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# Study and prediction of the impact of anaerobic digestion process parameters on the composition of digestates, and their effect on the structural stability of soils

Joshua Cooke

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COLLEGE ECOLOGIE

DOCTORAL GEOSCIENCES

BRETAGNE AGRONOMIE ALIMENTATION



# THESE DE DOCTORAT DE

Présentée par

**Joshua COOKE**

## ÉTUDE ET PREDICTION DE L'IMPACT DES PARAMETRES DE DIGESTION ANAEROBIE SUR LA COMPOSITION DES DIGESTATS ET LEUR EFFET SUR LA STABILITE STRUCTURALE DES SOLS

Thèse présentée et soutenue à Rennes, le 21/06/2023  
Unité de recherche : INRAE UR OPAALE / UMR SAS

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“Though the problems of the world are increasingly complex, the solutions remain embarrassingly simple.”

— Bill Mollison

“The master’s eye is the best fertiliser.”

– Pliny the Elder

“A good farmer is nothing more nor less than a handy man with a sense of humus.”

– E.B. White

## Foreword

The following PhD thesis manuscript is the result of work started on 1st November 2019 as a collaborative project between the IRSTEA “Optimisation des procédés en agro-alimentaire, agriculture et environnement” research unit (UR OPAALE) and the INRA-Agrocampus Ouest “Sol Agro-hydrosystem et Spatialisation” mixed research unit (UMR SAS). IRSTEA and INRA would merge in 2019 to become the French National Institute for Research in Food, Agriculture and Environment – INRAE, bringing the two research units under the same administration.

This PhD was co-directed by Safya Menasseri-Aubry (UMR SAS) and Anne Trémier (UR OPAALE), and supervised by Romain Girault (UR OPAALE). The project was co-financed by the Brittany Region (Region Bretagne) and INRAE. The members of the PhD coordination committee (CSI), chaired by Samuel Abiven (ENS Paris), were Julie Jimenez (INRAE LBE), Anne Jaffrezic (UMR SAS), and Laurent Thuriès (CIRAD).

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## Context of the thesis

## The consequences of mobilising anaerobic digestion in the transition to sustainable energy

Successive reports from the International Panel on Climate Change (IPCC, 2022) have stressed the urgent need for global action to curb atmospheric carbon emissions in order to mitigate climate change. At the same time, global energy consumption continues to rise, and the world stands on the brink of an energy crisis (IEA, 2022). As part of the strategy to tackle these issues, many nations have put forward targets to reduce consumption of fossil fuels and move towards a mix of renewable energy technologies. One such technology is anaerobic digestion (AD), the production of a mixture of methane and carbon dioxide, known as “biogas”, through the biological fermentation of organic matter. The technology of AD is interesting in that it can be used to partially reduce and sanitise waste biomass streams that would otherwise constitute pollutants, while also producing a combustible energy source that does not contribute towards net greenhouse gas emissions. Recent research has shown that widespread adoption of bio-methane production from AD of agricultural residues could lead to a 60%-85% reduction of greenhouse gas emissions from agricultural systems compared to a “business-as-usual” scenario without AD and using natural gas (Esnouf et al., 2021).



*Fig. 0.1 Small farm anaerobic digestion plant, Munchberg (Auvinet N., personal communication)*

Driven by the demand for greater valorisation of biomass resources for the production of energy, the AD sector has been growing rapidly. The number of AD plants in Europe increased by 18 % between 2013 and 2018, and many more projects are underway (European Biogas Association, 2018). As of 2022, over 1400 AD plants were in operation in France, with 47% based on farms (an image of a typical small-scale farm AD plant is given in Fig. 0.1), and almost 90% of the feed materials to the whole AD sector originating from agriculture (Chambres d’Agriculture France, 2022).

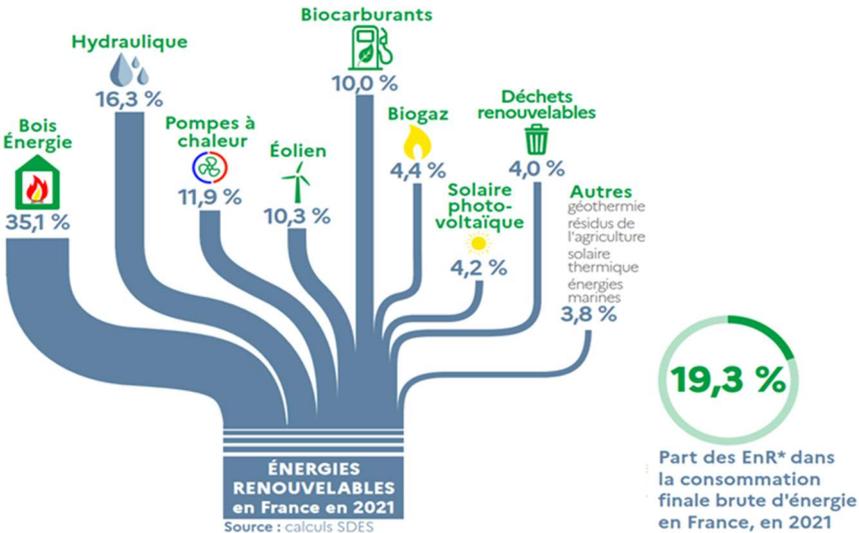


Fig. 0.2 The contribution of different sources towards renewable energy production in France in 2021 (SDES, 2022)

Though the current energy production from AD in France represents only a very small percentage (4.4%) of the renewable energy mix (see Fig. 0.2), equivalent to around 16 TWh, this production has seen a 20% increase over the previous year alone (SDES, 2022). Nonetheless, projections for a fully renewable gas supply by 2050 estimate that upwards of 130 TWh of production will need to come from anaerobic digestion in order to meet future demands (see Fig. 0.3), even when accompanied by an equally strong push towards better energy economisation (Association NegaWatt, 2022).

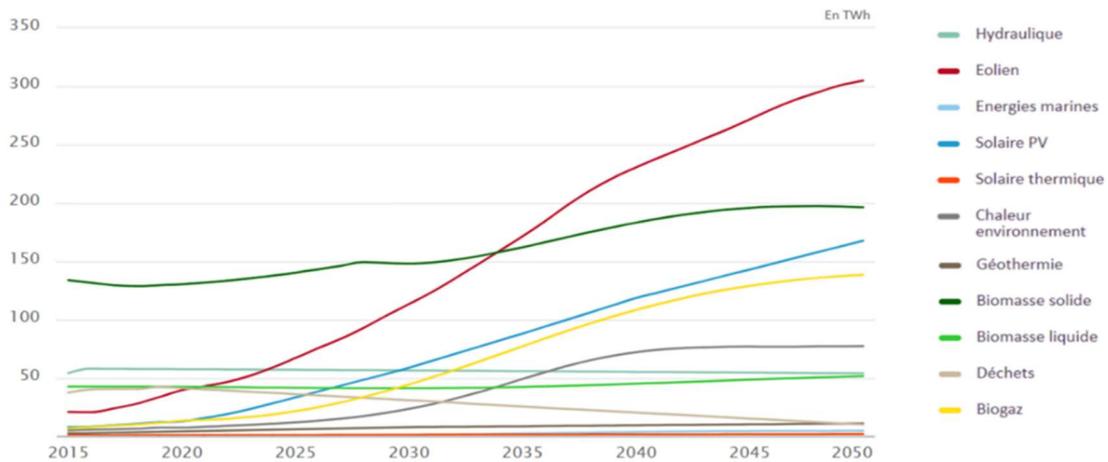


Fig. 0.3 Evolution of energy production from renewable sources from 2015 to 2050, projection from the scenario NegaWatt (Association NegaWatt, 2022)

In order to achieve this goal, much greater quantities of biomass will need to be redirected to feed anaerobic digesters (see Fig. 0.4). While a much of this biomass may be converted to biogas over the course of anaerobic digestion, most of the organic matter leaves the process as a by-product material known as “digestate”. Farm AD sites can receive a wide diversity of feed biomasses and organic wastes, follow a number of different design configurations, and operate under a range of process conditions (see Fig. 0.5). This leads to production of digestates of varying characteristics depending on the operational context of the AD plants (Guilayn et al., 2019a). Rather than be considered as a waste stream to be disposed of, these digestates may serve for a number of possible purposes in function of their quality (Sawatdeenarunat et al., 2016).

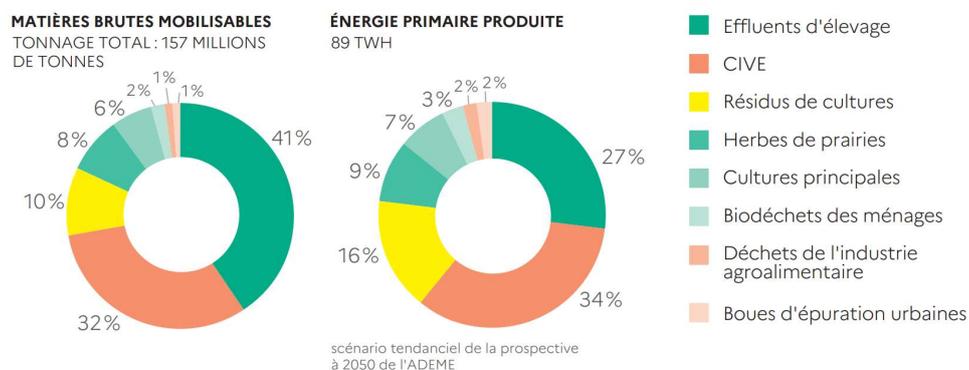


Fig. 0.4 Projection of the biomass directed to anaerobic digestion in France by 2050, in terms of total mass and total energy production (ADEME, 2021)

One of the most viable and sustainable uses of digestates is as organic fertiliser for crop and biomass production (Al Seadi et al., 2013), permitting to close the cycle of nutrients across the agroecosystem (Arthurson, 2009; Vaneeckhaute et al., 2013). Indeed, it is likely that digestates will gradually replace other organic matter streams previously returned to the soil as this biomass will be redirected, along with numerous other organic substrates, to AD in order to meet the targets for the vast increase in capacity anticipated by 2050 (ADEME, 2021) (as evidenced by Fig. 0.4). Digestates are widely valued as a rich source of plant-available nutrients, providing a viable alternative to imported mineral fertilisers due to their comparable impact on crop growth and yields (Alburquerque et al., 2012c; Bhogal et al., 2016; Frøseth et al., 2014; Möller and Müller, 2012). However, the wider impacts of the agricultural use of digestates on soil physical and biological health have, until recently, remained relatively unexplored, and many reviews stress the need for further research (Insam et al., 2015; Möller, 2015; Nkoa, 2014; Paolini et al., 2018).

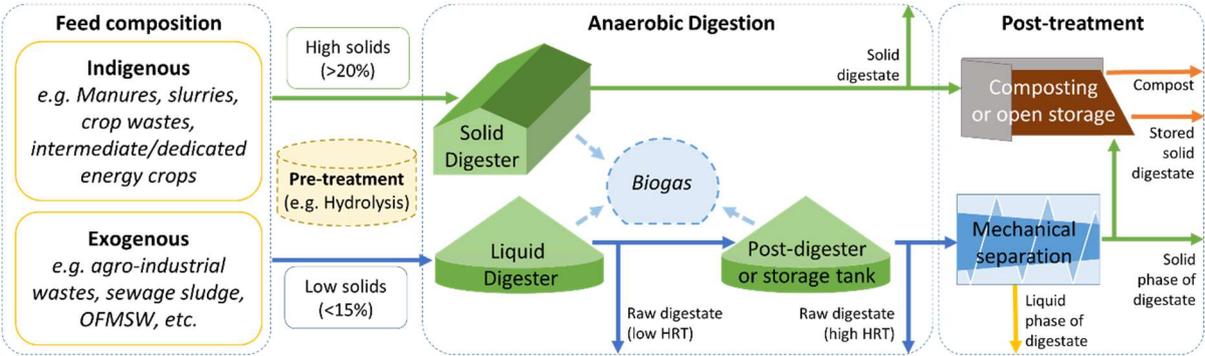


Fig. 0.5 Schematic of the possible feed substrates, plant configurations, and post treatments commonly used in on-farm AD (OFMSW = Organic Fraction of Municipal Solid Waste, HRT = Hydraulic Residence Time)

# Soil degradation and protecting soil structure through organic amendment

Healthy soils are central to supporting life on this planet, providing a myriad of important services, including production of the primary biomasses necessary for human survival. Around 95% of food for human consumption comes from terrestrial sources (European Commission, 2021). Soil management towards improving soil health could be vital in efforts to mitigate climate change via the regulation of greenhouse gas emissions (Initiative “4 pour 1000”, 2022). Indeed, it is estimated that almost three times as much carbon is currently stored in soils as organic matter than is contained in the atmosphere as CO<sub>2</sub> (Derrien et al., 2016a), and the annual cycling of carbon into and out of soils alone is far greater than the carbon being emitted by human activity (Lal et al., 2018). In addition, healthy soils provide a habitat for a huge range of native fauna and flora (Lehmann et al., 2017), as well as being critical to nearly all terrestrial biodiversity.

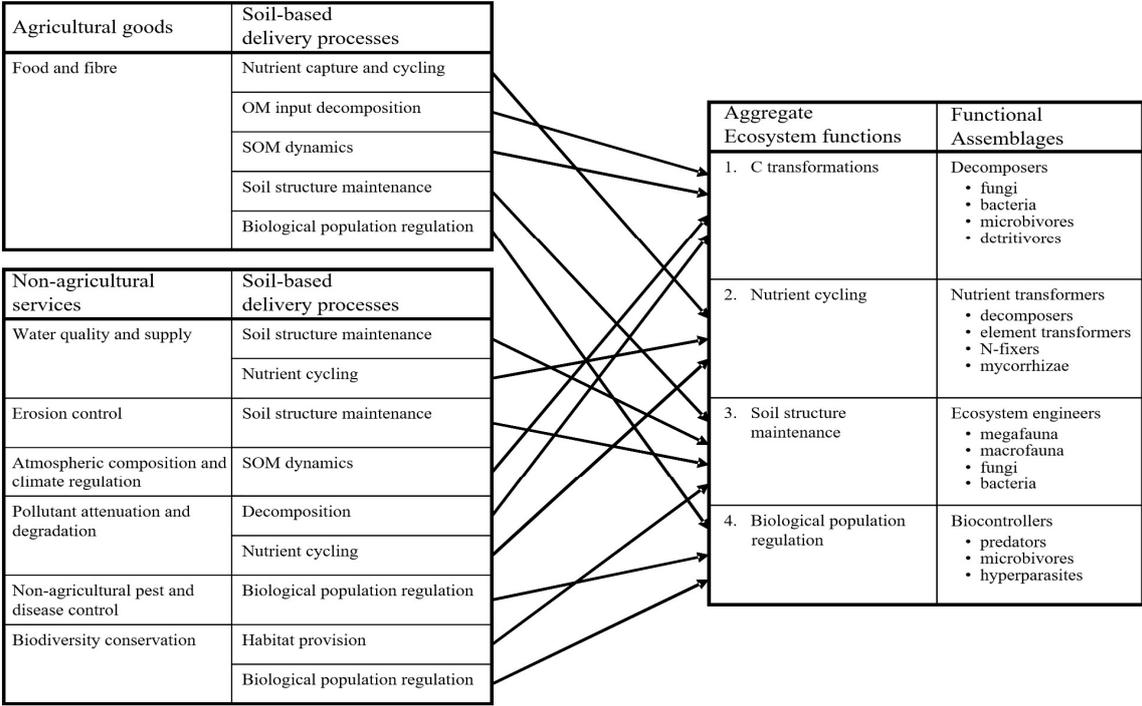


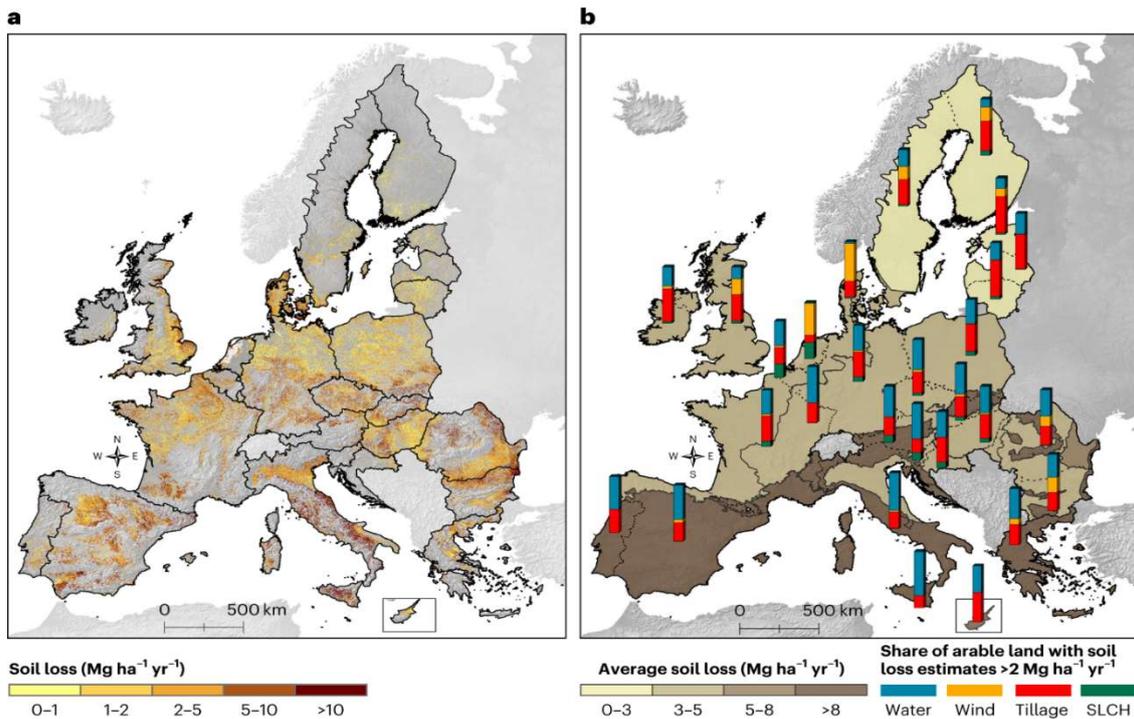
Fig. 0.6 Relationships between the principle soil health factors, the biological communities involved and a range of ecosystem goods and services expected from soils (Kibblewhite et al., 2007)

One of the four major functions central to soil health, involved in water quality and supply, erosion control, and provision of food and fibre (see Fig. 0.6), is the maintenance of the soil structure (Kibblewhite et al., 2007). Unfortunately, unsustainable agricultural practices, such as excessive tillage and overuse of chemical fertilisers, are responsible for widespread degradation of the soil structure (Diacono and Montemurro, 2010) (see examples in Fig. 0.7).



*Fig. 0.7 Some examples of soil degradation: a) rill erosion as a result of water movement over cultivated soil (© Dr D.Mulligan), b) surface sealing of a recently cultivated silt-loam soil due to rainfall (Brittany, France, © J.Cooke), c) compacted topsoil due to repeated ploughing and heavy vehicle passage (ESDAC, 2023), d) soil displacement by deep ploughing (Brittany, France, © J.Cooke)*

Over the past 40 years, approximately a third of the world's croplands have been abandoned due to soil degradation and, partly as a result, soil carbon is being lost from European cropland soil at a rate of approximately 0.5% per year (European Commission, 2021). Of the estimated 110 M ha of arable land in Europe, almost 43 M ha is considered to be vulnerable to soil erosion, with tillage and the action of water being the primary drivers (Borrelli et al., 2023) (see map in Fig. 0.8). Around 18% of soils in France are at a medium risk or higher of erosion (see map in Fig. 0.9), though projections estimate that this may increase to over 45% of the 2016 estimates by 2050 (Panagos et al., 2021).



*Fig. 0.8 European maps of (a) estimated soil loss rates in  $\text{Mg ha}^{-1} \text{yr}^{-1}$  and (b) the proportion of factors contributing towards erosion of arable land with soil loss estimates  $>2 \text{ Mg ha}^{-1} \text{yr}^{-1}$ . SLCH = Soil Loss due to Crop Harvesting (Borrelli et al., 2023)*

Among the many agricultural practices intended to restore and protect soil structure is the application of various types of organic material, including both primary biomasses (crop residues, manures, etc.) and transformed biomasses (composts, sewage sludges, biochars, etc.) (Abiven et al., 2009; Sarker et al., 2022). The incorporation of organic matter into the soil is a practice that dates back to the earliest times of agriculture as a means of maintaining the productivity of the agricultural land (Parr and Hornick, 1992). However, the application of different organic materials, particularly organic wastes, to the soil requires careful consideration of the benefits and risks in order to assure that they form part of a sustainable agricultural system (Diacono and Montemurro, 2010; Wezel et al., 2014). When correctly managed, addition of organic materials may contribute to the soil functions in several ways (Diacono and Montemurro, 2010), namely:

- They usually contain various minerals, particularly nitrogen, phosphorus or potassium and so restore concentrations of these nutrients in the soil that are taken up by crops, thus maintaining the **chemical fertility** of the soil,

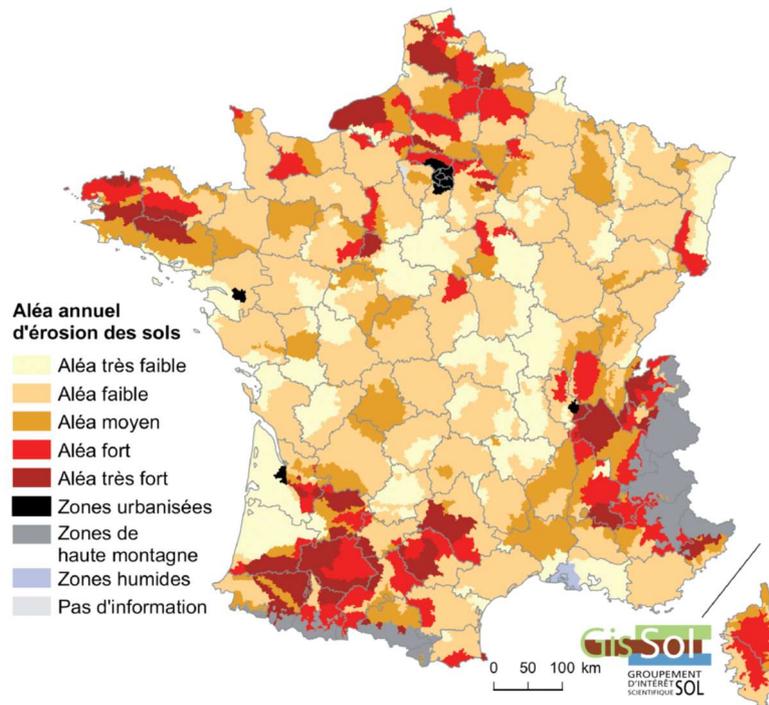
- They provide an important source of energy for soil organisms in the form of fresh organic carbon, as well as the other nutrients that help to maintain the soil's **biological fertility**,
- They may also provide recalcitrant forms of organic carbon that contribute eventually to the stable soil carbon. Coupled with the stimulation of soil biological activity turning over and generating new forms of organic matter, these processes are integral to the development and protection of the soil structure, and therefore its' **physical fertility**.

