Additional investigations were also conducted to study specific aspects of microbial community composition on the soil-plant-grain continuum, in collaboration with the Natural History Museum of Paris. In order to close the loop from "farm to fork", our research went as far as bread making, in order to potentially highlight the effects of these contrasting agricultural practices on the final consumer product.

Our study highlighted more or less high levels of agroecological performance within the CA and CONV groups, leading to variable effects on soil health. In general, we demonstrate for this network the positive effects of CA systems compared to CONV systems on the main functions that contribute to soil health, i.e. nutrient cycling, carbon transformation, structural stability of aggregates and biological regulation. The effects on grain quality were more difficult to highlight, due to a significant effect of the year of study. However, we were able to observe the positive effects of CA on the nutritional qualities of the grain (higher concentration of P, K and ergothioneine). Finally, we show, under our study conditions, that the practice of CA is carried out without loss of yield while reducing diesel consumption and the workload for the farmer. We also show that it is possible for the most efficient CA systems to limit dependence on mineral fertilizers and synthetic pesticides compared to their CONV counterparts, which is encouraging for the development of this practice from an environmental and public health point of view. All these results suggest positive effects of the practice of CA on the environment and on the health of consumers (humans or animals).

This study is the result of collaborative work on several scales: first with farmers, who took part in the creation of the study network, then with the multiple technical, scientific and industrial partners, who allowed this study to be carried out horizontally. This work ultimately made it possible to investigate methodological and analytical aspects of soil health, plants and the nutritional quality of wheat in real conditions.