While these different aspects of soil fertility are interlinked, it is the soil physical fertility and the maintenance of the soil structure that is the area of interest in this thesis project. A commonly used definition proposed by Kay (1990), defines the instantaneous soil structure or the "soil structural form" to be "*the heterogeneous arrangement of solid and void space that exists in soil at a given time*". A healthy, well-structured soil is a spatially complex arrangement of solids, interspersed with a rich network of liquid- and gas-filled pores, traversed by plant roots and occupied by a host of soil organisms. The soil structural form is important in numerous processes (Bronick and Lal, 2005; Sarker et al., 2022), including:

- Offering an extensive and varied habitat for soil micro-organisms, micro- and macro-fauna,
- Allowing the aeration and gas exchange through the soil profile necessary for supporting extensive biological activity,
- Enabling rapid water infiltration from rainfall, whilst also retaining adequate water to support biological activity and plant growth during dry periods,
- Providing access for plant roots down through the soil to access nutrient sources, and thus assisting in healthy plant growth and high crop yields,
- Facilitating the sequestration of soil carbon through physical protection of organic matter from mineralisation by soil microorganisms.

The ability of the soil to preserve its structure when exposed to various stresses, such as mechanical stresses due to heavy machinery or the disruptive action of water, is referred to as the 'structural

stability' (Chenu and Cosentino, 2011). The structural stability is therefore critical to the maintenance of the various processes supported by the soil structure, and also has more direct benefits, through reducing the risk of formation of surface crusts (Fig. 0.7b) and soil erosion (Fig. 0.7a) (Le Bissonnais, 1996).



*Fig. 0.9. Erosion risk by small agricultural region in France (GIS Sol-INRA-SOeS, 2011)*

Soils high in silt and low in organic matter content generally have a low structural stability, and are susceptible to the processes of crust formation and erosion (Le Bissonnais & Arrouays, 1997). These soils predominate across much of North-Western Europe (Ballabio et al., 2016), including in the Brittany Region of Northern France where, due to the wet coastal climate, risks to agricultural soils from soil crusting and erosion are also high (see Fig. 0.9). Brittany is an area of high agricultural activity in terms of animal rearing, with the highest rate of animal waste production in France (ADEME, 2021). As a result, it has also been undergoing a significant development in the on-farm AD sector in recent years (AILE, 2023). This places the locality at the forefront of the transition towards widespread digestate usage in agriculture, and gives rise to a considerable need for guidance on how best to

manage their use while equally protecting soil health. Correct management of organic amendments in agriculture could help in efforts to prevent soil degradation and maintain soil fertility, particularly through their contribution to the protection of the soil structure (Abiven et al., 2009; Diacono and Montemurro, 2010; Sarker et al., 2022). However, digestates represent a relatively recent addition to the range of organic materials available for use to this end, and we still lack a clear understanding of their impacts on soil physical health.

## Operational Objective of the Thesis

In light of the above, the following operational objective was defined for this doctoral thesis project:

*To improve our understanding of the effects on soil structural stability of the diversity of digestates produced and used in agriculture, with a view to guiding decision making in the conception and operation of anaerobic digestion sites.*

# 1 State of the art

## 1.1 Soil aggregate stability

### 1.1.1 Introduction

One branch in the wider field of research into soil structure is the study of soil “aggregates”, or “naturally occurring clusters or groups of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent clusters” (Martin et al., 1955). Aggregates exist at many different scales in the soil structure (Emerson, 1967), and exist in a hierarchy with larger aggregates composing of smaller aggregates down to the level of soil particles (Tisdall and Oades, 1982) (see Fig. 1.1). Small micro-aggregates (<250  $\mu\text{m}$ ) are generally considered to be very stable and resistant to soil disturbance (Six et al., 1998). Macro-aggregates (>250  $\mu\text{m}$ ) are formed and held together by weaker forces, and are therefore more susceptible to climatic forces and cultivation (Six et al., 1998; Tisdall and Oades, 1982). Up to 85% of soil aggregates in cultivated systems may be made up of larger macro-aggregates (Six et al., 2000b).

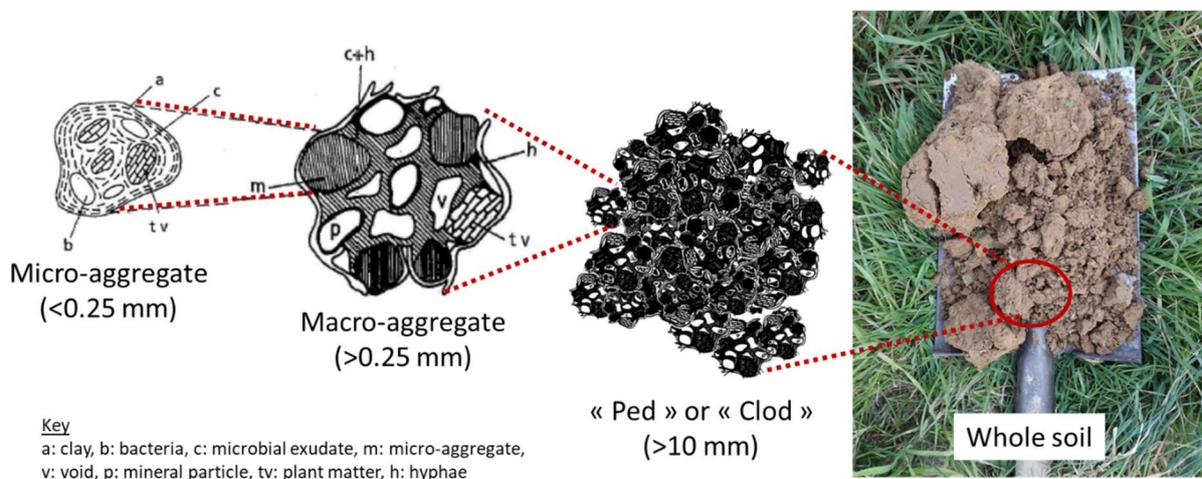


Fig. 1.1 The composition of the soil structure, from clod down to a macro- and micro-aggregate (adapted from (Duchaufour et al., 2018))

When regarded from the perspective of aggregates, the overall soil structural stability may be considered as a function of the stability of these individual elements. The resistance of soil aggregates to breakdown from forces such as rainfall or tillage is known as the soil aggregate stability (AS). In temperate climate soils, it is the resistance of macro-aggregates to breakdown by water that is

generally considered (Emerson, 1967; Le Bissonnais, 1996), and this is the meaning by which the term “soil AS” will be used for the rest of this document unless otherwise stated.

Soil AS, as a measure of soil structural stability, is critical to the maintenance of the soil structural form and its protection from degradation such as water erosion (Amézketa, 1999; Bronick and Lal, 2005; Le Bissonnais et al., 2007; Rabot et al., 2018). Almost 90% of soil organic matter (SOM) is located in soil aggregates (Jastrow, 1996) with macro-aggregates in silt soils may contain 13% more organic C than is contained in micro-aggregates (Puget et al., 1995). Incorporation of OM into soil aggregates may be a key mechanism in its’ protection from breakdown by soil organisms (Lehmann and Kleber, 2015), and so the stability of aggregates is also important in soil carbon storage (Derrien et al., 2016b).

The study of aggregates and soil AS has its’ origins reaching far back in the history of agronomy (Warkentin, 2008). Despite our continuously evolving understanding and interpretation of the soil structure (Six et al., 2004; Warkentin, 2008), the soil AS remains a key indicator of changes in soil physical health resulting from agricultural practices (Bottinelli et al., 2017; Hudek et al., 2021; Redmile-Gordon et al., 2020; Sarker et al., 2018).

### 1.1.2 The mechanisms of aggregate breakdown by water

When studying the effects of aggregate breakdown by the action of water, four distinct primary mechanisms are recognised, namely: slaking, differential swelling, mechanical breakdown from raindrop impact and physical-chemical dispersion (summarised in Fig. 1.2) (Chenu and Cosentino, 2011). Measurement of soil AS is carried out by subjecting the soil aggregates to a treatment simulating one or more of these forces to which it would typically be subjected in the field (Kemper and Rosenau, 1986; Le Bissonnais, 1996).

### 1.1.2.1 Slaking

Slaking occurs when dry aggregates are wetted in such a way that air contained in the pores of the aggregate are trapped and compressed by the incoming water and eventually force apart the aggregate into smaller micro-aggregates (Yoder, 1936). The more air that is trapped within the aggregate - as a function of its porosity, initial moisture content, and the rate of wetting - the greater the effect of slaking.

### 1.1.2.2 Differential swelling

Although dependent on the same properties as slaking, the mechanism of differential swelling is different, and increases with clay content (Le Bissonnais, 1996). Dry clay expands on contact with water and, where clay content is high enough, this can cause small fissures to form which may break apart the aggregate (Emerson, 1967).

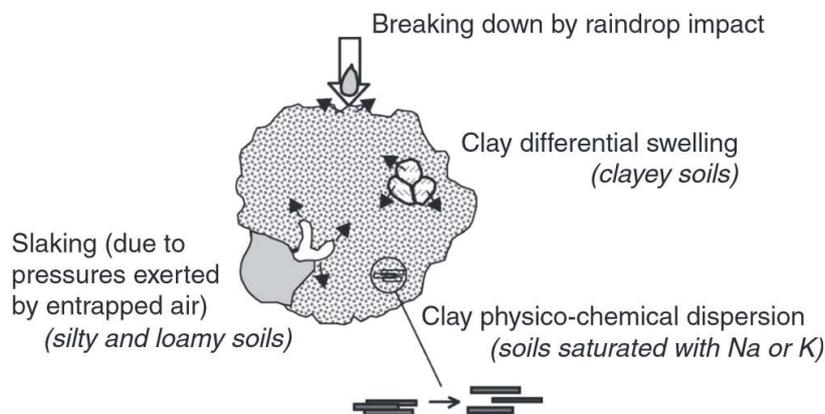


Fig. 1.2 Mechanisms of aggregate breakdown by water (Chenu and Cosentino, 2011)

### 1.1.2.3 Mechanical breakdown by raindrop impact

If the kinetic energy of a raindrop is high enough, then the impact on the soil's surface is enough to detach and project clay particles and very small fragments. This is an effect that will occur in conjunction with other mechanisms, and is enhanced by the fact that already wetted aggregates are much weaker (Le Bissonnais, 1996).

#### 1.1.2.4 Dispersion

Physico-chemical dispersion of aggregates occurs due to disruption of the attractive forces between colloidal particles upon wetting. This is dependent on the cations present in the soil and their valency, as well as the strength of the attractive forces holding together clay particles (Emerson, 1967). Dispersion of aggregates results in elementary particles of clay, silt (and very small micro-aggregates), and is as such the most effective process of aggregate breakdown, greatly increasing the effect of the other processes.

### 1.1.3 The principal factors affecting soil aggregation and stabilisation

The climatic context in which a soil is found also has an important bearing on the factors that lead to the formation of aggregates and their stabilisation against these mechanisms of breakdown (Emerson and Greenland, 1990). Several principal factors govern the formation and stabilisation of aggregates in temperate climate soils (Amézketa, 1999; Le Bissonnais, 1996), of which the most significant may be considered to be the soil texture and clay content, the soil exchangeable mineral content, the soil organic matter, and the soil microbiological activity.

#### 1.1.3.1 Soil texture, clay content, and exchangeable minerals

The mixture of clay, silt and sand particles that make up a soil's texture are determinant in the formation of a soil structure as they provide the fundamental building blocks of soil aggregates (Edwards and Bremner, 1967; Tisdall and Oades, 1982). The soil texture equally governs the way in which other factors interact to form and stabilise soil aggregates. Very sandy soils (Chaplot and Cooper, 2015) and silty soils (Annabi et al., 2011) frequently show low aggregate stability. In the case of silt soils, factors such as the soil organic matter content, become highly important in the formation and stabilisation of aggregates (Le Bissonnais and Arrouays, 1997). Conversely, for soils with high clay contents (>30%), aggregate stability is generally very high (Kemper and Koch, 1966), and the arrangement of clay particles and the electrostatic forces between them are the dominant factor in

the formation of stable aggregates (Six et al., 2000a). However, this may also depend on the clay mineralogy, and the presence of aggregating minerals in the clay structures of the soil (Denef and Six, 2005; Emerson, 1967). Many clays swell and shrink depending on their moisture content, and it is believed that wetting-drying cycles, induced by rainfall and evaporation, will draw together clays and organic matter to form aggregates held by strong inter-particle bonds (Emerson and Greenland, 1990). Repeated freeze-thaw cycles may also have the same effect in soils with a reasonably high clay content (>12%) (Lehrsch et al., 1991).

Soil clays are made up of lamellar sheets held together by weak intermolecular bonds and cation bridges. Polyvalent metal cations, particularly  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , form strong poly-cationic bridges between negatively charged clay lamellae and organic matter particles and so are important binding agents for soil micro-aggregates (Amézketa, 1999; Bronick and Lal, 2005). Al and Fe, due to their polyvalent nature, can exist as amorphous poly-cations, which can coat and bind clay and organic matter molecules together (Oades, 1984). When present in excess, certain monovalent cations - particularly  $\text{Na}^+$  and  $\text{K}^+$  can replace larger poly-cations between the lamellae of certain clay types (e.g. smectites, illites), generating a double-layer around the clay particles and facilitating their dispersion (Tessier, 1990). The interference of monovalent cations may subsequently disrupt the stability of macro-aggregates (Emerson, 1967; Levy and Torrento, 1995). For this reason, the soil pH and the concentration of exchangeable minerals can be critical factors affecting soil aggregate stability (Amézketa, 1999; Emerson and Greenland, 1990).

#### 1.1.3.2 Soil organic matter

It has been understood for a long time that the soil organic matter (SOM) is key in the formation and stabilisation of soil aggregates (Haynes, 2005; Tisdall and Oades, 1982), with increasing levels of SOM well correlated with increased soil aggregate stability (Chaplot and Cooper, 2015; Chenu et al., 2011). It is likely that specific fractions of SOM play a much more significant role in stabilising aggregates than others (Tisdall and Oades, 1982). However, we still lack a complete picture of the composition of SOM

to this day (Lützow et al., 2006), and research is still ongoing to isolate particular organic “binding agents” in soil aggregates (Jensen et al., 2019; Redmile-Gordon et al., 2020).

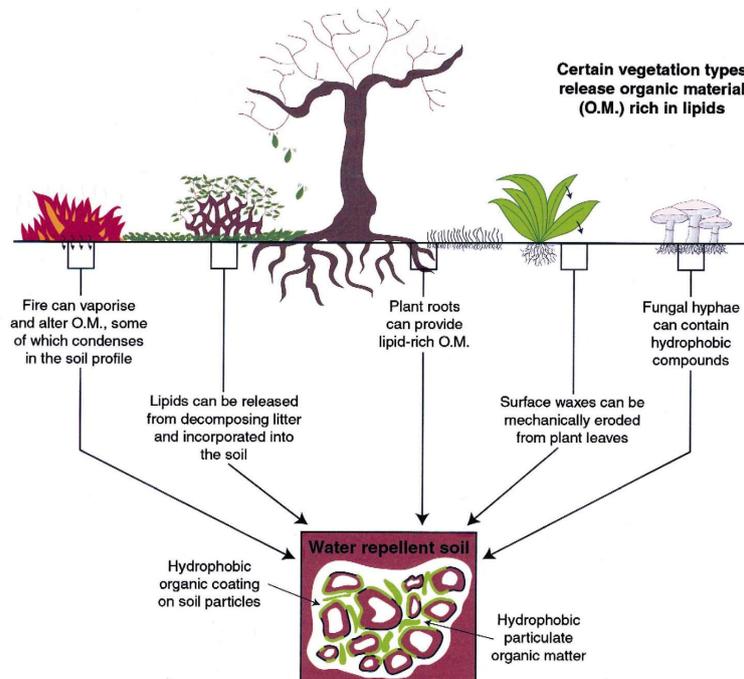
#### *1.1.3.2.1 Humic substances*

The soil “humic” fraction, made up of recalcitrant organic matter of large apparent molecular weight, may be important in long-term stabilisation of soil aggregates (Bronick and Lal, 2005). It is believed that large apparent molecular weight molecules, known as humic substances, may overlap across clay mica and stabilise these particles into soil aggregates (Edwards and Bremner, 1967; Oades and Waters, 1991). Where differentiation between different humic substances is made, it is suggested that larger “humic acids” are important in aggregate stabilisation, while more mobile “fulvic acids” show little effect on soil AS (Tejada and Gonzalez, 2007). However, recent developments in our understanding of the organic matter turnover in soil and the mechanisms for its stabilisation have changed how we view the soil as a whole (Lehmann and Kleber, 2015). The existence of ‘humic’ substances is disputed as a relic of fractionation analysis methods (Lehmann and Kleber, 2015). Analysis of the older SOM through pyrolysis and solid state <sup>12</sup>C NMR has shown it to be primarily composed of polysaccharides and proteins (Lützow et al., 2006), and what were previously identified as humic substances likely comprise complexes of various microbial and plant biopolymers (Kelleher and Simpson, 2006). Nonetheless, recalcitrant bio-macromolecules identified as humic and fulvic acids, continue to be investigated for their potentially useful properties (Salati et al., 2011).

#### *1.1.3.2.2 Hydrophobic organic matter*

Hydrophobic OM, originating from both plant and microbiological sources may attach to soil aggregates and generate a hydrophobic coating that reduces soil wettability (Doerr et al., 2000). Reduced aggregate wettability has been linked to increased soil AS (Piccolo and Mbagwu, 1999). Studies have shown that organic matter associated with clay also improves the hydrophobicity of clay sized (<2µm) micro-aggregates and in turn increased stability of macro-aggregates (Dal Ferro et al., 2012). While hydrophobicity of aggregates may reduce water entering into an aggregate and slaking,

very hydrophobic soils show increased susceptibility to mechanical breakdown from raindrop splashing, plus reduced infiltration and increased channelling of water, thus accelerating erosion (Doerr et al., 2000).



*Fig. 1.3 Source of hydrophobic OM contributing to reduced soil wettability and, in extreme cases, water repellent soil (adapted from (Doerr et al., 2000))*

### 1.1.3.3 Plants and plant roots

Overall the presence of plants has a noticeable positive effect on soil aggregate stability, with their roots and symbiotic mycorrhizal fungi creating a network of filaments that hold macro-aggregates together (Angers and Chenu, 2018). The growth of plant roots force soil clay particles into alignment, to create new aggregates (Bronick and Lal, 2005; Dorioz et al., 1993; Lynch and Bragg, 1985). Furthermore, plant roots also generate exudates that may act as binding agents (Dorioz et al., 1993; Martins et al., 2009).

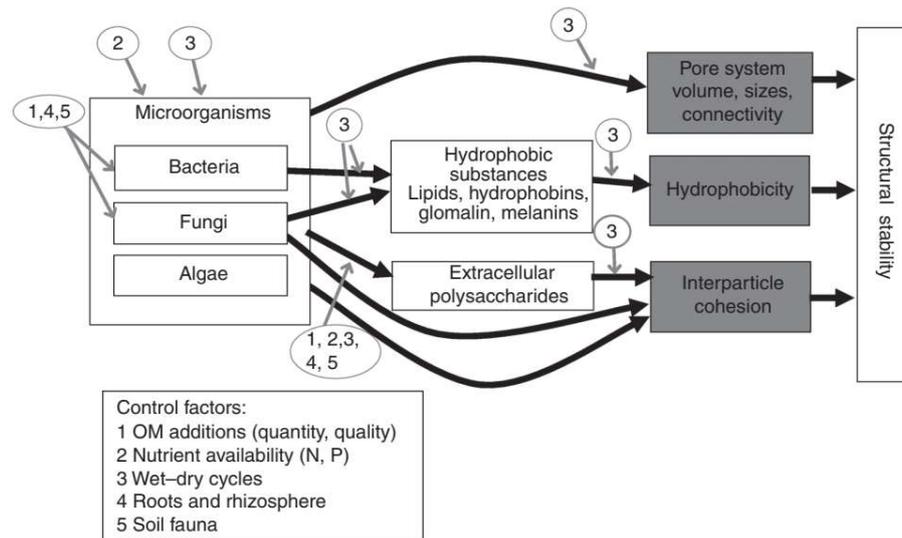
The dominant source for most soil OM is determined to be of plant origin (Six et al., 2001), including from the remains and residues of cover vegetation. Structural polysaccharides (e.g. cellulose and hemi-

cellulose) and storage polysaccharides (e.g. starch), that form a large part of organic matter in most plants, are subsequently found in abundance in all soils bearing plant life. The quantity and quality of plant residues determines the formation and stabilisation of aggregates (Blanco-Canqui and Lal, 2004). The roots of plants are again particularly important, and root derived POM may well contribute more towards the formation of water stable aggregates than surface inputs of organic carbon (Blanco-Canqui and Lal, 2004). Though not directly involved in the stabilisation of soil structure, light fraction and coarse inter-aggregate particulate organic matter (POM) derived from plant litter and root deposits (Puget et al., 1998; Six et al., 2001) acts as the nucleus for microbial activity (Tisdall and Oades, 1982).

#### 1.1.3.4 Soil microorganisms

Microorganisms are central to the dynamics of SOM in the soil, metabolising and mineralising various organic molecules and contributing their necromass and deposited residues in turn. This activity means that they play a vital role in the formation and stabilisation of aggregates (Chenu and Cosentino, 2011), (see Fig. 1.3). Many soil microorganisms release sticky exudates in order to adhere to the surface of soil structures, and to provide a protective coating around cell walls. Stable macro-aggregates may form around soil POM, with clay, silt and micro-aggregates adhering to the decaying particle as it is covered in microbial exudates (Angers and Chenu, 2018; Martens, 2000; Oades, 1984). As the POM acting as the centre of the macro-aggregate is decomposed, the macro-aggregate may break down to form micro-aggregates of exudate coated POM (Golchin et al., 1994; Oades, 1984). In some cases simple polysaccharides and multifunctional organic ions in association with soil minerals may form highly stable micro-aggregates (Blanco-Canqui and Lal, 2004; Tisdall and Oades, 1982). The extra-cellular polymeric substances (EPS) produced by soil microbes are much less abundant in the soil than plant-derived OM, but are nevertheless believed to be highly effective and important aggregate binding agents (Martin et al., 1955; Redmile-Gordon et al., 2020). Readily extractible carbohydrate fractions, associated with EPS, have been shown to be closely correlated with soil AS (Haynes and Swift, 1990; Puget et al., 1998), though more recent research suggests that only specific types of

polysaccharides and proteins extractible from the soil may be responsible for the stabilisation of aggregates (Redmile-Gordon et al., 2020, 2015).



*Fig. 1.4 Schematic representation of the main mechanisms by which microorganisms stabilize soil aggregates (Chenu and Cosentino, 2011)*

Certain microbial exudates may be hydrophobic, particularly those generated by certain fungi during their colonisation of the soil (Chenu and Cosentino, 2011). These products deposited on the aggregate surface may reduce wettability and protect against water entry (Chavez-Rico et al., 2023). The accumulation of microbial residues as well as accretions of microbial biomass in the pores of aggregates may also serve to impede water entry into the aggregates (Chenu and Cosentino, 2011). In some cases, organic acids exuded by plants, bacteria or fungi may disperse soil clays by increasing negative charge on clay surfaces (Oades, 1984). However, this is potentially offset by the beneficial binding action of exuded polysaccharides.

Specific soil microbes are probably much more involved in the process of stabilising soil aggregates than others (Lynch and Bragg, 1985), and it is believed that fungi may generally play a more important role in macro-aggregate stabilisation than bacteria (Bossuyt et al., 2001; Lehmann et al., 2017; Lucas et al., 2014). Fungi generate exudates that glue aggregates together, while their mycelia also act as a protective net around macro-aggregates (Lehmann et al., 2017; Oades, 1984). Addition of fungicides

to soil incubation have been shown to severely impede water stable aggregate formation (Denef et al., 2001), suggesting that the growth and activity of soil fungi provide the primary mechanism(s) by which macro-aggregates are held together.

The texture and mineralogy of the soil may govern which taxonomic group of soil microbes will predominate in the aggregation of soil following incorporation of plant residues, with bacterial markers more correlated with aggregation in clay soil, and fungal markers more correlated with aggregation of sandy soil (Zhang et al., 2021). In silty and sandy soils low in clays, water stable aggregate formation correlates well with fungal hyphae length suggesting that these provide an important mechanisms for holding soil particles together into macro-aggregates (De Gryze et al., 2006). Conversely, bacterial populations and their exudates are better correlated to soil AS in finer textured, clay soils (Zhang et al., 2021).

From this section, it can be seen that aggregates are formed and stabilised by the interaction between soil minerals, organic matter, plant roots and microorganisms. Agricultural activities such as crop growth, tillage, fertilisation and organic amendment can have a significant impact on soil aggregate formation, stabilisation and destruction, via their direct and indirect alteration of soil chemistry, organic matter and microbiology.

#### 1.1.4 Agricultural practices affecting soil aggregate stability

##### 1.1.4.1 Tillage

Disturbance of soils through cultivation is known to reduce aggregation and the ability of soil to sequester carbon (Jensen et al., 2019; Lynch and Bragg, 1985). The breakdown of macro-aggregates through ploughing may expose the SOM protected within aggregates to rapid mineralisation by soil microorganisms, and inhibit the formation of further micro-aggregates through disruption of the aggregation process (Six et al., 1998). Comparison of different tillage practices has shown that

aggregate stability is higher for soils under no-tillage and surface tillage when compared to soil subjected to mouldboard ploughing (Bottinelli et al., 2017). The passage of heavy vehicles during field intervention also negatively impacts the soil AS due to the stresses and shear forces applied to the soil by the vehicle wheels (Abdollahi et al., 2014; Barik et al., 2014). Furthermore, the soil disturbance by continuous vehicle passage and ploughing may encourage formation of larger but weaker soil aggregates that lack the internal structure of more naturally formed aggregates (Horn et al., 1995).

#### 1.1.4.2 Crop diversification

As already discussed, plants are an important source of organic material for promoting soil microbiological activity, and their roots may provide stabilisation through enmeshment of soil aggregates and generation of root exudates (Martins et al., 2009). Increasing the variety of crops grown above-ground increases the diversity of leaf litters, plant exudates and rooting patterns below-ground, thus encouraging a richer soil biota and improved soil health (Gliessman, 2014). Certain crop plants have a more beneficial action on soil AS, in terms of their greater production of fine roots and stabilising agents (Chan and Heenan, 1996). Plant residues of different quality will also have different effects on soil AS (Martens, 2000). The planting of cover crops between main crops in order to maintain soil cover increases soil OM, reduces soil erosion through protecting the surface from raindrop impact, improves water infiltration and retention, and reduces soil surface temperatures (Hartwig and Ammon, 2002). However, in cultivated systems, the positive effects on soil AS from crop growth are often short-lived (Chan and Heenan, 1996) due to the negative effects of repeated tillage.

#### 1.1.4.3 Mineral fertilisation and liming

Correct fertilisation should assist healthy crop growth and development, therefore potentially increasing the direct contribution of plant roots to soil stabilisation, and also the eventual contribution of plant matter to the soil carbon pool (Albuquerque et al., 2012b) (see Fig. 1.4). Nitrogen fertilisation over the long term, when coupled with organic amendment in the form of straw residues, has been shown to produce positive effects of soil AS (Blair et al., 2006). However, some negative effects on soil

structure from over-fertilisation have been observed (Haynes and Naidu, 1998). Large quantities of mineral nitrogen may reduce the positive impact of added organic matter, as this creates a flush of bacterial activity, in preference to fungal activity, that rapidly consumes the soil OM (Le Guillou et al., 2011; Vandevivere et al., 1990). Over the very long-term (up to 70 years), repeated nitrogen fertilisation with ammonia containing fertilisers may lead to severe soil acidification and a subsequent decline in soil aggregate stability due to loss of soil carbon (Buthelezi and Buthelezi-Dube, 2022). This suggests that the issue may lie in the correct management of mineral fertilisation.

Addition of minerals to the soil may alter the exchangeable cation composition of the soil as well as its pH, potentially leading to clay dispersion and reduced soil structural stability (Rengasamy, 2018). It is possible that free ammonium ions ( $\text{NH}_4^+$ ), as with other mono-cations may have a negative effect on soil clay dispersion (Haynes and Naidu, 1998; Whalen and Chang, 2002). Conversely, the use of lime or gypsum, that are rich in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  may significantly improve soil AS by increasing polycation bridging across soil particles (Bronick and Lal, 2005). However, increasing soil pH through liming may precipitate exchangeable Al and Fe ions into oxides and increase the negative surface charge on certain types of clay particle, thus repelling them from one another (Chorom et al., 1994). A review by (Haynes and Naidu, 1998) of the effects of liming on soil aggregate stability noted that for many studies, a shift in pH from acid to neutral conditions brought about a short term increase in dispersible clay (1-3 months). On the other hand, this was followed by a more long lasting increase in aggregate stability as the soil soluble minerals eventually reached a new equilibrium and the soil structure reorganised (>1 year).

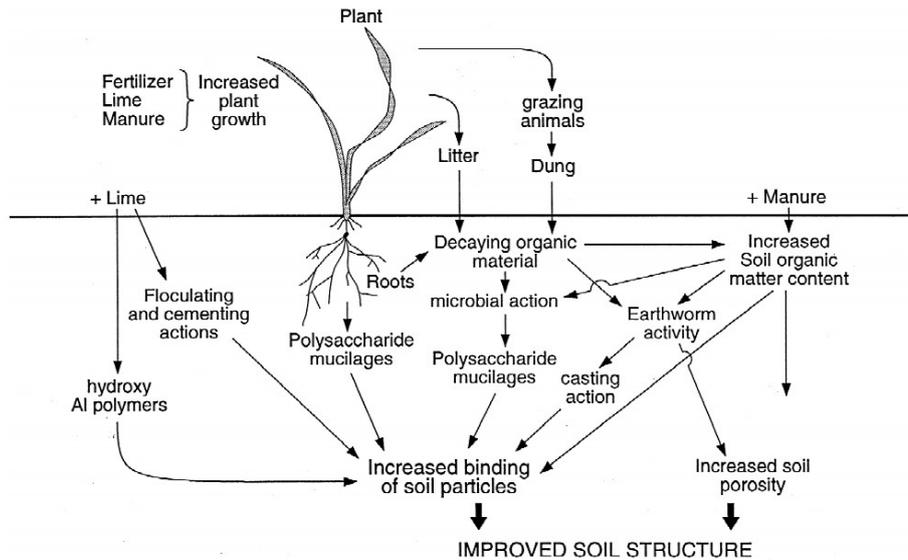


Fig. 1.5 Schematic of the major effects that mineral fertilisers and manure (organic amendments and fertilisers) have on improving aggregation and soil structural stability (adapted from Haynes and Naidu, 1998)

#### 1.1.4.4 Incorporation of organic soil amendments and fertilisers

The term “organic amendment” is applied throughout this thesis work to refer to the “wide array of organic materials, wastes, and residuals of either rural or urban origin that can improve soil properties and plant growth, and impart such beneficial effects over time that will improve soil productivity” (Parr and Hornick, 1992). Organic amendments contribute to the SOC and are key in the improvement of soil aggregation and stabilisation (Diacono and Montemurro, 2010; Sarker et al., 2022) (see Fig. 1.5), but the scale and duration of their impact depends on the organic matter quality (Abiven et al., 2009). The organic matter contained in an organic amendment, depending on its physical and chemical structure, is more or less available to soil microorganisms and soil fauna (Haynes, 2005). This in turn modulates the response in soil microbiological activity leading to production of various organic binding agents (described in section 1.1.3). Amendment with materials rich in readily biodegradable organic components have been shown to rapidly and significantly increase microbial activity and thus improve aggregate stability (Hansen et al., 2016; Sarker et al., 2017). Fresh plant residues are particularly labile and will initiate a rapid response in soil aggregation, associated with simultaneous rapid C mineralisation (Zhang et al., 2021). Depending on the content and quality of the organic carbon in

organic amendments, soil fungi may be more important in aggregate stabilisation than other soil microbes (Lucas et al., 2014). Using fungi or bacteria selective pesticides, (Metzger et al., 1987) were able to establish that the majority of the contribution towards aggregate stability following sludge addition to soil was attributed to stimulation of fungal activity.

Addition to the SOM by organic amendment may also have more direct, abiotic effects on soil AS. Certain OM components in organic amendments may interact directly to stabilise soil aggregates through increasing particle cohesion or modifying their wettability (Annabi et al., 2007; Voelkner et al., 2019). Pre-humic substances present in plant materials may contribute to the development of soil humic substances responsible for long-term soil stabilisation (Martens, 2000). Conversely, some research has shown that manuring may reduce the level of humification of the soil, while increasing the amount of particulate OM (Aoyama and Kumakura, 2001). Addition of organic amendments such as manures may not add to the soil humic substances, but rather results in accumulation of other substances that also stabilise the soil (Paré et al., 1999). Indeed, in clay rich soils, the humic and fulvic fractions of slurries or sewage sludges may even disrupt soil aggregates by displacing clays bound by poly-cations (Mbagwu et al., 1991).

Complex lipids, waxes and resins derived from plant matter, and not easily broken down by soil microorganisms may form a hydrophobic coating on the surface of soil aggregates (Piccolo and Mbagwu, 1999). While investigating the impact of soil application of oily wastes on soil structure (Plante and Voroney, 1998) observed both short-term and medium-term improvement of soil AS, attributing this effect to hydrophobic coating of aggregates by lipids. However, they were unable to ascertain if the binding action came directly from the waste itself or whether the simple lipids added were rapidly degraded and replaced by more stable microbial lipids. Modification of aggregate wettability may occur either by deposition of hydrophobic molecules on the surface of the aggregates, or else by a modification of the aggregate pores by accretion of organic material or soil microbes (Chenu and Cosentino, 2011). Indeed, pore modification may be a more important mechanism in

aggregate stabilisation from organic amendment than hydrophobic coating (Dal Ferro et al., 2012). Heavy doses of manure followed by drying of the soil may help to precipitate a hydrophobic coating around aggregates, but this can also have negative impacts resulting from blocking of the pore structure and reduction of the soil hydraulic conductivity (Haynes and Naidu, 1998).

It is often suggested that some direct negative effects will be observed from the addition of highly mineral amendments to soil that may alter the balance of cations and disrupt soil micro-aggregates (Rivier et al., 2022). Whalen and Chang (2002) proposed that repeated applications of manures may cause aggregate dispersion through the accumulation of monovalent cations such as  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NH}_4^+$  and disruption of soil clays linkages. However,  $\text{K}^+$  ions would appear to have little impact on clay dispersion at low concentrations (10-15 % Exchangeable K), and may even counteract exchangeable sodium to some extent (Levy and Torrento, 1995).

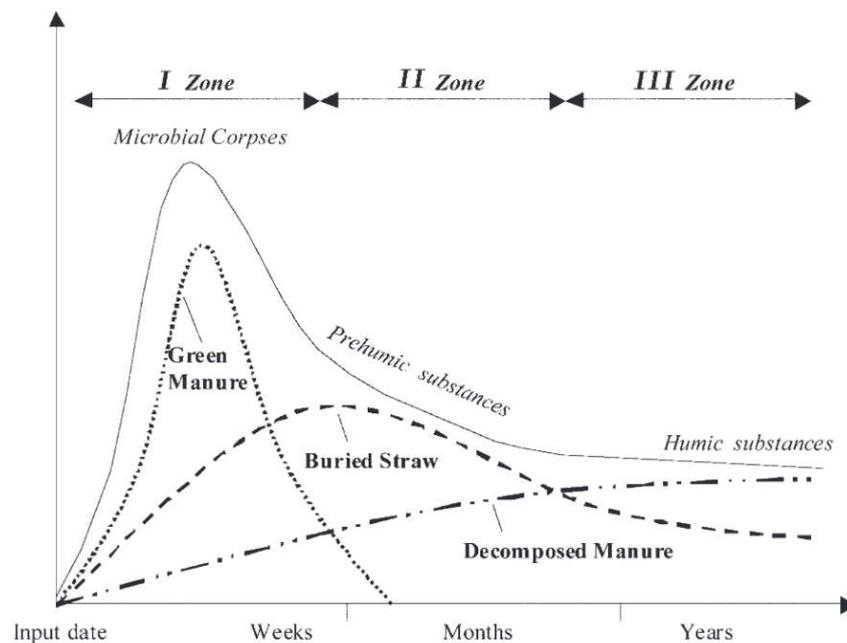
### 1.1.5 The dynamic nature of soil aggregate stability

#### 1.1.5.1 Aggregate stability dynamics following incorporation of organic amendments

The processes described in the previous sections can occur simultaneously, at varying strengths and over differing time scales, so that soil aggregate stability is characterised by both a high sensitivity to organic amendment and by a pronounced “dynamic” in its evolution over time.

As all organic matter in the soil is subject to slow decay, the effects on soil AS induced by the incorporation of fresh organic matter and stimulation of soil biological activity will only last for a limited period. The strength and duration of the effect depends on the different organic binding agents produced and the time they take to be broken down along with the added organic matter (Abiven et al., 2007; Sarker et al., 2018). In general, microbial and plant exudates are believed to be relatively quickly broken down by continued microbial activity and so have only a transient (days to weeks) effect on soil AS (Le Guillou et al., 2012). Fungal hyphae and plant roots are considered to be temporary

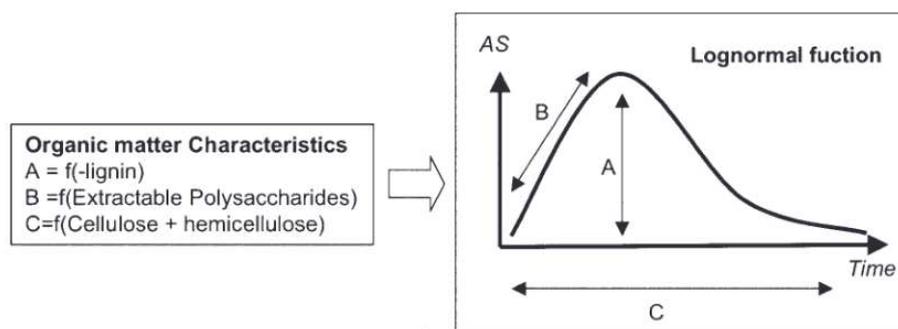
binding agents (weeks to months), lasting as long as the organisms themselves before succumbing to eventual decay (Tisdall and Oades, 1982). Finally, the OM highly resistant to microbial breakdown, or else physically protected in OM-mineral structures, that make up the soil “humus”, are considered to be recalcitrant binding agents (years-), providing long-term improvements to soil AS (Bronick and Lal, 2005; Sarker et al., 2022).



*Fig. 1.6 The conceptual model of temporal variations in aggregate stability following organic substrate incorporation proposed by Monnier (1965) (Image from Abiven et al. (2008))*

In order to describe the dynamic changes in soil AS following organic amendment, a conceptual model was originally postulated by Monnier (1965), who suggested that the effects depended on the level of “maturity” of the organic matter, or its biodegradability by soil microorganisms (see Fig. 1.6). He assumed that aggregate stability dynamics were due only to microbial activity degrading fresh OM and depositing binding agents in the short-term, while longer-term effects were due to the formation of humic substances through the process of humification. The “Pouloud” model proposed by Abiven et al. (2008), represented a first attempt to directly apply Monnier’s conceptual model. Using chemical fractionation of organic matter as per the “Van Soest” method to determine water soluble,

hemicellulose, cellulose and lignin fractions, the model allows a prediction of the temporal variation in soil AS resulting from the microbial breakdown of these fractions following addition of a given organic amendment (see Fig. 1.7). Furthermore, the Pouloud model also attempts to take into account the modulating effects of environmental factors such as changing soil moisture content, soil temperature and nitrogen availability, on organic amendment decomposition and - as a consequence – on the soil AS. However, Pouloud requires pre-testing and setting of parameters based on the type of organic amendment and soil used, and does not take into account factors such as the interaction with plants and plant roots, the presence of abiotic aggregate binding agents (e.g. hydrophobic OM, humic substances poly-cations), or possible disruptive agents (e.g. mono-cations).



*Fig. 1.7 Description of the Pouloud model for describing soil aggregate stability dynamics under controlled conditions. A, B, and C are the magnitude, scale and shape parameters of the lognormal function obtained from laboratory results (Abiven et al., 2008)*

The CANTIS-STAB model developed during the thesis of Cosentino (2006) uses the CANTIS model for modelling C mineralisation of organic amendments in soil based on their OM fractions (Garnier et al., 2003), and attaching a statistically derived equation for estimating soil AS dynamics from both predicted C mineralisation rates and microbial biomass dynamics. The model was relatively successful, and even took into account a possible direct abiotic effect of an organic amendment on soil AS (Chenu et al., 2011). However, it was only developed and parametrised for amendments with straw, and has yet to be applied to other organic amendments.

### 1.1.5.2 Cumulative dynamics of aggregate stability in cultivated systems

On the basis of Monnier's model, Annabi et al. (2007) proposed a further conceptual model for the impact of successive compost amendments over multiple years (see Fig. 1.8), where short-term (intra-annual) soil AS dynamics are governed primarily by microbial activity while, longer-term (multiple years) dynamics are determined by the accumulation (or loss) of SOC. This "cumulative" model was partially proven to be the case in a later study by the same authors (Annabi et al., 2011), and other long-term studies have shown significant improvements in soil AS associated with application of certain organic amendments (composts, manures) (Bhogal et al., 2018; Tejada et al., 2009). However, depending on the type of organic amendment, high quantities of OC may need to be incorporated in order to observe effects after only several years. Furthermore, while aggregation may increase with increasing organic matter content, there may well be a certain level of OM content after which soils show no significant increase in aggregation (Tisdall and Oades, 1982), and therefore an upper limit to the stabilisation of soil structure that can be achieved.

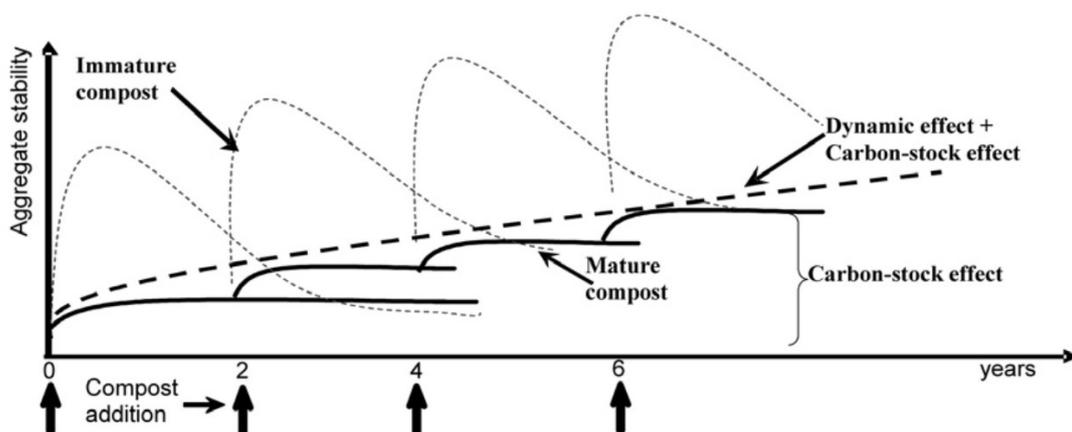


Fig. 1.8 Conceptual evolution of soil aggregate stability over the course of repeated applications of immature or mature compost (Annabi et al., 2007)

#### 1.1.5.2.1 Aggregate stability dynamics following incorporation of complex organic wastes

The models so far developed have limited predictive power for the impacts of more complex organic amendments. Attempts to apply the Poloud model to various composted and anaerobically digested

materials met with limited success where the dynamics in AS observed following amendment with digestates, composts and some animal waste slurries did not follow a model ‘lognormal’ curve (Dénès et al., 2014). Complex dynamics in AS following incorporation of organic wastes have been observed elsewhere. For example, in a study by Metzger et al. (1987), two “phases” of aggregate stabilisation in soil amended with sewage sludges were observed: firstly, a rapid organisation of stable aggregates followed by a rapid decline, then a second steady rise in AS towards the end of incubation (see Fig. 1.9). Although no attempt was made to apply an appropriate model to this data, it was hypothesised that microbial (fungal) decomposition of organic matter of varying biodegradability along with abiotic (chemical) stabilisation occurred at various times over the course of incubation. To conclude, it would appear necessary to develop a better understanding of the different mechanisms that may act to stabilise aggregates depending on the organic and inorganic characteristics of a particular organic amendment before advancing to the development and application of a predictive model of short-term AS dynamics.

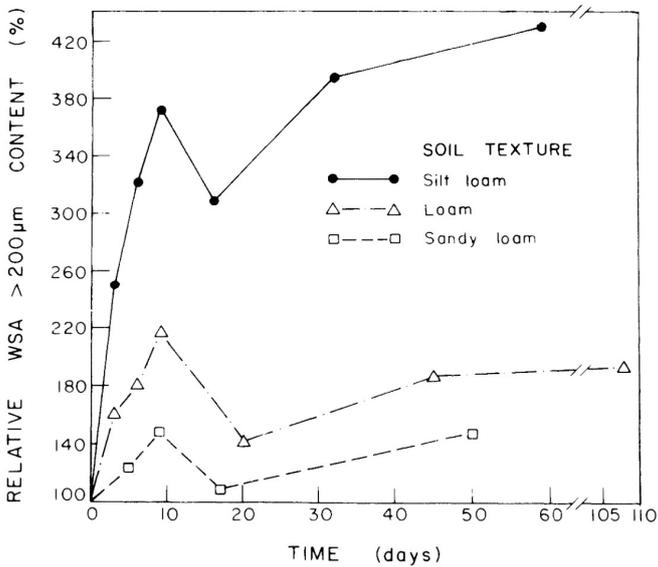


Fig. 1.9 Aggregate stability dynamics for three soils amended with sewage sludge (Metzger et al., 1987)

### 1.1.5.3 Seasonal dynamics in soil aggregate stability

In the field, soil aggregates will undergo seasonal variations in stability, an effect attributed largely to moisture content of the soil (Perfect et al., 1990) as well as the impact of temperature and rainfall on soil microbial activity (Dimoyiannis, 2009). The scale of this variation may even be greater than the effects observed from agricultural practices (Bottinelli et al., 2017; Perfect et al., 1990). The moisture content of the soil has a significant impact on the measurement soil aggregate stability. For instance, dry aggregates will tend to more quickly take up water on wetting, leading to greater slaking effects (Le Bissonnais, 2000). At the same time, drying and wetting cycles in the soil will increase the influence of added organic matter on aggregate stability (Cosentino et al., 2006), possibly by increasing the surface hydrophobicity from OM precipitated on the aggregate surface (Bottinelli et al., 2017). In addition to the above, seasonal cycles of plant growth in terms of root development and canopy cover, as well as variations in crop planting and selection, will also alter the soil AS from one growing season to the next (Chan and Heenan, 1996; Dimoyiannis, 2009). All of these interacting factors can make observation of the changes to soil AS induced by organic amendments difficult to separate from the inherent seasonal variations in the field.

### 1.1.6 Conclusion

In section 1.1, we have established that the protection of soil physical fertility is linked to the stability of the aggregates of which it is composed. Although governed by intrinsic soil properties, aggregate formation and stabilisation are heavily affected by plants and soil microorganisms through their deposition of certain forms of soil organic matter, referred to as binding agents. The soil microorganisms that deposit binding agents are also responsible for their eventual destruction, and so their activity leads to a dynamic process of aggregate creation, stabilisation and breakdown. Human agricultural activities and climatic conditions in turn have a considerable influence on these processes and therefore generate further dynamic changes in the soil AS.

An important method of positively affecting the soil AS is the incorporation of various organic amendments that add into the soil OM and stimulate microbiological activity. The quality of the OM in an organic amendment, in terms of its biodegradability, is important in regulating the microbial response by which aggregate binding agents are deposited, and by which they are eventually broken down over the short- to medium- term. The various biotic mechanisms (production/destruction of biological binding agents) and abiotic mechanisms (introduction of binding agents or disruptive agents in the added material) by which organic amendments may affect soil aggregate stability have been identified in the literature, though it is not always clear which mechanisms predominate for a given organic amendment and in a given context.

A number of models have been developed and refined over recent years intended to predict the dynamics in soil AS in response to organic amendment of varying quality. However, these mostly mechanistic models have been developed for typical organic amendments such as straws, crop residues, and composts, and they have yet to be successfully applied to more complex organic residues such as sewage sludges or digestates, where AS dynamics do not necessarily follow easily predictable patterns. Furthermore, they are not yet able to take into account the direct effects that the mineral component as well as certain OM forms in the amendment may have on soil AS. The impact of an organic amendment is also clearly modulated by the soil initial physical properties (texture, OM content, pH, exchangeable ions), biological properties (microbial quantity, activity and diversity), and, in a field context, the climatic conditions (seasonal temperature variations, wetting-drying cycles). It appears necessary to develop a greater understanding of the reasons behind complex dynamics in soil AS following organic amendment, and how they may be regulated by other factors depending on the agricultural context.

## 1.2 Digestate production and valorisation in agriculture

### 1.2.1 Introduction

In oxygen-free environments such as within swamps, deep-sea sediments, and the guts of ruminant animals, organic matter may naturally be broken down and metabolised by various microorganisms leading to the release of a mixture of methane and carbon dioxide, known as “biogas”. This process is called anaerobic digestion, and may be harnessed for the treatment of organic wastes and production of biogas for combustion and energy recovery through the use of purpose built “digesters”. In industrial scale anaerobic digestion, one or more different feed biomasses or “substrates” are fed into a tank held under anaerobic conditions, where a stable community of facultative anaerobic bacteria, obligate anaerobic bacteria, and archaea metabolise this material, and the produced biogas is collected and either stored or directly combusted to generate heat and electricity. The remaining material at the end of this process is commonly known as “digestate”, and this is the term hereby used to refer to all the by-products from anaerobic digestion, including those produced from subsequent post-treatments (see Fig. 1.10).

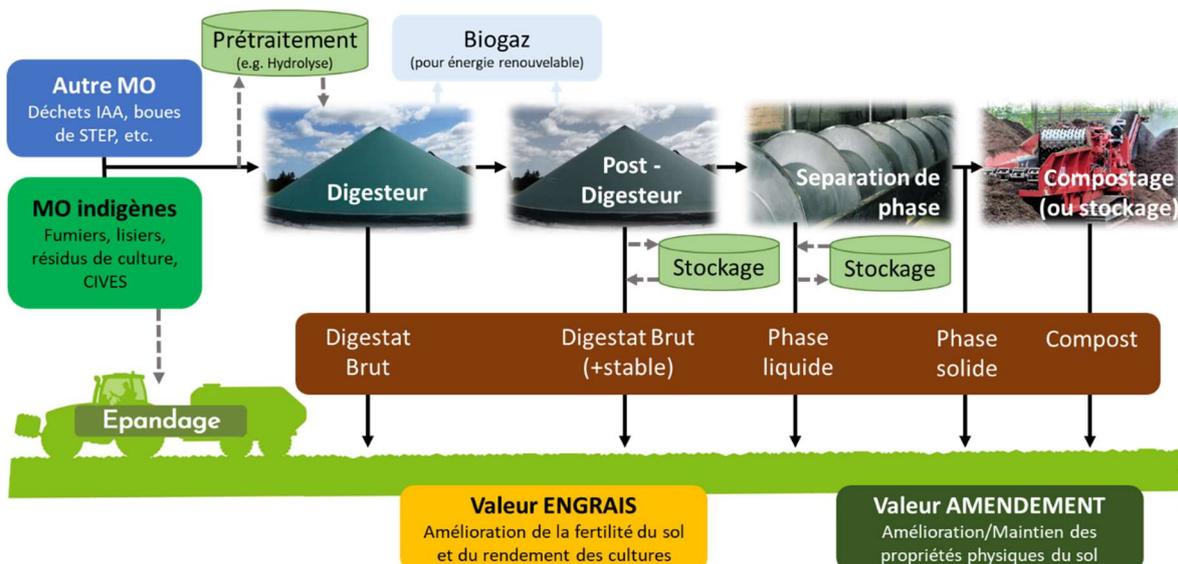


Fig. 1.10 Conceptual schematic of an on-farm anaerobic digestion site with two-stage wet digestion, plus pre- and post-treatment of digestate. The digestate may, in theory, be taken at various steps in the process and used in agriculture as either organic fertiliser or amendment

## 1.2.2 Matter transformation in anaerobic digestion

### 1.2.2.1 Disintegration and solubilisation

The process of AD begins with the breakdown and disintegration of larger molecules and dead cells that make up the majority of most substrates, in order to become accessible to microorganisms (and their enzymes) in the digester. This is considered to be a non-biological step, consisting of lysis, non-enzymatic decay, phase separation and physical breakdown (e.g. from shear forces by pumps, conveyors and mixers) (Batstone et al., 2002). The structure and available surface area of the material being digested is important for determining the rate and efficiency of hydrolysis, as it is reliant on the ability of enzymes to access and adsorb onto biodegradable sites (Azman et al., 2015). It is suggested that, while initial particle size of the substrate does affect the rate of solubilisation, after a certain minimum time the degree of solubilisation stabilises (Jash and Ghosh, 1996). However other studies propose that reducing particle size does lead to increased methane yields (Hartmann et al., 2000; Sharma et al., 1988).

### 1.2.2.2 Organic matter conversion to biogas

The biological conversion of OM into biogas occurs through four broad transformation steps, namely: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Moletta, 2011) (see Fig. 1.10).

#### 1.2.2.2.1 Hydrolysis

In order for microorganisms to absorb molecules, they must be sufficiently small to pass the cell wall. This occurs through extracellular hydrolysis of complex organic polymers into their component molecules by bacterial exuded enzymes. The linkages between the monomers forming long chain polymers (such as polysaccharides, proteins and lipids) are broken down in the presence of water to form simple molecules (such as sugars, amino acids, peptides, volatile fatty acids and long chain fatty acids). A large variety of bacteria can be involved in hydrolysis under anaerobic conditions, including both strict and facultative anaerobes, though the physiology and biochemistry of these organisms is still little understood (Azman et al., 2015).

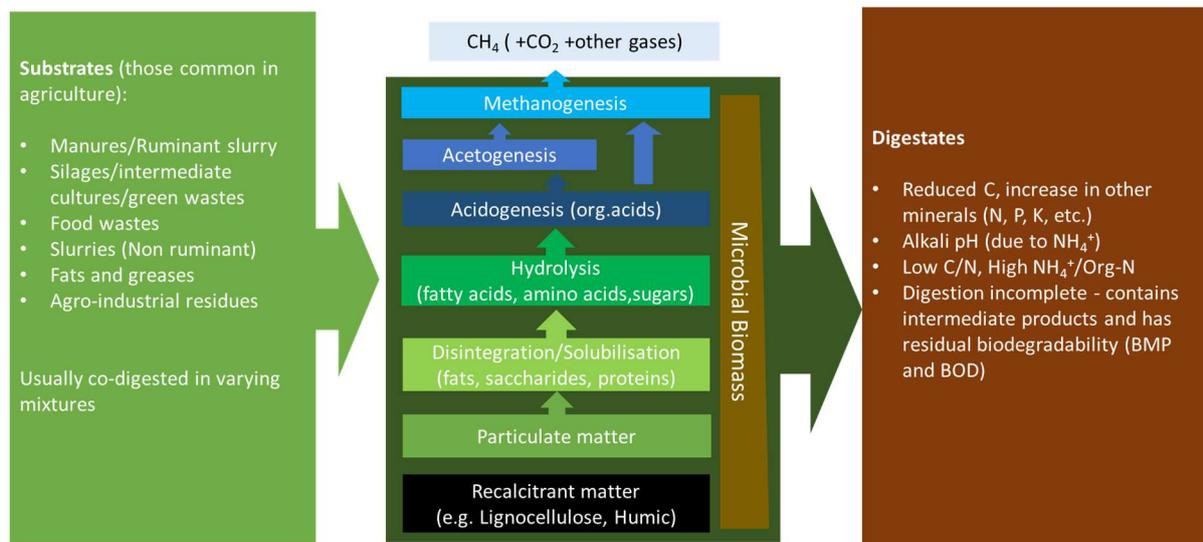


Fig. 1.11 The transformation of organic matter over the course of anaerobic digestion

#### 1.2.2.2.2 Acidogenesis

Once complex polymers have been broken down to a sufficient extent, a wide range of bacteria can take up these components for further transformation (Dewil et al., 2011). The monosaccharides and amino acids produced are metabolised into volatile fatty acids, alcohols, organic acids, organic nitrogen and sulphur compounds, and producing gases such as hydrogen, carbon dioxide and ammonia. Lactic acid is produced as an intermediary compound, which can strongly reduce the pH of the digester leading to inhibition (acidosis) if not broken down as quickly as it is produced (Weiland, 2010).

#### 1.2.2.2.3 Acetogenesis

Volatile fatty acids are in turn taken up along with long chain fatty acids by two groups of acetogenic bacteria – obligate hydrogen producing acetogenic bacteria produce acetate, hydrogen and carbon dioxide, while another group of specific bacteria convert the hydrogen and carbon dioxide back into acetate (Dewil et al., 2011).

#### 1.2.2.2.4 Methanogenesis

The dissolved acetate is then taken up by acetotrophic methanogens (Archaea) to produce methane and carbon dioxide, or else hydrogenotrophic methanogens may also take up dissolved hydrogen and carbon dioxide to produce water and methane (Dewil et al., 2011).

#### 1.2.2.3 Other organic matter transformations

Aside from the above-described processes, OM may undergo a number of simultaneous transformations in anaerobic digestion, not necessarily culminating to biogas production (Bareha, 2018; Fernández-Domínguez et al., 2021). Around 5-10% of the incoming organic matter may be converted to microbial biomass that will subsequently remain in the resulting digestate as microbial necromass and various microbial residues (Moletta, 2011). There is some difficulty in tracking changes to OM from transformation in AD, particularly when using non-quantitative spectral methods, as it is not possible to determine whether products are simply accumulated or transformed (Shakeri Yekta et al., 2019). Bareha (2018) used chemical fractionation techniques to trace the conversion of protein and other organic N forms over the course of AD, and noticed a gradual accumulation of inert OM in the soluble fractions of digestates.

#### 1.2.2.4 Nitrogen mineralisation in anaerobic digestion

During the biological breakdown of proteins, amino acids and other nitrogen containing organic compounds in the oxygen free conditions of the digester, ammonium ( $\text{NH}_4^+$ ) is released as a product of this mineralisation. The accumulation of  $\text{NH}_4^+$  means that digestates are usually mildly alkaline (pH 7-9) (Kirchmann and Witter, 1992; Riva et al., 2016). Mineral nitrogen ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$ ) may equally be taken up by microorganisms and reorganised into amino acids and proteins. However, overall, the anaerobic digestion process increases the quantity of mineral nitrogen relative to organic nitrogen (Kirchmann and Witter, 1992). The resulting digestate will usually contain a mixture of ammonium, proteins and amino acids deriving from microbial activity, as well as complex organic

nitrogen compounds that were contained in the feed material but were not mineralised (Möller and Müller, 2012).

#### 1.2.2.5 Chemical alterations to other minerals

Minerals in the feed substrates, as they are generally not volatile, are retained in the digestate. In a similar manner to nitrogen, these minerals may be released into more soluble forms from organic materials broken down during anaerobic digestion or reorganised into new organic structures (Laera et al., 2019). While dynamics of particular minerals in anaerobic digestion depend on multiple factors, evidence suggests that many minerals in digestates are actually less easily extractable than in the substrates (see Fig. 1.11), and are possibly bound up with more recalcitrant OM forms.

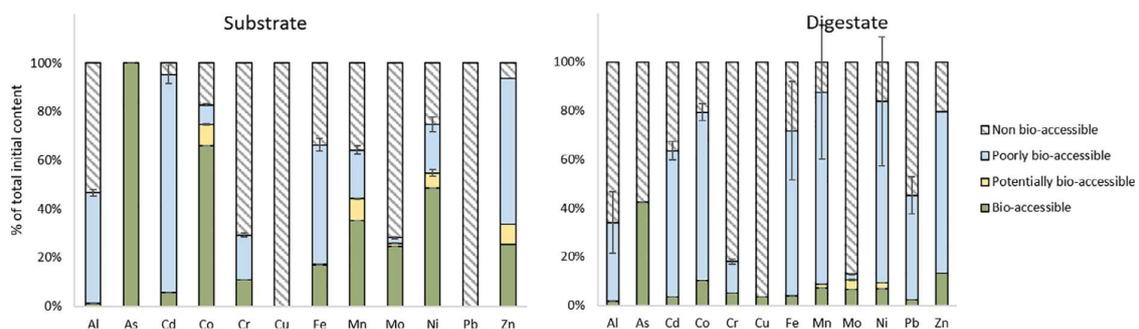


Fig. 3. Interpretation of metals fractionation in terms of potential bio accessibility in substrate and digestate.

Fig. 1.12 Average bioaccessibility of trace minerals for a number of substrates and digestates (Laera et al., 2019)

#### 1.2.2.6 Recalcitrant and poorly bioaccessible organic matter

A fraction of organic matter passing through a digestion process will remain relatively unchanged, either due to its inability to be broken down by microbes under anaerobic conditions, or through the structure of the surrounding recalcitrant organic material rendering it resistant to attack by microbial enzymes (Jimenez et al., 2014). In particular, substrates high in ligno-cellulosic material prove difficult to hydrolyse during the digestion process due to the recalcitrance of lignin structures that surrounds more biodegradable OM (Shrestha et al., 2017). Spectral analysis of digestates indicate that chemically recalcitrant organic compounds, such as long-chain aliphatic compounds, complex proteins, and large

molecular weight aromatic compounds (e.g. polyphenols), are unchanged over anaerobic digestion and remain in the digestate (Kovačić et al., 2022; Provenzano et al., 2011; Shakeri Yekta et al., 2019; Tambone et al., 2013). Furthermore, biochemically recalcitrant lignin and cellulosic structures may shield more biodegradable material from breakdown (Bareha et al., 2018). Jimenez et al. (2015) have demonstrated that the biodegradability of substrates in digestion is controlled by the “bioaccessibility” of different forms of organic carbon present in the substrates.

#### 1.2.2.7 The organic matter composition of digestates

Digestates share a number of properties in common as a group of organic residues. Due to the conversion of organic matter to biogas during the digestion process, they are generally lower in organic C and have therefore lower C-N ratios than their undigested substrates. They are also poorer in organic N, though richer in ammonium nitrogen ( $\text{NH}_4^+$ ). Digestates from agricultural sources may equally share common OM characteristics, with a high content in aromatic compounds, high molecular weight olefinic compounds, short-chain organic acids, amines and low molecular weight amides (Iocoli et al., 2019). Bareha (2018) proposed that the OM in digestates is likely to be comprised of:

- Organic compounds remaining from the chain of conversion of OM into biogas (as summarised in section 1.2.1.4.2) (e.g. partially hydrolysed polysaccharides, proteins, and lipids, volatile fatty acids, other organic acids, etc.),
- Microbial products from the growth and deactivation of biomass (i.e. cell structures, necromass),
- Recalcitrant organic compounds and structures, resistant to breakdown under anaerobic conditions, and therefore unchanged over AD (e.g. lignin, hemicellulose, complex proteins, polyphenols),
- Organic compounds residing in complex structures that protected them from breakdown during AD (e.g. proteins and simple molecules trapped inside ligno-cellulosic plant material).

The prevalence of these different OM fractions in the digestate may be governed by the process operating parameters applied in AD, including the feed substrate composition, reactor design, process temperature and residence time in treatment, which are discussed in section 1.2.4. The raw digestate may also undergo various post-treatments that will alter the content in OM forms as well as other characteristics of the resulting digestate, and this is discussed in section 1.2.5.

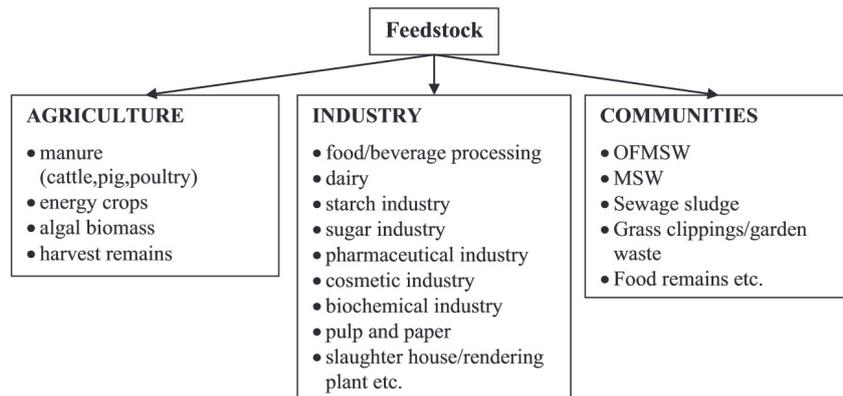
### 1.2.3 Digester operating parameters and their impact on digestate characteristics

#### 1.2.3.1 Feed substrates to anaerobic digestion

A wide range of organic materials may be fed into anaerobic digestion (see Fig. 1.11), including both waste streams such as manures, slurries, sewage sludges, food wastes, industrial wastes, and municipal solid wastes, and specifically produced biomass such as cover crops or dedicated energy crops (Moletta, 2011). Organic inputs, even those coming from the same source, may vary considerably in quality depending on their source, their storage and the season (Weiland, 2010). A large proportion of the substrates currently used in anaerobic digestion in France come from animal wastes, crop residues, and dedicated energy crops or cover crops (see Fig. 0.4). This is equally the case from a European perspective (Weiland, 2010), though with an increasing interest in the productivity of dedicated energy crops in AD.

France is one of the largest producers of animal manures in the EU, and manures or slurries are more often the principal feedstock used in on-farm anaerobic digesters (Kovačić et al., 2022). Manure has a number of advantageous properties, including its concentration in important micronutrients for optimal microbial growth, and an elevated buffering potential that prevents acidification of the digester (Béline et al., 2010). The methane production potential of animal wastes is low, however, and so various feed materials are frequently combined in “co-digestion” systems (Lukehurst et al., 2010),

with the intention of improving biogas yields, balancing nutrients required by anaerobic microbes, and to stabilise process operation (Weiland, 2010). In an agricultural context, anaerobic co-digestion therefore represents not only the transformation of manures and plant matter that are produced on site, but the introduction of “novel” waste streams, not previously destined for use in agriculture, into the system.



*Fig. 1.13 Types of feed substrates for anaerobic digestion. OFMSW = organic fraction of municipal waste, MSW = municipal solid waste (Kothari et al., 2014)*

Digestates may retain a number of characteristics of their feed substrates. Digestates from manures are rich in nutrients such as P and K (Lukehurst et al., 2010), as well as many other trace minerals that may be supplemented into animal feed (Albuquerque et al., 2012c). Substrate source is believed to be a major contributor to digestate OM characteristics, with studies showing similarities between substrate and digestate spectral profiles, both for NMR spectroscopy (Shakeri Yekta et al., 2019) and FTIR and fluorescence spectrometry (Provenzano et al., 2011). Aromatic and therefore recalcitrant components found in substrates are preserved in the digestate (Fernández-Domínguez et al., 2021; Tambone et al., 2013). Fulvic acid-like, glycolated protein-like, melanoidin-like and humic acid-like substances present in cow manure remain in the liquid fraction of digestate (Akhiar, 2017).

#### 1.2.3.1.1 Pre-treatment of substrates

Before entry into the anaerobic digester, feed materials may undergo pre-treatment, generally intended to improve organic matter breakdown and biogas yields. For wet digestion processes, the

feed material must inevitably be brought to a liquid consistency via addition of wet materials or water coupled with mechanical breakdown of large solids (Weiland, 2010). More advanced processes such as steam explosion, saponification, enzymatic or thermal hydrolysis may be used to initiate and accelerate the breakdown of OM structures otherwise more slowly broken down in the digester, thus speeding up the biogas production and reducing the required substrate treatment time in AD (Carrere et al., 2016).

Little is currently known about the impact of substrate pre-treatment on the quality of OM in the digestate, and much of existing research on pre-treatments is mainly focussed on the improvement of biogas yields rather than the digestate. It is possible that, beyond accelerating the breakdown of OM in anaerobic digestion, and therefore reducing labile OM content compared to recalcitrant OM, these processes change little of the quality of the digestate.

#### 1.2.3.2 Reactor design

Anaerobic digesters are broadly divided into two types, those operating on low solids (<15%) regimes known as “wet” digesters, and those operating on high solids regimes (20-40%) known as “dry” digesters (Moletta, 2011; Weiland, 2010). Dry anaerobic digestion plants can be configured as a series of “batch” reactors or, less commonly, as “plug-flow” type reactor. In “plug-flow” reactors, solid feed substrates are mixed with some treated digestate used as inoculum, fed in one end of a long reactor, conveyed horizontally through, and digestate fed out at the other end. Batch reactors are loaded with solid substrate mixed with a predetermined proportion of treated inoculum, then sealed to allow anaerobic conditions to be established. Liquid leachate pooling at the bottom of the reactor is often recirculated and sparged over the top of the substrate to improve digestion (Kothari et al., 2014). The decision on installing a dry reactor configuration is normally based on the type of substrate to be digested. Digestate from dry reactors have a number of benefits, such as the relative ease of transportation due to lower water content, and their high content in OM (Kothari et al., 2014). Dry AD reactors are not widespread, due to a number of operational issues around inhibition and the long

retention times required to reach similar digestate stability and biogas yields when compared to wet systems (Kothari et al., 2014).

The most frequently occurring type of reactor in agricultural AD is the wet digester under a continuously stirred tank reactor (CSTR) operation (Béline et al., 2010). In a continuous, wet-phase anaerobic digester, pre-mixed substrates are pumped into a sealed tank, covered by either a solid cap connected to a separate biogas storage bag or covered by a single or double membrane roof that expands to store the produced biogas (Weiland, 2010). Tanks may be placed in series as a primary and secondary digestion step to allow accumulation of digestate in the secondary tank while maintaining biogas production. These tanks must be stirred using one of several possible systems, the most common being mechanical stirrers (Weiland, 2010), in order to improve contact between substrate and inoculum, facilitate biogas bubble rise in the liquid, and to maintain homogeneous temperature throughout the reactor.

Apart from the obvious differences in the moisture content of digestate produced from either wet or dry AD, the differences in the types of feed substrates placed in these systems and the efficiency with which OM is broken down in the reactor will lead to differences in digestate quality. Digestates from dry systems will tend to have higher C-N ratios, while those from wet systems tend to be more concentrated in minerals such as N, P and K (Guilayn et al., 2019a). Little information could be found on comparison of the differences in OM quality of digestates from various reactor types.

### 1.2.3.3 Temperature

Maintaining a relatively constant sludge temperature inside the digester is crucial for its stable operation, as the microbial communities prevalent in digesters are only able to metabolise efficiently within certain temperature ranges. Anaerobic digesters can be operated within one of three temperature regimes – psychrophilic (10-25°C), mesophilic (30-40°C), and thermophilic (45-60°C) (Moletta, 2011). Psychrophilic digesters are not very common as generally very slow to produce biogas thus requiring much higher lengths of time in digestion (Kovačić et al., 2022) and so are not considered

further in this research. Thermophilic digesters are generally the most efficient, able to operate at higher solids loading rates and lower lengths of time in digestion to obtain similar biogas yields (Weiland, 2010).

While higher operating temperatures increase the rate of breakdown of organic matter into biogas, mesophilic processes are more stable than thermophilic, and so most anaerobic digesters operate at this temperature regime (Béline et al., 2010; Kovačić et al., 2022). Little information is available on the comparison of digestate from agricultural AD plants operating at different reactor temperature regimes (Gebreyessus and Jenicek, 2016). Comparison of thermophilic and mesophilic AD of sugar beet pulp has shown mesophilic digestates to contain higher concentration of volatile solids, including a large proportion of soluble microbial products that were not present in the thermophilic digestate (Suhartini et al., 2014).

#### 1.2.3.4 Residence time

In AD, the average time that material is subjected to anaerobic treatment in the digester is referred to as the hydraulic residence time (or retention time) (HRT). Assuming a homogeneously mixed system in wet digestion systems, the HRT can be calculated as the approximate volumetric feed rate of substrate divided by the functional volume of the digester. The same principal may equally be applied to batch or plug flow digesters operating in the solid phase, though in this case referred to as the “solids retention time”. For the sake of simplicity, residence/retention times in wet or dry systems are hereby referred to as HRT. The HRT is normally set to optimise digester stability in terms of biogas production rate vs. feed input rate, but may also include considerations such as pathogen destruction in the digestate (Moletta, 2011).

The longer the HRT, the more “stable” the OM in the digestate (Nyang’au et al., 2022), as anaerobically biodegradable OM will continue to be broken down. Biogas production drops towards the end of anaerobic treatment of a volume of digestate, and so, where the objective is to maintain the highest methane production rates, the HRT may be shorter than the time needed to fully stabilise the

digestate. This will increase the undigested organic matter content of the digestate and equally its volatility, toxicity and pathogen content (Nkoa, 2014). As the digestion process rarely arrives at 100% completion within a set HRT, many of the products generated from the breakdown of the substrate OM to biogas, such as volatile fatty acids, may be present in the fresh raw digestate. Longer HRTs may lead to increased solubilisation of humic- and fulvic- like substances in the digestate (Bareha et al., 2018). However, the effects of varying HRT on digestate quality from anaerobic digestion of different substrates are still little understood and difficult to determine amongst the other factors affecting the OM quality of the digestate (Fernández-Domínguez et al., 2021).

## 1.2.4 Post-treatments and their impact on digestate characteristics

### 1.2.4.1 Secondary digestion and storage of liquid digestate

Often, due to limitations in the timing of digestate spreading on agricultural land, digestate will undergo a considerable amount of time in closed or open storage before use (Al Seadi et al., 2013; Lukehurst et al., 2010). From the primary anaerobic digester, the resulting digestate therefore may be passed to a secondary tank, which often serves as a point for accumulating and storing treated digestate prior to other post-treatment or direct application to the soil. This tank may be covered and heated to act as a secondary digester, in which case the anaerobic digestion process continues and biogas may be produced and collected. In this case, the transformation of the digestate is equivalent to that which occurs in the primary digester and the HRT is increased proportionally to the volume of the secondary digester. In other cases, the secondary tank may be covered but unheated, in which case biogas production is either reduced or lost, although other changes in OM may continue to occur (Jimenez et al., 2020a). Digestate may also be transferred to open-air storage prior to field application. Open-air storage of digestate leads to nitrogen losses through ammonia volatilisation as well as the release of greenhouse gases ( $\text{CO}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ), with losses of total N from 14-20% for raw liquid

digestates (Perazzolo et al., 2015). However, the effects on OM of raw liquid digestate exposure to the pseudo-aerobic conditions of a storage pond are not well documented in the literature.

#### 1.2.4.2 Dewatering or phase separation

The basic principle of dewatering, or phase separation, is to extract liquids from the raw digestate while retaining the solid material, thus obtaining two separate “phases”. Phase separation of digestate is a fairly common process in AD, as it allows separation of a nutrient-rich (N, K) liquid phase from a more P and OM-rich (TS = 20-30 %) solid phase (Lu and Xu, 2021; Nkoa, 2014), which can be used separately depending on fertilisation requirements in agriculture. Phase separation can be carried out on an industrial scale via a number of different means, most commonly through use of a screw press, but occasionally using a centrifuge, belt press, vibrating screen, or rotary drum (Guilayn et al., 2019b). Different phase separation processes exhibit varying levels of efficiency in terms of the total solids, OM and nutrients transferred into either the solid or the liquid phase (Akhiar, 2017; Lukehurst et al., 2010) (see Fig. 1.14). The separation process used is to some extent determined by the feed input to AD, with “low-performance” (mainly screw presses) taking principally digestate from fibrous inputs such as cow manure and silage, and “high-performance” separators (e.g. decanting centrifuges) used principally to separate non-fibrous digestate from inputs such as pig slurry, sludge and agro-industrial waste (Guilayn et al., 2019a).

Solid fractions of digestates are typically characterised by high concentrations of readily biodegradable OM, as they are concentrated in the plant fibres not broken down over AD (Maynaud et al., 2017; Teglia et al., 2011). High-efficiency decanting centrifuges leave relatively little in the liquid phase except nitrogen and soluble OM, while low-efficiency screw presses tend to lead to a majority of nutrients, excluding P, Mg and Ca, remaining in the liquid phase (Guilayn et al., 2019b) (see Fig. 1.14). The OM in the liquid phases of digestates from screw-press separation is highly stable, with a recalcitrance to biological breakdown similar to the OC in composts (Tambone et al., 2019). However,

in a separate study the liquid fraction from low efficiency separators taking cow manure digestate was found to still contain large amounts of degradable OM (Akhiar et al., 2021).

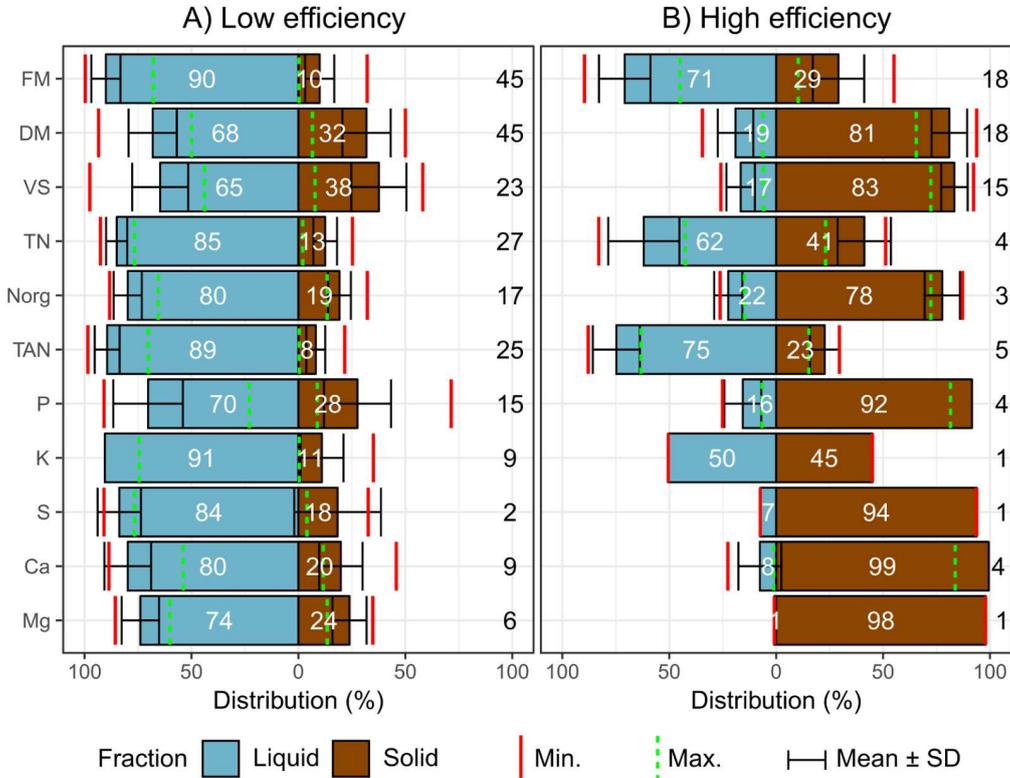


Fig. 1.14 The repartition of digestate components following a) “low efficiency” phase separation (mainly screw presses) and “high efficiency” separators (mainly decanting centrifuges) (Guilayn et al., 2019)

Other processes for further dewatering and stabilisation of the digestate are possible, though less common, such as thermal drying or bio-drying that heat the digestate and drive off moisture (Lu and Xu, 2021). These processes are fairly innovative and costly, and not widespread in on-farm AD, so they are not considered further in this study.

1.2.4.3 Composting or open-air storage of solid digestate

Composting of the solid digestate or solid phase of digestate from phase separation is required in order to qualify as a true “organic amendment” as defined by French standard NF U44-051 (2006). This frees the use of the produced compost from the restrictions in place on the land application of manures and slurries that also apply for uncomposted manure-based digestates. Aerobic post-treatment may

equally be desired to reduce phytotoxicity or gas emission potential (Teglia et al., 2011). Organic matter resistant to breakdown during anaerobic digestion may still be biodegradable under aerobic conditions so that, upon composting, this organic matter will be lost to mineralisation (Maynaud et al., 2017; Teglia et al., 2011). Digestate may need to be composted with other waste streams, such as woody wastes, to improve composting conditions (Al Seadi et al., 2013), which will alter the composition of the final composted digestate considerably.

Open-air storage and curing of digestate, as opposed to composting, are uncontrolled processes involving pseudo-anaerobic conditions where not all of the digestate will typically be stabilised and some volatile organic compounds may continue to be formed (Drennan and DiStefano, 2010). Nonetheless, organic C and N may be rapidly lost to volatilisation during open-air storage (Hansen et al., 2006), though the speed of transformation will depend on the ambient temperature and level of aeration. Thus, for digestate in unregulated storage tanks or heaps, the transformation of organic matter is likely to be highly uneven and difficult to predict, and to date there is little published data on the subject.

### 1.2.5 Conclusion

In this section, we have considered the anaerobic digestion process from the perspective of the conversion of organic materials into digestate. The biological process of anaerobic digestion is relatively well understood from the point of view of production of biogas. However, the complex organic matter transformations occurring in parallel within a digester, and their impact on the remaining digestate OM quality, are still not fully understood. Anaerobic treatment of a diversity organic materials including manures, slurries, crop residues, and agro-industrial wastes may be conducted using a variety of digester configurations, operating at differing temperatures and hydraulic residence times, which will affect the resulting composition of the raw digestate. Further to this, raw digestate may undergo further transformation using post-treatments, such as phase separation to

generate a solid and liquid phase, or composting to produce composted digestate. The composition of a given digestate is therefore a function of the numerous feed materials used, the operating parameters of the digester, and the subsequent treatments applied to it. The composition of the digestate can in turn be expected to affect its impact when used in agriculture in various ways, and this is discussed in the next section.

## 1.3 The agricultural use of digestates as organic fertiliser and amendment

### 1.3.1 Introduction

While most digestates are undeniably valuable for their rich content in soluble and plant-available nutrients, they are also an important source of organic matter for return to the soil. This means that they may be considered as both an organic fertiliser and amendment (see Fig. 1.12), though their impact as one or the other depends largely on their physico-chemical properties (Total solids, C-N ratio,  $\text{NH}_4^+$  content, etc.). In this section, we consider the existing research around the various impacts of digestate application in agriculture, taking into account their effects on soil chemical fertility, soil organic carbon, soil microbiology, and the soil structure.

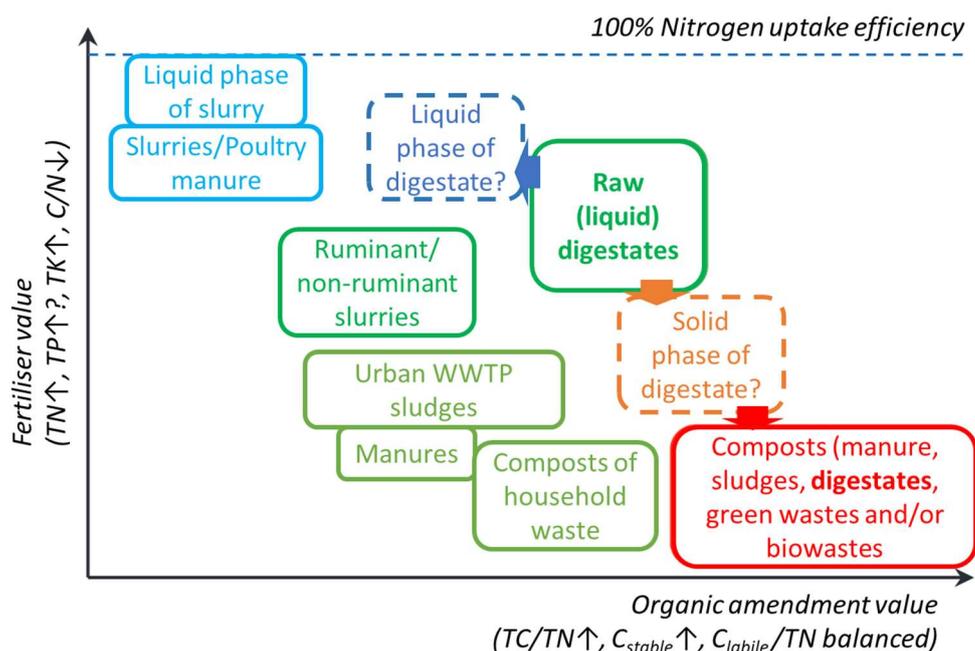


Fig. 1.15 The relative fertiliser and amendment value of various “exogenous” organic materials, including raw digestate, with the expected changes in their value following phase separation and composting. Some desirable physico-chemical properties in each case are listed on the axes (Translated and adapted from (Houot et al., 2014))

### 1.3.2 Digestate impact on soil chemical fertility

Raw digestates provide N, P, K and S to the soil, leading to higher crop yields and nutrient contents (Bhogal et al., 2016). Due to their high mineral nitrogen content (in the form of  $\text{NH}_4^+$ ), field application of raw liquid digestates may give crop yields equivalent to synthetic nitrogen fertilisers (Albuquerque et al., 2012b; Walsh et al., 2012). However, a portion of the mineral N in digestate will always be lost directly after spreading due to ammonia volatilisation. Overall nitrogen uptake efficiency (compared to N fertiliser) of digestate has been found to vary widely depending on the digestate type, application methods, crop types and soil conditions falling generally between 42 and 65% (Le Roux and Michaud, 2021).

Though raw digestates may be used directly as fertiliser, the balance of nutrients contained in digestate is not always matched to crop requirements, and so there are risks of nutrient accumulation (Lukehurst et al., 2010). There is considerable published research, including several in depth studies, investigating the nutrient content of digestate with a view to their use as fertiliser for crop growth (Albuquerque et al., 2012c; Guilayn et al., 2019a; Jimenez et al., 2020b; Zennaro et al., 2022). In order to better define the potential fertiliser value of a wide variety of digestates (Guilayn et al., 2019a) proposed a typology dividing digestates into six groups (see Fig. 1.13) depending on the type of feed substrate used and the type of AD process used (wet or dry).

Post-treatment of digestates provides a means of altering the nutrient profile of digestates, as well as improving/reducing characteristics that may otherwise be potentially harmful to the soil (Albuquerque et al., 2012c) (see Fig. 1.14). Liquid phases of digestate from phase separation are proportionally very high in N, with low C-N ratios, and so make potentially better substitutes to mineral-N fertilisers (Tambone et al., 2017). However, the distinction between a “nutrient-rich” liquid phase and “fibre-rich” solid phase is not always clear, as the efficiency of the separation process used is an important aspect in determining how materials are divided between the two phases (Akhlar et al., 2021; Guilayn et al., 2019a). Recent in-depth study of phase separated digestates from a number

of sites has revealed that a significant fraction of dry solids (and therefore organic matter) may be retained in the liquid phase, while a significant concentration of N and P remains in the solid (Akhiar, 2017; Tambone et al., 2017). The typology developed by (Guilayn et al., 2019a) also took into account the redistribution of solids, C, N and other nutrients into digestate phase from mechanical separation (the same dataset was used in the analyses of phase separator efficiency in Fig. 1.14, though not in terms of the typology).

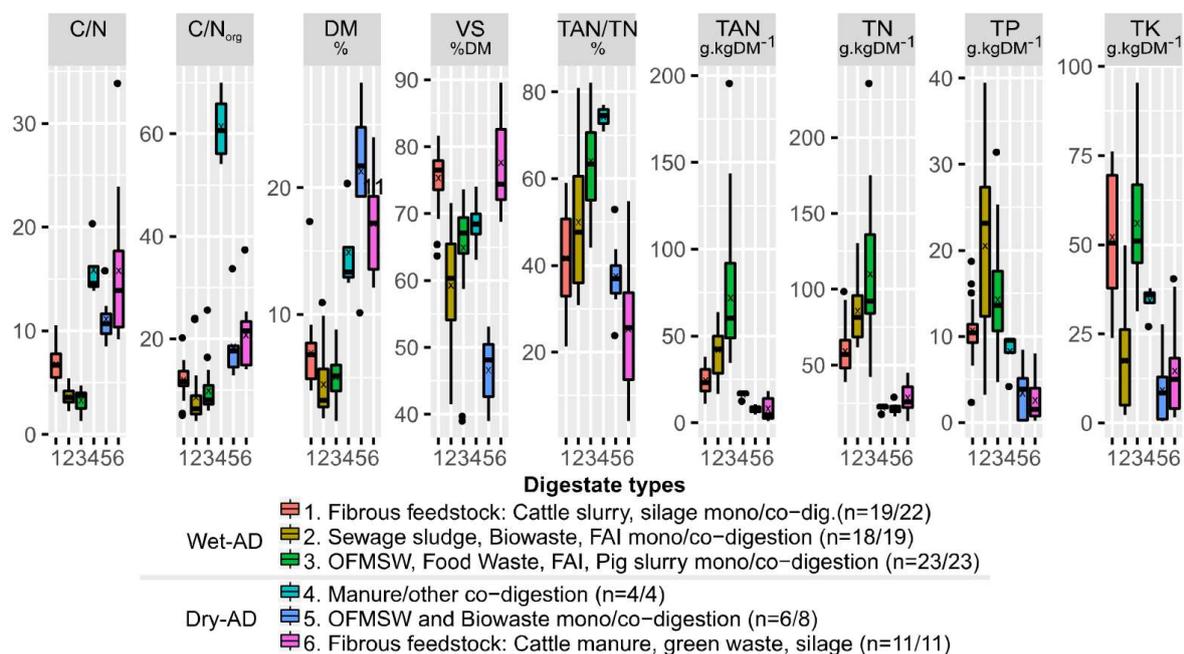


Fig. 1.16 Characteristics of a panel of 91 raw digestates produced under varying conditions (Guilayn et al., 2019a). Numbers represent the class in the first-fertilising typology developed by the authors, as described in the key above.

### 1.3.2.1 Environmental impacts of fertilisation with digestate

Though mineral N rich digestates are potentially powerful fertilisers, their application also presents a higher risk of ammonia emissions when compared to addition of the undigested substrates (Albuquerque et al., 2012c). Soil incorporation of poorly stabilised digestate may also lead to rapid mineralisation and reorganisation of the added mineral N, thus decreasing its availability for crop growth (Albuquerque et al., 2012a). A balance of minerals is essential for plant growth, though the high levels of certain minerals (e.g. Cu, Zn, Mn) observed in some digestates means that repeated

applications may lead to accumulation and toxicity over time, and may even stop biological functions of the soil leading to compaction, reduced drainage and OM accumulation (Nkoa, 2014). However, longer term observation of successive digestate additions on soil total metals have not revealed increases in concentrations in soil or in crops (Bhogal et al., 2016). A panel of agricultural derived digestates analysed by Tambone et al. (2017), showed acceptable levels of heavy metals when compared to EU recommended levels and compared to other organic materials used in agriculture.

Concerns equally exist around the potential harmfulness of digestate application toward crop germination (Nkoa, 2014; Panuccio et al., 2021), and the presence of various residual organic acids in the digestate has been associated with phytotoxicity (Salminen et al., 2001). However it is argued that this may depend on the rate of digestate application and that, at the right dose, digestates may even promote better seed germination (Alburquerque et al., 2012c).

### 1.3.3 Digestate impact on soil organic carbon

As anaerobic digestion represents the conversion of labile organic matter contained in the feed materials, the produced digestate nearly always contains a higher proportion of recalcitrant OM (Thomsen et al., 2013). However, under the aerobic conditions in the soil, certain digestates may still show relatively high levels of C mineralisation, likely as a result of the breakdown of decaying microbial biomass produced over the course of AD as well as residual biodegradable compounds that could not be broken down under anaerobic conditions (Alburquerque et al., 2012a). Furthermore, digestate addition may lead to a positive “priming effect” where a portion of native soil C is also exposed and lost to mineralisation (Alburquerque et al., 2012a; Coban et al., 2015). This appears to depend on the digestate stability (low length of time in digestion, poorly digested feed substrates) leading to a high labile C content and/or a low C-N ratio (< 2?). Conversely, the addition of stable digestates (with long HRT) to soil may generate a “negative priming” effect through reduction of mineralisation rates of native SOC breakdown (Béghin-Tanneau et al., 2019; Nyang’au et al., 2022). For the latter case, the

use of digestates is recommended over the use of highly labile OM sources such as fresh plant matter that can produce positive priming effects.

The solid phase of digestate is often considered to be most effective as an organic amendment due to its proportionally higher concentration in OM (Nkoa, 2014; Panuccio et al., 2021). However, it has been found that the liquid phase contains recalcitrant, aromatic OM compounds that could also be useful in soil amendment (Akhiar, 2017; Tambone et al., 2019). Comparison of CO<sub>2</sub> emissions from digestates before and after different post-treatments suggests that soil amendment with composted or dried digestates will lead to greater increases in SOC, while raw digestates nearly always lead to greater CO<sub>2</sub> emissions and so reduced improvements to SOC (Askri et al., 2016). Outside of controlled incubation trials, very high CO<sub>2</sub> emissions have been observed from agricultural soil amended with digestate in the days following spreading, potentially leading to the loss of the majority of the added C (Czubaszek and Wysocka-Czubaszek, 2018).

Changes in SOC levels from digestate application are likely very slow and, even after several years, can prove difficult to detect in field trials (ADEME et al., 2016; Bhogal et al., 2018). The application of manures, including digestates produced from animal wastes, is restricted in Europe by the Nitrates Directive to around 170 kg<sub>N</sub> Ha<sup>-1</sup> (Al Seadi et al., 2013; Kovačić et al., 2022), in order to prevent excessive nitrogen application leading to pollution from infiltration and runoff. This limits the quantity of raw digestate that can be added to the soil, and, as their concentration in N is so high, limits the amount of OM that is added along with it (Bhogal et al., 2016). Furthermore, many of the studies mentioned above do not consider the wider context, in terms of the overall contribution of digestate to soil carbon storage. A study carried out during the thesis of Nicolas Malet (2022), modelled the impact on greenhouse gas (GHG) emissions of replacing soil return of untreated residues and farm effluents with AD and digestate spreading. It was determined from simulations that digestates application may lead to greater carbon storage than the untreated residues, though the overall carbon

emissions would be higher if biogas from AD was not injected and cogeneration heat was not recovered.

The study of net SOC increases or losses leads to the risk of neglecting the importance of labile carbon additions to the soil that may lead to increased soil biological activity and, consequently, increased physical SOM protection from degradation through soil aggregate formation and stabilisation. Indeed more recent soil carbon models place significant importance on microbial breakdown of OM to form compounds that can be protected throughout different levels of the soil structure and soil mineral matrix (see Fig. 1.14). Thus, the importance of the “recalcitrant” OM in digestate for soil organic amendment may be overstated, and it could be that a greater emphasis should be placed on stimulation of soil microbiological activity by the use of organic amendments.

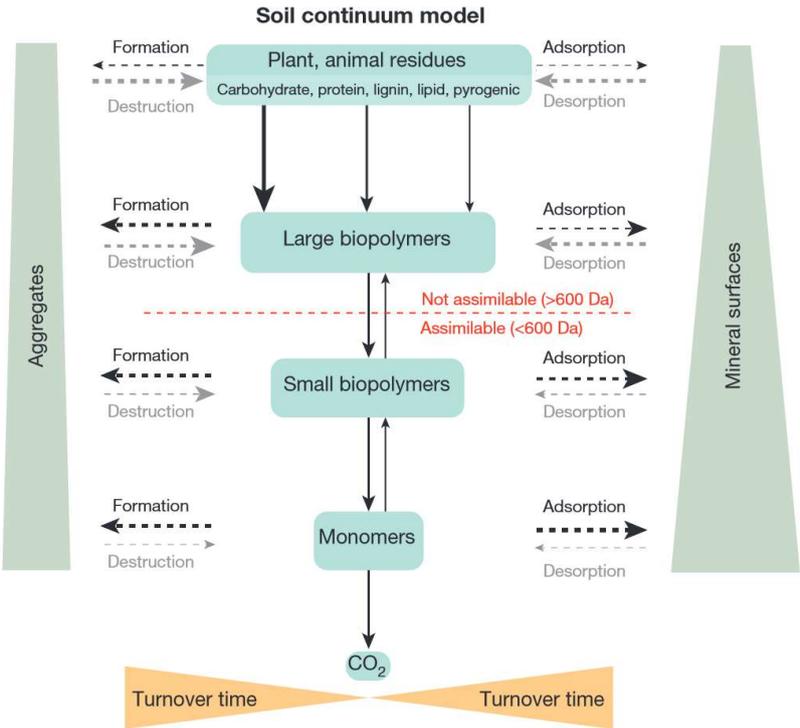


Fig. 1.17 The “soil continuum model” for the fate of organic debris added to the soil (Lehmann and Kleber, 2015)

What is clear from the existing literature is that addition of raw digestates to soil can cause significant increases in microbial activity and rapid carbon mineralisation, though this activity quickly subsides

(Alburquerque et al., 2012a; Risberg et al., 2017; Thomsen et al., 2013). This suggests that digestates contain a highly labile portion of OM that is readily available to aerobic soil microorganisms and which can be quickly consumed. The total C mineralisation from digestates added to the soil can vary greatly, even between digestates produced from similar substrates, with studies reporting predicted values from as little as 11 % up to 60.5 % of added C (Askri et al., 2016; de la Fuente et al., 2013; Häfner et al., 2022; Risberg et al., 2017). Digestates with very low C-N ratios, such as those based on food wastes may show much higher levels of C mineralisation (>60%) than manure digestates with higher C-N ratios (Häfner et al., 2022). Solid digestates contain large amounts of biodegradable OM (Maynaud et al., 2017), that may be interesting for stimulation of soil microbiological activity, but may limit their potential to increase SOC levels.

#### 1.3.4 Digestate impact on soil microorganisms

Digestate incorporation to soil stimulates soil microbiological activity over the short-term, as evident from the observation of C mineralisation discussed in the previous section. However, there is currently limited information into what changes occur to the soil microbial biomass and the soil microbial community dynamics. Short-term increases in soil microbial biomass have been observed (Cardelli et al., 2018; Cattin et al., 2021; Coelho, 2019; Panuccio et al., 2021), though it is believed that these effects may be only temporary. Some evidence points towards raw digestate application generally favouring bacterial communities (Rezacova et al., 2021). However, in other cases, no alteration of microbial communities was been observed shortly after raw digestate incorporation (Cattin et al., 2021; Johansen et al., 2013). Solid digestate incorporation has been shown to favour development of fungal communities (Cattin et al., 2021; García-Sánchez et al., 2015).

Over the medium term, the field application of several different digestates has been shown to have little effect on native soil microbial diversity, despite them carrying a very different mixture of microorganisms to the soil itself (Coelho et al., 2020). However, shifts in soil physical properties, such as pH, caused by highly alkali digestate may drive longer lasting changes in soil fungal populations

(Coelho et al., 2020). It is possible that dynamics in microbial communities following amendment may have more to do with the soil type (texture, pH, SOM content) than with digestate properties or dose (Panuccio et al., 2021), though it is unclear which soil properties are most important. A recent review suggests that almost as many studies report negligible or even negative effects of digestate application on soil biology as report positive effects (Karimi et al., 2022). However, this depends on their comparison with other organic fertilisers, whereas when compared with mineral fertilisation, there are much less reported negative effects (see Fig. 1.27). The same authors stress the need for further in-depth studies in order to better assess the impact of digestates from different production parameters and post-treatments on soil microbiology.

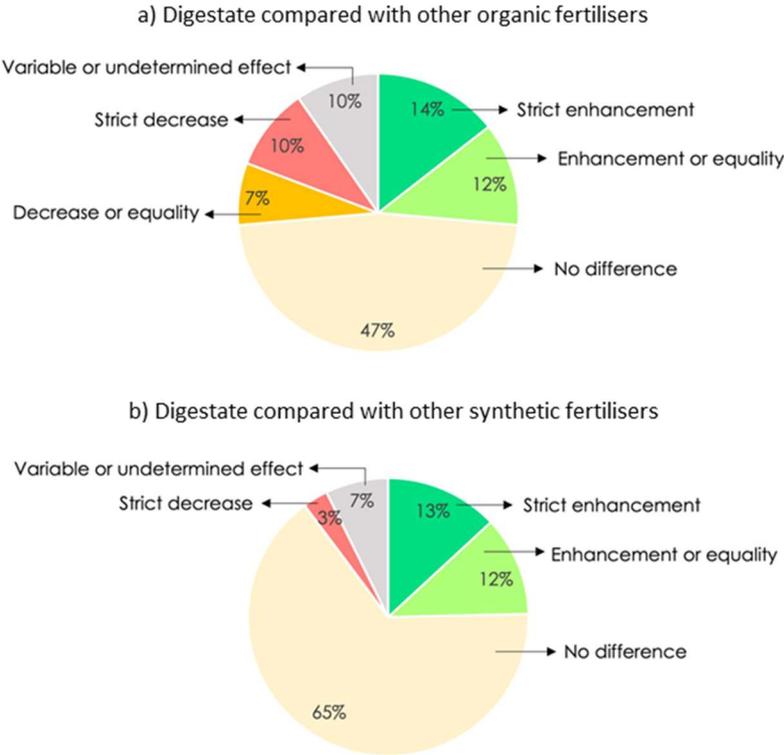


Fig. 1.18 Pie-charts summarizing the “global ecological impact of digestates application on the soil microbiological quality”, from 56 studies and measuring 23 microbial parameters, in comparison with: a) a synthetic mineral fertiliser; b) other organic fertilisers (adapted from (Karimi et al., 2022))

### 1.3.5 Digestate impact on soil structure and aggregate stability

There has been a marked lack of literature on the effects of digestate on soil physical properties, and in particular field-based studies, until recently (Insam et al., 2015; Nkoa, 2014). Where the physical properties related to the structure of the soil have been measured, application of digestate has been found to have positive impacts, reducing soil bulk density, increasing hydraulic conductivity and improving water retention (Beni et al., 2012; Garg et al., 2005; Singh et al., 2007; Tang et al., 2022). These effects are attributed to the OM in the digestate acting as a bulking agent to open up the soil structure (Garg et al., 2005), or else the fertilising effect of digestate nutrients increasing microbial activity (Tang et al., 2022). Direct measurement of soil porosity following digestate and sludge additions (at a rate of 4.5 - 9 t<sub>DM</sub> Ha<sup>-1</sup>) have shown that the decomposition of the added organic matter assists in the creation of new “structural porosity”, that should improve water infiltration and gas exchange (Skic et al., 2020). For this reason, digestates used as amendments have proven to be particularly effective in remediation of polluted soils (Gielnik et al., 2019) and restoration of fertility in marginal or degraded soils (Manasa et al., 2020; Nabel et al., 2017), by increasing water holding capacity and basal respiration rates.

Observation of repeated applications of raw food-waste digestate over 3 years has been shown to generate a minor increase in soil organic carbon and some increase in soil bulk density (Bhogal et al., 2016). However, compared to results from application of other organic amendments the benefits to soil structure were minimal, and even a small decrease in soil bulk density was observed. The lack of raw digestate impact on soil physical properties can be attributed to the low quantity of organic matter added at the application rates used (approx.. 10-250 kg<sub>N</sub> Ha<sup>-1</sup>), which is limited by the high nitrogen content of the digestate (ADEME et al., 2016; Bhogal et al., 2016).

There are still currently far less scientific publications on the impact of digestate on soil structure than exists for other groups of organic amendments (Web of Science topic search results: “Digestate” AND “Soil structure” = 19, “Compost” AND “Soil structure” = 196, “Manure” AND “Soil structure” = 404.

Accessed: 06/03/2023). A non-exhaustive review of the published literature from the previous decade of studies evaluating effects of soil AS following digestate incorporation is given in Table. 1.2, covering the digestate type, soil texture, effects observed, and the time-scale of the effects. Many studies cover either very short-term (<3 months) or medium- to long-term effects (>3 years). Only one short-term study considered the effects of different digestates (Dénès et al., 2014), and only two covered application to more than one soil type (Badagliacca et al., 2020; Sarker et al., 2018). The digestates used in these studies were generally poorly characterised, with only three providing detailed OM characterisation (Béghin-Tanneau, 2020; Chavez-Rico et al., 2023; Sarker et al., 2018). Equally, very few provide detailed information on the modes of production of the digestates tested.

Overall, the short-term and long-term effects of digestate reported in the literature are either positive or not significant. Where positive impacts on soil AS have been observed, a number of different biotic stabilisation mechanisms from the production of binding agents including: fungal hyphae (Alburquerque et al., 2012b; Badagliacca et al., 2020; Rezacova et al., 2021), microbial exudates (Béghin-Tanneau, 2020; Manasa et al., 2020), and “humic” substances (Rezacova et al., 2021). Abiotic mechanisms directly arising from the addition of digestate are less well discussed, though some consideration is given to their direct contribution to soil “humic” substances (Erhart et al., 2014; Rezacova et al., 2021) and hydrophobic OM (Badagliacca et al., 2020).

Long et al. (2015) found that regular applications of raw digestate to a silty loam soil at fairly high doses of carbon (2-3 t<sub>c</sub> Ha<sup>-1</sup>) over two years led to a slight (but non-significant) decline in soil AS. However, in this case mineral fertiliser was also applied with the already mineral rich digestate, meaning that large quantities of N, P and K were introduced to the soil. As discussed earlier in section 1.1.4.3, excessive mineral fertilisation may lead to reduced or negative effects on soil AS. Conversely, Tang et al. (2022) observed greater improvement to soil AS from digestate when accompanied by mineral fertilisation. The very high content of monovalent salts (K<sup>+</sup>, Na<sup>+</sup>) in certain digestates has been linked to clays dispersion (Voelkner et al., 2015b), which may in turn have an effect on soil AS through weakening of

Table. 1.1 Comparison of studies investigating the impacts of digestate application on soil AS. For the duration of effect on soil AS, we define “Transient” as an effect lasting over only several months and “Temporary” as an effect lasting upwards of several months to one year.

Study	Digestate type	Feed substrates	USDA texture class	Observed effect on soil AS (+duration)	Time scale
Rivier et al (2022)	Solid phase (centrifuged)	Food waste	(Loamy) Sand	Positive (1 measure)	Short-term (30 d)
			Loamy clay	Negative (1 measure)	Short-term (30 d)
Manasa et al (2020)	Raw digestate	Corn straw	Loam (saline soil)	Positive (Transient)	Short-term (60 d)
Dénès (2014)	Raw liquid	Cow manure	Silt loam	Positive / not signif.	Short-term (60 d)
	Raw liquid	Pig slurry		Positive (Transient)	
	Raw liquid	Bio-waste		Positive (Temporary?)	
Chavez-Rico et al (2023)	Solid phase (centrifuged)	Meadow hay, Food waste	Loamy sand	Not significant	Short-term (3 m)
Béghin-Tanneau (2019)	Raw liquid	Maize silage, dairy manure	Loam	Positive (Transient)	Short-term (9 m)
Sarker et al (2018)	Solid digestate	N/A	Loam	Positive (Temporary)	Short-term (10 m)
			Sandy clay loam		
			Sandy loam		
Badagliacca et al (2020)	Solid digestate	Animal manure, plant wastes, milk serum	Clay	Positive (2 d - 18 w)	Short-term (1 y)
			Sandy loam	Positive (7 w- 18 w)	
Alburquerque et al (2012)	Raw liquid	Pig slurry, agro-industry waste, biodiesel waste water	Sandy loam	Positive (Temporary)	Medium-term (6 m - 2 y)
Pastorelli et al (2021)	Raw liquid + Solid phase	Energy crops, cattle slurry, food wastes	Silty clay	Positive (Transient)	Medium-term (1 m -2 y)
Long et al (2015)	N/A (Raw solid? TS = 23%)	N/A	Silty loam	Negative / not significant	Medium-term (2 y)
Bhogal et al (2016)	Raw liquid	Food waste	Various (7 soils)	Not significant	Med/Long-term (3y)
Eich-Greatorex et al (2018)	Raw liquid	Food waste	"Loam" (not spec.)	Positive	Long-term (3-7 y)
			"Moraine" (not specified)	Not significant	
			"Silt" (not spec.)	Positive	
ADEME et al (2016)	Raw liquid	Pig slurry	Silty loam	Not significant	Long-term (6 y)
Tang et al (2022)	Stored liquid phase	Pig slurry	Silty clay	Positive (more + with mineral fertiliser)	Long-term (6 y)
Rézacova et al (2021)	Raw liquid	Corn silage, cattle slurry	Clay loam	Positive	Long-term (8 y)
			Sandy loam	Not significant	
			Sandy loam	Not significant	
	Raw liquid	Corn silage, pig slurry, barnyard manure	Clay loam	Positive	
			Sandy loam	Positive	
			Sandy loam	Positive	
Erhart et al (2014)	Raw liquid	Energy crops	N/A	Positive	N/A

aggregates on a molecular level. In a study by Rivier et al. (2022), where some minor but non-significant negative effects on AS of a loamy soil were observed, this was also attributed to dispersion of aggregates by the high levels of minerals added with the digestate.

### 1.3.6 Conclusion

The precise and combined effects of the different process parameters involved in AD on the organic matter in the digestate remain poorly understood, so that it is not always possible to predict their impact on soil properties. Nonetheless, studies of different digestates produced across the AD sector and mobilised in agriculture, have shown that they may possess wide range of characteristics conducive to their use as both organic fertilisers and amendments. What is clear from the available literature on the agronomic impacts of digestates, and in particular their impact on soil physical properties such as AS, is the need for many more comparative studies taking into account a larger number of distinctly different digestates.

Based on the high variability of digestate quality, and the reports of digestate impacts on soil OM and soil microbiology, it is very likely that investigation will reveal both positive and negative effects on soil AS. Overall, the current research into digestate impact on soil AS shows a positive but less marked impact over the medium- to long-term when compared to other organic fertilisers and amendments (Long et al., 2015; Sarker et al., 2018). The effects of digestates on soil AS dynamics over the short-term are largely unknown, though appear to vary considerably between existing studies. Nonetheless, based on our current knowledge of the following:

- the mechanisms involved in soil aggregate stabilisation (section 1.1.3),
- the impact of various organic amendments on soil AS dynamics (sections 1.1.4.4 and 1.1.5.1),
- the characteristics and agricultural impacts of digestates produced in AD (sections 1.2 and 1.3),

It is possible to hypothesise the mechanisms through which certain digestates characteristics may affect soil AS, as summarised in Table. 1.2. It is likely that, as for other organic amendments, the stimulation of biotic production of aggregate binding agents will play an important role, depending on

the biodegradability/accessibility of the OM contained in the digestate (points 1-3 of Table. 1.2). These biotic mechanisms may be altered, and even negatively affected, by the mineral composition of the digestate or the presence of toxic compounds (points 4-5). Furthermore, organic (points 6-7) and mineral (points 8-10) components of the digestate may have abiotic effects on the soil AS. Finally, in soil systems with crop production, the positive fertilising effects of digestate on crop growth and their subsequent impact on soil AS may be significant (point 11). The precise scale and duration of these different mechanisms is yet to be established in many cases.

Table. 1.2 Digestate characteristics and their proposed impact on soil AS via various mechanisms of aggregate stabilisation

Possible digestate characteristics	Proposed mechanism of action on soil aggregates (Positive / Negative / Unknown)	Category of mechanism	Possible time lapse and/or duration	Sources (either providing evidence of effect or attributing effect to digestate application)
1. Remaining undigested labile OM (simple carbohydrates, amino acids, soluble proteins) + Nmin	Proliferation of microorganisms modifying aggregate pore size and wettability	Biotic	Unknown (Depends on nature of change)	(Chenu and Cosentino, 2011; Tang et al., 2022; Vandevivere et al., 1990)
	Stimulation of bacterial activity producing exudates, increasing cohesion and hydrophobicity	Biotic	Rapid - transient	(Abiven et al., 2007; Annabi et al., 2007; Martens, 2000; Sarker et al., 2018)
2. Slowly biodegradable OM (ligno-cellulose, complex proteins)	Stimulation of fungal activity increases number of fungal hyphae that improve aggregate cohesion	Biotic	Slower than above - temporary	(Abiven et al., 2007; Annabi et al., 2007; Martens, 2000; Sarker et al., 2018)
3. All biodegradable OM	Humification / Formation of complex OM by soil microbial activity (+see 6)	Biotic	Unknown - long-term	(Muscolo et al., 2017; Rezacova et al., 2021)
4. Low C-N ratio / Highly labile C + N	Acceleration of OM decomposition by soil microorganisms that may deposit aggregate binding agents	Biotic	Rapid - transient (short-term)	(Abiven et al., 2007; Le Guillou et al., 2011; Vandevivere et al., 1990)
	Positive priming effect leading to reduced native SOC (Attenuation of 1 and 2)	Biotic	Rapid + long-term	(Albuquerque et al., 2012a; Coban et al., 2015)
5. Toxins or heavy metals	Accumulation and suppression of microbial activity – affects all biotic mechanisms	Biotic	Unknown – possibly immediate and long-term	(Nkoa, 2014; Nwuche and Ugoji, 2008)
6. Large stable OM (Microbial residues, High M <sub>R</sub> aromatic compounds, “Humic” substances)	Formation of stable mineral-OM complexes	Abiotic	Unknown (time taken to formation?)	(Bronick and Lal, 2005; Oades and Waters, 1991; Rezacova et al., 2021)
	Hydrophobic coating of aggregates	Abiotic	Unknown – possibly immediate/recalcitrant	(Annabi et al., 2007; Bronick and Lal, 2005; Dal Ferro et al., 2012; Piccolo and Mbagwu, 1999)
7. Hydrophobic OM (Lipids, amphiphilic molecules, microbial residues, extracellular polysaccharides)	Hydrophobic coating of aggregates – but eventually broken down by microorganisms unless physically protected	Abiotic/Biotic	Immediate - transient or recalcitrant depending on molecule	(Chavez-Rico et al., 2023; Paré et al., 1999; Plante and Voroney, 1998; Voelkner et al., 2019)
8. Polyvalent cations (Ca <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>3+</sup> , Al <sup>3+</sup> )	Formation of cation bridges between OM and clay particles, reinforced bonding between soil clays and OM	Abiotic	Unknown – possibly immediate	(García-Orenes et al., 2005; Haynes and Naidu, 1998; Tejada and Gonzalez, 2007)
9. Monovalent cations (Na <sup>+</sup> , K <sup>+</sup> , NH <sub>4</sub> <sup>+</sup> )	Substitution in clay structure, dispersion of clays, interference with bonding between soil clays and OM	Abiotic	Unknown – possibly immediate	(Haynes and Naidu, 1998; Rivier et al., 2022; Voelkner et al., 2015b; Whalen and Chang, 2002)
10. Digestate pH (> or < than soil pH)	pH change – change in availability and/or absorption of minerals (affecting 8 and 9)	Abiotic	Unknown – possibly immediate but transient	(Chorom et al., 1994; Haynes and Naidu, 1998)
11. Minerals / Plant nutrients (N, P, K, etc.)	Improved plant growth and root formation + mycorrhizal development + increased exudates	Biotic	Temporary	(Chan and Heenan, 1996; Dorioz et al., 1993; Frøseth et al., 2014)

## 1.4 Scope of the thesis

Given the wide number of variables involved in this research, in terms of soil types, digestate types, and pedo-climatic conditions, it was not considered feasible to explore all possibilities within the time available. As such, the majority of the investigation has been focussed on digestate application to silt loam soils that, as stated in the “Context of the thesis”, are typical of the Brittany region and for which organic matter has a central role in soil AS regulation (Le Bissonnais and Arrouays, 1997). In order to facilitate observation of changes in the soil aggregate stability due to digestate incorporation independent of variations from uncontrolled climatic factors, much of the experimental work was also conducted in laboratory trials rather than in the field. Furthermore, given the time constraints surrounding a thesis project, it was decided to dedicate our efforts to the study of short-term dynamics in aggregate stability following digestate amendment, rather than investigating long-term effects. There is a considerable knowledge gap in this area, as already highlighted in section 1.2.2.4. The effects of digestate on soil AS over the short-term (<12 months) may also contribute toward prediction of the longer term impacts (>12 months) through the estimation of cumulative effects from multiple applications over successive years (Chenu et al., 2011).

In summary, the current thesis work is centred on the study of short-term dynamics in soil aggregate stability following amendment of a silt loam soil with a range of digestates produced under varying anaerobic digestion process conditions.

## 1.5 Scientific objectives

Based on the information gathered over the course of our bibliographic research discussed over the course of this chapter, we have defined several scientific objectives for the following thesis work:

- ❖ Understand and compare the impact of digestate amendment on short-term dynamics in soil aggregate stability, taking into account their diversity,

- ❖ Assess the influence of various anaerobic digestion process parameters (feed substrates, digester operating parameters, post-treatments including phase separation and composting) on the digestate impact on soil aggregate stability,
- ❖ Explore the connections between digestate characteristics and observed soil aggregate stability dynamics following amendment, with a view to identifying the mechanisms by which digestates may stabilise soil aggregates (as proposed in Table. 1.2), as well as establishing useful indicators for assessment of their agronomic value,
- ❖ Study how the findings can be applied to digestate application in-field, and ascertain how the impacts of digestates on soil aggregate stability are likely affected by other controllable factors (dose, tillage, plant cultivation) and modulating factors (climate, soil type).

## 1.6 Organisation of the thesis

The work undertaken to respond to each of the scientific objectives is presented over the course of the following chapters of the thesis manuscript. In chapter 2, we present the development and selection of the experimental methods used to carry out the thesis objectives. Chapter 3 takes the form of an article recently published in the review “Waste and Biomass Valorization”. We present the results from the incubation and monitoring of soil aggregate stability dynamics following amendment of a silt loam soil with a diverse panel of digestates, which led to the proposition of a first digestate typology of impact on soil aggregate stability. In chapter 4, using further results from the incubation trials reported in chapter 3, we investigate the link between digestate characteristics and their observed impact on soil AS, with the development of a statistical model using partial least squares regression (PLS-R). This is presented in the form of an article to be submitted to the review “Soil Use and Management”. Chapter 5 is also in the form of an article to be submitted to the review “Soil Science Society of America”. This article reports on the findings of a separately conducted field trial, using one of the digestates initially characterised over the course of chapter 2, and was intended to

validate the findings obtained in the laboratory and discuss their relevance in the context of “real world” conditions (i.e. seasonal climatic variations, heterogeneous soil conditions, limited digestate application rates, etc.). Finally, chapter 6 is a short report on the findings from a final experiment, conducted towards the end of the thesis, investigating the interactions between varying soil types and digestates representative of the groups in the typology of impact proposed in chapter 3.

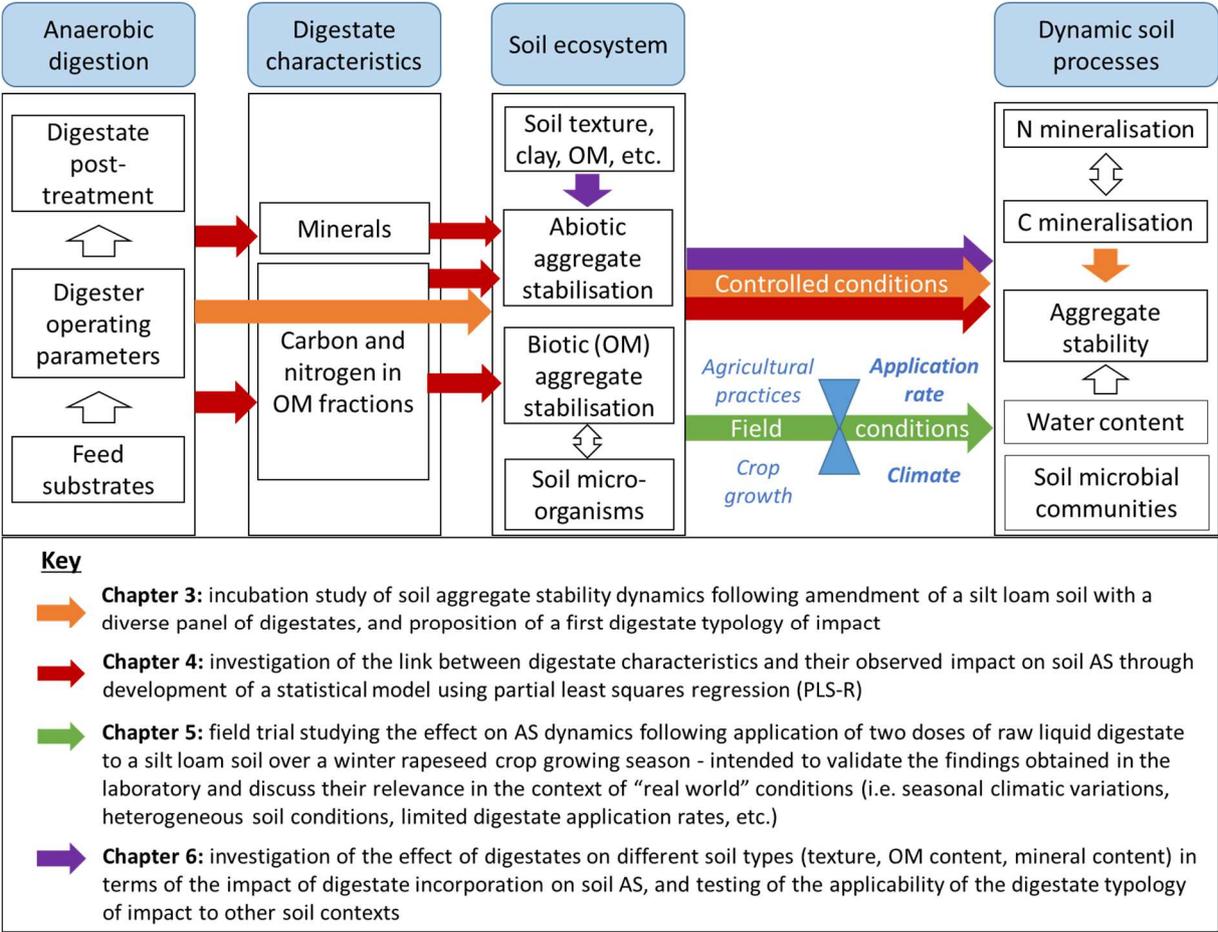


Fig. 1.19 Schematic of digestate production, resulting digestate characteristics, their impact on soil properties when used as soil organic amendment, and the principal factors involved in aggregate stabilisation.

## 2 Development and selection of experimental protocols

In this chapter, we describe the analytical methods adopted in order to answer to the scientific objectives presented at the end of the previous chapter. Two preliminary experiments were conducted: The first one, intended to devise a means for creating digestates under laboratory conditions that would allow for more precise control of various AD production parameters, is described in section 2.4. A second experiment was carried out to establish a protocol for agronomic trials involving the incubation of digestate with soil and is described in section 2.5. The findings and conclusions from this preliminary incubation trial were used to decide the experimental protocol for all subsequent experimentation.

## 2.1 Digestate sampling and characterisation

### 2.1.1 Digestate sampling

In order to test the characterisation methods and protocols selected during this chapter, a digestate of a mixture containing cow slurry (DCS<sub>FS</sub>), and another digestate of a mixture containing pig slurry (DPS<sub>FS</sub>) were sampled from two full-scale on-farm anaerobic digestion facilities in Brittany. The operational parameters of the two digesters are given in *Table. 2.1*.

*Table. 2.1 AD process parameters involved in the production of two raw liquid digestates sampled and tested during the development of experimental protocols*

Digestate	DCS <sub>FS</sub>	DPS <sub>FS</sub>
Predominant feed substrate	Cow Slurry	Pig Slurry
Digester type	Continuous stirred tank	Continuous stirred tank
Primary digester volume (m <sup>3</sup> )	1000	1200
Secondary digester volume (m <sup>3</sup> )	1350	1800
Operating temp (°C)	39-40	38-40
Estimated HRT (days)	130-160	90-117

### 2.1.2 Physico-chemical characteristics

Throughout this thesis project, the following characterisation methods were used for digestates and other organic substrates unless otherwise stated. Total solids (TS) and volatile solids (VS) content were determined through weighing before and after drying at 105°C, and after combustion at 550°C, respectively. Total carbon (TC) was determined by analysis of the whole digestate, and inorganic carbon (IC) by analysis of the remaining dry ash after combustion at 480°C, using a Thermo Fisher® CN Flash 2000, as per the manufacturer's instructions. Total organic carbon (TOC) of the digestates was calculated by as the difference between the TC and IC. Total Kjeldahl Nitrogen (TKN) and ammonium nitrogen (N-NH<sub>4</sub><sup>+</sup>) were analysed by automatic distillation using a Gerhardt Vapodest 50, as per manufacturer's instructions. To perform elemental analyses of minerals in the digestate, a solution was first obtained by mineralising the ashes obtained from combustion at 550°C in a mixture of HCl (37%) and HNO<sub>3</sub> (65%) followed by digestion in an Anton Paar® HVT50 Microwave Reactor. Concentrations of various elements in the solution (K, S, Ca, Mg, Al, Cu, Fe, Mg, Zn, Al, Na) were

obtained through microwave plasma atomic emission spectrometry using an Agilent® 4200 MP-AES, while total phosphor (P) was obtained by spectrophotometry using a Thermo Fisher Gallery® discrete analyser, as per the manufacturers' instructions.

### 2.1.3 Characterisation of organic matter by sequential extraction

It was decided to characterise digestate OM through sequential extraction as per the “EPS” method developed by Jimenez et al. (2015) and adapted by Bareha et al. (2018) (see Fig. 2.1). This method of chemical fractionation is considered to be less aggressive than the “Van Soest” method (Jimenez et al., 2015), and has proved effective in prediction of both substrate anaerobic degradability (Bareha et al., 2018) and digestate aerobic degradability (Fernández-Domínguez et al., 2021). Briefly, 0.5 g<sub>TS</sub> of digestate was placed in a centrifugation tube and subjected to OM extraction using a number of solutions:

- **Extractable Soluble from Particulate Organic Matter (SPOM):** 30ml of 10 mM CaCl<sub>2</sub> solution was versed into the tube, then agitated for 15 min at 30°C on a rotating plate set to 300 rpm. This was followed by centrifugation at 11400g at 4°C for 20 min, after which the supernatant was decanted into a container, and the pellet retained in the tube. This procedure was repeated 2 times, with the supernatants from each step being mixed together. The SPOM represents the most accessible fraction and essentially contains water-soluble proteins and sugars.
- **Readily Extractable Organic Matter (REOM):** 30ml of 10 mM NaOH and 10 mM Na<sub>2</sub>CO<sub>3</sub> solution was versed into the tube, then agitated for 15 min at 30°C on a rotating plate set to 300 rpm. This was followed by centrifugation at 11400g at 4°C for 20 min, after which the supernatant was decanted into a container, and the pellet retained in the tube. This procedure was repeated 4 times, with the supernatants from each step being mixed together. The REOM represents an accessible fraction of mainly proteins and lipids.

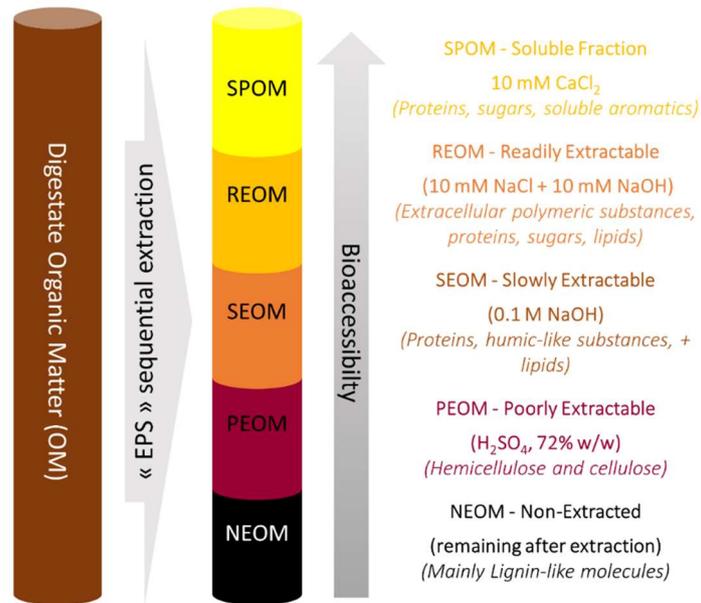


Fig. 2.1 The “EPS” sequential extraction method for characterisation of digestate organic matter

- **Slowly Extractable Organic Matter (SEOM):** 30mL of 0.1 M NaOH solution was versed into the tube, then agitated for 4 hr at 30°C on a rotating plate set to 300 rpm. This was followed by centrifugation at 11400g at 4°C for 20 min, after which the supernatant was decanted into a container, and the pellet retained in the tube. This procedure was repeated 4 times, with the supernatants from each step being mixed together. The SEOM mainly contains humic-like and fulvic-like acids and complex proteins (i.e. glycolated proteins) as well as certain lignocellulosic compounds which can be soluble under strongly basic conditions.
- **Poorly Extractable Organic Matter (PEOM):** 25 mL of 72% H<sub>2</sub>SO<sub>4</sub> was dosed into the tube using a calibrated dispensing pump under a ventilation hood then agitated for 3hr at 30°C on a rotating plate set to 300 rpm. This was followed by centrifugation at 11400g at 4°C for 20 min, after which the supernatant was decanted into a container, and the pellet retained in the tube. This procedure was repeated 4 times, with the supernatants from each step being mixed together. The PEOM extraction targets holocellulose (i.e. hemicellulose and cellulose).
- The remaining pellet was considered to be **Non-Extractable Organic Matter (NEOM)**, which contains lignin-like compounds and non-extractable humic-like acids.

Extracted solutions were filtered at 0.45 µm then analysed for TOC, TC, IC, and TN using a Shimadzu® TOC/N analyser, as per the manufacturer's instructions. The NEOM was calculated as the TOC, TC or TN (taken as equal to the TKN) of the whole sample minus the values analysed on all other extractions.

## 2.2 Analysis of soil aggregate stability

### 2.2.1 Existing methods of analysis of soil aggregate stability

There are numerous protocols for analysis of aggregate stability (Le Bissonnais, 1996). Different methods have been developed to suit local conditions, depending on the predominant soil types, and principal causes of soil erosion (wind, rainfall, ploughing, etc.) in the geographical area of study. In the context of the study of the disaggregation by water of soils in temperate climates, several broad aggregate separation and calibration techniques may be used: wet sieving (Kemper and Rosenau, 1986; Le Bissonnais, 1996), rainfall simulation (Almajmaie et al., 2017; Barthès and Roose, 2002; Kimble et al., 2000), or ultrasonic dispersion (Almajmaie et al., 2017; Imeson and Vis, 1984). These various test methods of varying force and acting by various mechanisms of aggregate disruption, as well as the different metrics used to represent the result, mean that it is often difficult to draw direct comparison between different studies. Some methods have been used to make a qualitative comparison between results from these different test methods across numerous studies (Abiven et al., 2009), but cannot directly compare between individual studies. Difference in aggregate starting moisture content (Haynes and Swift, 1990), operator experience (Rabot et al., 2018) and the multitude of different methods that can be used (Almajmaie et al., 2017), can also lead to differences in results and a lack of comparability between similar studies, and so should always be carefully controlled or taken into account.

Rainfall simulation is arguably the test most closely resembling the breakdown of aggregates on the soil surface under natural conditions (Almajmaie et al., 2017), as they subject the aggregates to all four mechanisms of aggregate breakdown (see Fig. 1.2 on page ). However, for this reason rainfall simulators are relatively destructive and not always suited for differentiating between mechanisms of

breakdown on fragile silt soils (Lax et al., 1993). Use of ultrasonic vibration to disrupt aggregates, while being highly precise, is also a fairly aggressive method (Almajmaie et al., 2017). In temperate climate countries, wet sieving is arguably the most common method used, and a large number of different protocols have been devised (Elliott, 1986; Kemper and Rosenau, 1986; Le Bissonnais, 1996; Yoder, 1936). Aggregates are often calibrated to a certain size range, and may be air dried before testing, and may be subjected to various pre-treatments simulating the different mechanisms of aggregate breakdown before sieving (Le Bissonnais, 1996). A detailed review of existing test methods by Le Bissonnais (1996) led to the development of a new test protocol adapted from a long-established method developed by Hénin (1958).

### 2.2.2 The “Le Bissonnais” method for analysis of soil aggregate stability

The full protocol for the Le Bissonnais method consists of three different “pre-treatments” that vary the force applied to the aggregates, and are intended to distinguish between the different mechanisms of soil aggregate breakdown caused by rainfall and water ingress. This is useful when dealing with different textured soils where inherent differences in soil AS can be great, while still permitting to detect subtle differences that may be due to varying management practices. The three tests are:

- 1) **The “fast wetting” test:** Five grams of calibrated aggregates are gently immersed in a 250 cm<sup>3</sup> beaker filled with 50 cm<sup>3</sup> of deionized water for 10 min. The water is then removed delicately using a pipette, and the soil aggregates are transferred to a 50 µm sieve previously immersed in ethanol. This method simulates rapid immersion of aggregates in water as caused by intense rainfall or submersion, and emphasizes disaggregation by slaking over other mechanisms.
- 2) **The “slow wetting” test:** Five grams of calibrated aggregates are spread on a filter paper and placed on a tension table at a matric potential of -0.3 Wa for 30 min. Soil aggregates are then transferred to a 50 µm sieve previously immersed in ethanol. This capillary rewetting of aggregates is intended to simulate the action of wetting under gentle rain. It is less destructive than fast wetting and may allow a better discrimination between unstable soils.

- 3) **The “ethanol” or “mechanical breakdown” test:** Five grams of calibrated aggregates are gently immersed in a 250 cm<sup>3</sup> beaker filled with 50 cm<sup>3</sup> of ethanol for 10 min. The ethanol is then sucked off with a pipette and the soil material transferred into a 250 cm<sup>3</sup> Erlenmeyer flask filled with 50 cm<sup>3</sup> of deionized water. The water content is adjusted to 200 cm<sup>3</sup>, and the flask is then corked and agitated end over end 20 times. The flask is left for 30 min for sedimentation of coarse fragments before excess water is sucked off using a pipette. The soil aggregates are transferred to a 50 µm sieve previously immersed in ethanol. This test simulates the mechanical resistance of aggregates, independent of slaking, by removal of the air in the aggregate pores before immersion.

The resulting samples on the 50 µm sieve are gently submerged five times in ethanol using a “Hénin” or “Yoder” apparatus in order to stabilise and separate the remaining aggregates. These are then dried for 24h at 40°C before dry sieving by hand on a column of six sieves: 2, 1, 0.5, 0.2, 0.1 and 0.05 mm, each of which are subsequently weighed. The weight of each size fraction allows calculation of the mean weight diameter (MWD), as per the method of (Kemper and Rosenau, 1986) as the sum of the mass fraction of soil remaining on each sieve after sieving multiplied by the mean aperture of the adjacent mesh. (Calculated MWDs therefore range between a minimum of 25 µm and a maximum of 3.5 mm).

Aside from direct comparison of MWD measurements for soils under different treatments, a further method of interpreting the measurements is a classification of stability and crustability given in *Table 2.2*. The differences in MWD values measured by the different tests proposed above correspond to the varying soil behaviour resulting as a result of initial water content and the rainfall characteristics (Le Bissonnais, 1996).

*Table. 2.2 The classes of stability and crustability as defined by measurement of the MWD using Le Bissonnais' method (Le Bissonnais, 1996)*

<b>Class</b>	<b>MWD (mm)</b>	<b>Stability</b>	<b>Crustability</b>
1	< 0.4	Very unstable	Systematic crust formation
2	0.4-0.8	Unstable	Crusting frequent
3	0.8-1.3	Medium	Crusting moderate
4	1.3-2.0	Stable	Crusting rare
5	> 2.0	Very stable	No crusting

The “Le Bissonnais” method is the standard method used in France (NF X31-515, 2005) and forms the basis of the European standard method (ISO 10930, 2012). It has been used in a number of studies into the effects of various organic amendments on soil AS (Abiven et al., 2007; Annabi et al., 2007; Béghin-Tanneau, 2020; Grosbellet et al., 2011; Zhou et al., 2019), as well as for studies into the impacts of other agricultural practices (Bottinelli et al., 2017; Papadopoulos et al., 2009). It was therefore considered to be the best adapted to the requirements of this thesis work.

## 2.3 Testing a protocol for production of digestates under laboratory conditions vs. full-scale AD

### 2.3.1 Introduction

The assessment of the agricultural value of digestates is more often carried out on samples obtained from commercially operated digesters (Albuquerque et al., 2012b; Tambone et al., 2013; Teglia et al., 2011). However, digestates collected from these full-scale AD plants are the product of a specific combination of conditions, limited by commercial and legislative constraints governing their operation. This limits the possibility of distinguishing between the effects of precise factors that may alter the digestate quality. Pilot or laboratory-scale anaerobic digesters may be operated in a manner that process conditions can be varied at will, allowing production of a wide range of digestates. However, the considerable differences between these small-scale laboratory set-ups and the operation of full-scale digesters leads to questions around the representativity of digestate from laboratory digesters (see *Fig. 2.2*).

A reasonable quantity of organic material is needed in order to perform detailed physical and chemical characterisation. Agronomic trials involving incorporation into the soil may equally consume substantial quantities of the tested material, depending on the size and format of the experiment. Obtaining sufficient volumes of digestate at a small scale may sometimes prove difficult due to a number of constraints. Pilot-scale digesters are able to provide an abundance of digestate, but represent a higher cost of investment and their operation can be difficult, approaching that of a full-scale digester. A considerable amount of time can often be required to bring them to stable 'equilibrium' conditions following a change in substrate or operating conditions, particularly where long hydraulic residence times (HRT) are desired (e.g. a digester with a HRT of 60 days would typically require  $3 \times \text{HRT} = 180$  days to reach stable conditions).

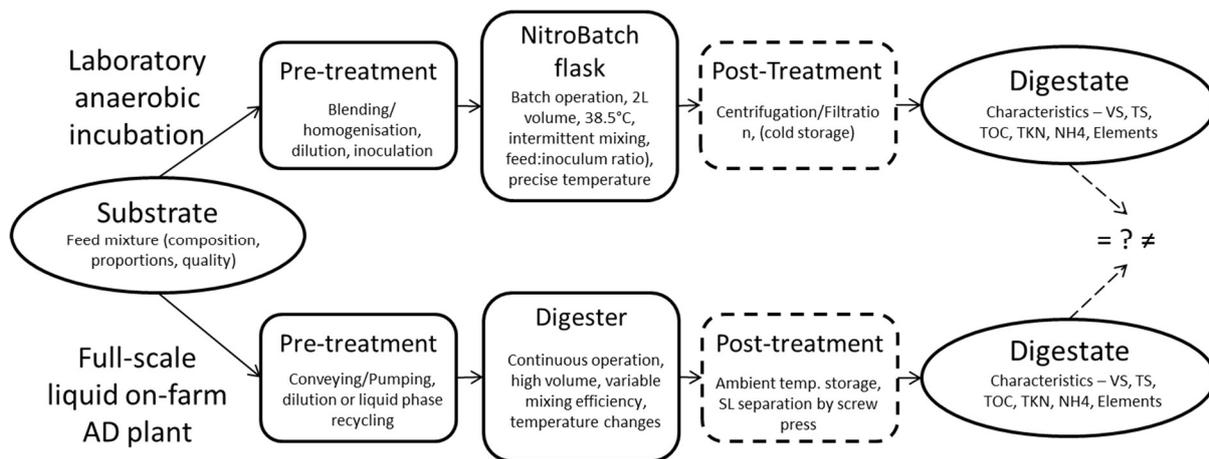


Fig. 2.2 Schematic comparing conditions for production of digestate by laboratory-scale anaerobic incubation and full-scale on-farm AD

One solution for obtaining a diverse range of digestates in the shortest length of time is thus to run a series of laboratory-scale batch digestions in a sealed flask or container. This makes it possible to follow the evolution in organic matter quality of a specifically chosen substrate under controlled conditions, and to run numerous tests in parallel with ease and at very low cost, potentially giving greater insight into the digestion process. However, at this scale the digester volume is often very small (1-2L) and the quantity of raw material for further analysis is limited. Because of this, testing of a laboratory protocol is essential so that the volume of digestate obtained may be verified as sufficient for use in further experimentation, particularly if this would involve agronomic pot trials or incubations.

It was decided to test a protocol called the “NitroBatch”, devised by Bareha (2018) over the course of his thesis work in order to follow the biochemical changes of nitrogen over the course of digestion.

This experimental phase was intended to respond to the following questions:

- Is it possible to reproduce digestate of similar quality to that produced from a full-scale AD plant using a laboratory batch type pilot?
- Can sufficient quantities of digestate be produced in the laboratory to allow their characterisation and use in the subsequent phases of experimentation?

## 2.3.2 Materials and method

### 2.3.2.1 Preparation of feed substrate mixtures

Samples of substrates being fed to the anaerobic digesters were also sampled from the same two sites as the digestates  $DPS_{FS}$  and  $DCS_{FS}$ . Feed mixtures for the laboratory AD trials were created by mixing the same proportion of each substrate fed to the full-scale digesters daily. In reference to the principle substrate within each feed mixture, they were named, respectively, “CS” - Cow Slurry mixture, and “PS” - Pig Slurry mixture. The percentages of each substrate in the two feed mixtures are given in *Table 2.1*, along with measured TS and VS contents. The feed substrates were blended together using a Robotcoupe® stick blender to obtain a homogeneous liquid slurry before characterisation (see *Table 2.3*).

### 2.3.2.2 The “NitroBatch” protocol

The experimental design for the NitroBatch (pictured in *Fig. 2.3*) is adapted from the bio-methane potential (BMP) of Raposo et al. (2011) by Bareha (2018). Briefly, 1L flasks were filled with 60  $g_{FM}$  ( $\approx 3.6 g_{VS}$ ) of an “inoculum” digestate, followed by the substrate added at a ratio of  $3.6 g_{VS} g_{VS, inoculum}^{-1}$ , and the flask topped up to 750 mL total volume using distilled water. The inoculum, intended to introduce the correct mix of anaerobic microbes to ensure a good digestion of the substrate, was produced on site using a 100 L digester fed with a mixture of pig slurry and horse feed granules and operating under stable conditions (38°C). Flasks were sealed hermetically and the headspace purged with nitrogen at  $1.5 L min^{-1}$  for 10 minutes. The main difference between the NitroBatch and the BMP protocol is that the former uses a higher substrate-inoculum ratio so that a reasonable quantity of digestate derived mainly from the substrate (and not significantly comprising the inoculum) might be obtained for detailed analysis (Bareha, 2018).

Despite the potential risk of acidification of the reactor due to the high substrate concentration, no buffering solution was used, as it was considered that the addition of salts to the mixture would most likely affect future soil incubations (due to a high content of  $Na^+$  ions). Four repetitions were prepared

in the same way for each of the two prepared mixtures (Pig slurry – PS, and cow slurry – CS), as well as two flasks containing only inoculum and distilled water to be taken as a controls. All flasks were placed in a temperature controlled heating cabinet set at 38°C and incubated for 63 days, with shaking at daily intervals in order to maintain a ‘well-mixed’ system. At the end of the experiment, the digestate was frozen at -20°C until characterisation could be carried out.

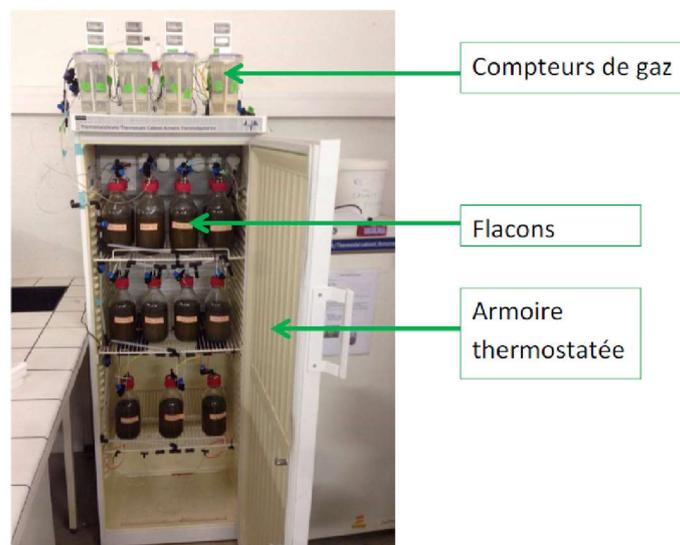
*Table. 2.3. Characteristics of the individual feed substrates, the predicted feed substrate mixtures (calculated from proportion of feed) and actual feed substrate mixtures placed in AD*

Sample Name	TS	VS (g kgFM <sup>-1</sup> )	TOC	% of feed mixture
<u>CS mixture</u>				
Cow slurry	100	84	49*	55
Cover crop silage	167	154	90*	14
Waste animal feed	693	616	358*	7
Turkey manure	510	423	246*	9
Road verge clippings	229	218	127*	14
Potato waste	208	159	93*	2
<b>Predicted mixture</b>	<b>205</b>	<b>179</b>	<b>104*</b>	
<b>Actual mixture</b>	<b>212</b>	<b>185</b>	<b>108</b>	
<u>PS mixture</u>				
Pig slurry	36	28	16*	45
Solid waste fats	287	274	160*	15
Liquid waste fats	238	227	132*	18
Maize silage	185	177	103*	6
Food waste	249	230	134*	12
Waste animal feed	855	723	420*	4
<b>Predicted mixture</b>	<b>181</b>	<b>164</b>	<b>96*</b>	
<b>Actual mixture</b>	<b>175</b>	<b>162</b>	<b>94</b>	

*TS = Total Solids, VS = Volatile solids, FM = Fresh Matter, TOC = Total Organic Carbon, \*Values estimated from TOC  $\approx$  VS/1.72 (Nelson and Sommers, 1983)*

Biogas samples were taken from each flask daily for the first week then roughly twice a week up to the end of the experiment, and analysed using gas chromatography in order to calculate methane production. One flask of the CS and two flasks of the PS mixtures, as well as one control flask, were connected to a volumetric gas flow meter so that the volume of biogas production could be monitored.

This was to allow an assessment of the digester operation when compared to an ideal measure of biogas production (the BMP) and provide a means of diagnosing inhibition of the digestion process without need for invasive monitoring. For the PS mixture, due to a high content in waste fats, the risk of overloading the reactor with a very rich substrate was offset through adding this component of the mixture slowly to the digesters over the course of the experiment. Approximately one third of the total quantity was added every 12 days (one third at day 0).



*Fig. 2.3 Picture taken of the “NitroBatch” laboratory-scale anaerobic digestion experiment (Bareha, 2018)*

### 2.3.3 Results and discussion

#### 2.3.3.1 Indicators of performance of the NitroBatch protocol

Monitoring of the methane production from three of the lab scale reactors (represented in *Fig. 2.4*) indicate a relatively constant production of biogas during the first several weeks, with the majority of biogas production occurring before day 36. The methane production from PS was much higher than for CS. However, biogas production from CS peaked more rapidly before levelling off. The shape of the curve for the PS mixtures was due to the intermittent feeding of the remaining grease at days 9 and 22.

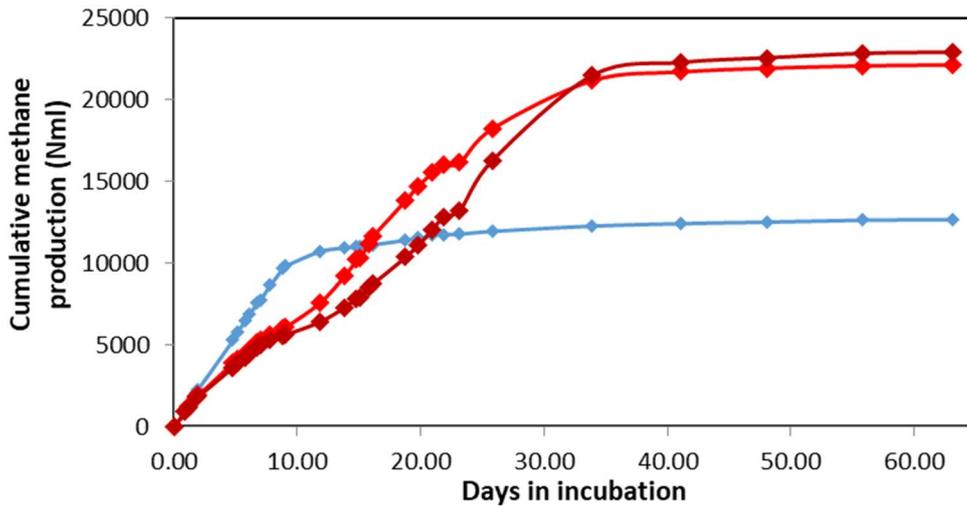


Fig. 2.4 Cumulative methane production over the course of “NitroBatch” batch anaerobic incubation trial for three reactors, one containing cow slurry (CS) mixture (blue line) and two containing pig slurry (PS) mixture (red lines).

From the measured biogas production from the monitored flasks in the NitroBatch trial, the biomethane yield for one CS flask was estimated to be  $366 \text{ NmL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$ , while for two PS flasks the average yield was estimated to be almost  $792 \text{ NmL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$ . Using the SYSmétha prediction tool (Bareha et al., 2021) to estimate potential biogas yields for the two feed substrate mixtures, a biomethane potential of  $348 \text{ NmL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$  for CS and  $740 \text{ NmL}_{\text{CH}_4} \text{ g}_{\text{VS}}^{-1}$  for PS was calculated. Therefore, it can be presumed that the NitroBatch pilot functioned well in terms of conversion of substrate to biogas.

### 2.3.3.2 Comparison of laboratory produced digestate with farm digestate

Comparing the operating parameters of the two full-scale digesters (given in *Table. 2.3*), against the operating parameters of the NitroBatch, it is clear that the HRT was significantly shorter (only 63 days) for the latter. For the digestates from full-scale AD, the time spent in primary digestion was similar (40-50 days), but this time was more than doubled (90-160 days) when including the time spent in the secondary digester. A significant decline in biogas production after 36 days for both mixtures in the NitroBatch digesters would suggest that the digestates had quickly reached a relatively stable state. It might be assumed that the AD process under laboratory conditions were more efficient than for full-scale AD plants, where mixing and temperature gradients lead to suboptimal conditions. Despite the

lack of biogas production after 50 days, however, it cannot be ruled out that other organic matter transformations would have occurred over the course of longer substrate residence times (Bareha, 2018).

*Table. 2.4 Characteristics of digestates produced under laboratory- and full-scale conditions, as well as the inoculum used in laboratory-scale production*

Parameter	Units	INOC	DINOC	DCS <sub>LAB</sub>	DPS <sub>LAB</sub>	DCS <sub>FS</sub>	DPS <sub>FS</sub>
TS	g kg <sub>FM</sub> <sup>-1</sup>	27. ± 0. 3 1	11. ± 0. 0 2	22. ± 0. 7 0	20. ± 0. 6 7	103. ± 0. 2 3	40. ± 0. 4 7
VS	g kg <sub>TS</sub> <sup>-1</sup>	589 ± 6	529 ± 12	594 ± 3	598 ± 38	616 ± 5	697 ± 17
TC	g kg <sub>TS</sub> <sup>-1</sup>	309 ± 11	440 ± 11	445 ± 41	435 ± 36	337 ± 26	392 ± 3
TOC	g kg <sub>TS</sub> <sup>-1</sup>	300 ± 11	381 ± 11	413 ± 41	398 ± 36	330 ± 26	390 ± 3
TKN	g kg <sub>TS</sub> <sup>-1</sup>	128 ± 2. 9	194 ± 25	150 ± 14	166 ± 3. 0	67 ± 0. 6	123 ± 3. 7
N-NH <sub>4</sub>	g kg <sub>TS</sub> <sup>-1</sup>	90 ± 1. 4	112 ± 1. 4	86 ± 1. 2	108 ± 2. 2	43 ± 0. 2	89 ± 1. 4
C-N ratio		2.3 ± 0. 1	2.0 ± 0. 3	2.7 ± 0. 4	2.4 ± 0. 2	4.9 ± 0. 4	3.2 ± 0. 1
pH		7.8	7.8	7.8	7.8	8.4	8.4

The global physico-chemical characteristics (TS, VS, TC, etc.) of all digestates, including the inoculum before and after the NitroBatch trial are given in *Table. 2.4*. The characteristics of the laboratory digestates were not comparable to that of the digestates sampled from the full-scale digesters, and indeed bore more similarities to the digested inoculum (DINOC). All of the NitroBatch produced digestates had been diluted with distilled water as part of the protocol, so the differences in dry matter content between the laboratory and full-scale digestates were unsurprising. It would appear that the added inoculum had a significant effect on the pH, TKN and N-NH<sub>4</sub> of the laboratory digestates, especially for the DCS<sub>LAB</sub>, which was much higher in nitrogen than DCS<sub>FS</sub>. The TC of laboratory digestates was higher, though the VS was lower, which might indicate that OM transformations in the NitroBatch were not similar to those in the full-scale digesters, or at least not at the same state of advancement. Despite the increased N-NH<sub>4</sub> content of all laboratory digestates, their pH was slightly lower than for the full-scale digestates, which suggests they had a higher alkalinity (though this was not tested

directly), also provided by the inoculum. This was somewhat confirmed by the mineral content of the digested inoculum and laboratory-scale digestates (see *Table. 2.5*), that contained significantly more Ca, K, and Na.

*Table. 2.5 Trace mineral contents of digestates produced under laboratory- and full-scale conditions, as well as the inoculum used in laboratory-scale production*

Characteristic	Zn	Ca	Fe	Cu	K	Mg	Al	Na	P
	(g kg <sub>TS</sub> <sup>-1</sup> )								
DCS <sub>FS</sub>	0.29	30.4	4.42	0.06	52.5	13.6	4.00	8.84	16.29
DCS <sub>LAB</sub>	0.42	42.3	2.18	0.16	83.7	6.56	2.52	26.6	15.7
DPS <sub>FS</sub>	0.37	30.4	13.0	0.11	41.9	5.15	3.04	17.1	17.31
DPS <sub>LAB</sub>	0.43	42.1	4.52	0.17	82.9	4.59	2.48	29.7	16.9
DINOC	0.41	52.9	1.77	0.21	104.3	3.14	2.23	40.9	17.6

#### 2.3.4 Conclusions

The testing of a protocol for laboratory production of digestate reported in this section demonstrated that the “NitroBatch” protocol is an effective means of converting substrate materials to biogas and digestate under non-rate-limiting conditions. Both laboratory-scale digestates were not particularly comparable to digestate from full-scale digesters but rather had characteristics closer in similarity to the inoculum used. Without the use of an inoculum, the correct digestion of the substrates is not guaranteed. Therefore, it was decided not to proceed with production of digestates under laboratory conditions, but rather to prioritise sampling and characterisation of digestates from full-scale AD plants.

## 2.4 Testing of a protocol for digestate incubation in soil

### 2.4.1 Introduction

In order to assess the impact of a panel of different digestates on soil AS dynamics under controlled conditions, a suitable incubation protocol needed to be developed and tested. A preliminary incubation trial was carried out in order to compare between the effects of varying several parameters of the protocol, namely: the method of preparation of the digestate, and the dose of digestate applied to the soil.

The application rate of organic amendment may be selected based on various characteristics. In simulation of agronomic doses based on constraints around the maximum allowable rate of N, P or K addition to the soil, the total nitrogen content (Locoli et al., 2019), or the mineral nitrogen content (Risberg et al., 2017), is frequently selected. For studies considering dynamics in soil AS and carbon mineralisation dynamics, the quantity of organic carbon added is of primary importance and so either the fresh matter (Albuquerque et al., 2012b; de la Fuente et al., 2013), or the C content (Abiven et al., 2007; Annabi et al., 2007) is selected. Higher doses of C may be added than would realistically be incorporated in the field in order to better evaluate the dynamics involved. It has been suggested that a single large dose of organic matter may have an impact equivalent to multiple smaller inputs of organic matter over time (Hansen et al., 2016). There is limited information on the effect of varying the dose of an organic amendment. Higher doses of organic amendment appear to lead to a higher response in soil AS (Özdemir et al., 2007), however this is not always the case in certain studies (Paré et al., 1999), most likely depending on complex interactions between elevated concentrations of aggregate stabilising and aggregate disrupting components in the organic material. From a practical point of view, the dose needs to be high enough that the heterogeneity of the organic material does not interfere with the repeatability of the result. It is also desirable to use a quantity of amendment high enough that a response in the measured variables can be easily observed, but not too much as to cause inhibitory phenomena related to lack of oxygen, toxic effects, or overdosing of certain minerals.

One issue arising from the application of large doses of C in the form of raw wet digestates is the quantity of water also added at the same time. Risberg et al. (2017) encountered issues when incorporating wet digestates into soil (dosing based on their mineral nitrogen content), and some modalities in their respirometry trials had to be discounted as they exceeded the desired moisture content. In order to prevent excessive moisture accumulation on addition of the digestate to the soil leading to collapse of the soil aggregates, the digestate may be prepared beforehand by drying or freeze-drying. Drying of organic amendments prior to incubation is a relatively common practice (Abiven et al., 2007; Annabi et al., 2007; Béghin-Tanneau et al., 2019; Sarker et al., 2018). Drying allows materials with varying moisture content to be more easily homogenised (through grinding) and more accurately analysed.

The objective of this phase of experimentation, apart from testing the protocol parameters discussed above (digestate dose and preparation), was to assess and compare the effects of incorporation of the two digestates DCS and DPS on soil aggregate stability dynamics and carbon mineralisation.

## 2.4.2 Materials and method

### 2.4.2.1 Soil sampling and preparation

Soil was sampled in late springtime (April 2020) as recommended by Le Bissonnais (1996). However, due to restrictions linked to the COVID-19 outbreak, it was not possible to sample soil from the intended site (EFELE SOERE PRO, Le Rheu), and instead soil was sampled from an arable field (under oilseed rape cover crop at time of sampling) located in Muel, Brittany, France. Soil was removed by shovel to a depth of 0-30 cm, then covered lightly with plastic bags (not airtight) and stored in cool, dark conditions until it could be processed. The soil was classified as a brunisol derived from siltstone deposits, containing 19.4% clay, 59.7% silt, 20.9% sand, and 2.3% OM, with a pH of 6.03, CEC of 6.73 cmol+ kg<sup>-1</sup>, and C-N ratio of 10.

In order to meet the requirements for analysis of soil aggregate stability (Le Bissonnais, 1996), prior to incubation the soil was broken gently into aggregates along lines of greatest weakness and sieved to

produce a batch of aggregates at 3.15-5 mm diameter. The factors that lead to formation of new aggregates and stabilisation of existing aggregates are very similar, and so it is often difficult to separate between these two effects in the whole soil (Cosentino, 2006). However, in the laboratory it is possible to study only the stabilisation of aggregates through the preparation of aggregates within a calibrated size range by carefully separating and sieving the whole soil. The aggregates were left for a period of two weeks to allow soil microbial activity to stabilise following the disturbance.

#### 2.4.2.2 Incubation protocols

##### 2.4.2.2.1 Incubation of dried digestate

A sufficient quantity of each digestate (DCS and DPS) was dried slowly at 40°C for 5-7 days (the low temperature was intended to reduce losses of volatile carbon and avoid chemical changes in the OM) and ground down to <2mm using a rotary mill. Two doses of each dried digestate were selected for incorporation into the soil, so that the experiment was broken down into incubation jars of the following modalities:

- 20 jars containing DCS<sub>DRY</sub> at a dose of 5,5 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> (=0,42 g<sub>N</sub> kg<sub>dry soil</sub><sup>-1</sup>)
- 20 jars containing DCS<sub>DRY</sub> at a dose of 10,9 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> (=0,83 g<sub>N</sub> kg<sub>dry soil</sub><sup>-1</sup>)
- 20 jars containing DPS<sub>DRY</sub> at a dose of 5,8 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> (=0,41 g<sub>N</sub> kg<sub>dry soil</sub><sup>-1</sup>)
- 20 jars containing DPS<sub>DRY</sub> at a dose of 11,6 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> (=0,82 g<sub>N</sub> kg<sub>dry soil</sub><sup>-1</sup>)
- 28 jars containing soil but no digestate (as controls)

Jars were placed in an incubation chamber in the dark at 20°C for 98 days. Soil incubations are frequently conducted at a temperature that will accelerate mineralisation and allow rapid observation over a shorter length of time than would be necessary at similar temperatures to field conditions (Levvasseur et al., 2022a). However, in the interest of conserving soil microbial activity at a rate similar to that observed in the field, and therefore maintain any related response in soil AS, it was decided to conduct incubation trials at 20°C. Average soil temperatures in Brittany rarely exceed this temperature

<https://fr.climate-data.org/europe/france/bretagne-268/>) and so there was less risk of potentially favouring different microbial populations that support higher temperatures.

The soil moisture content was maintained at around 80% (minimum >60%) of the water holding capacity ( $0.26 \text{ g g}_{\text{dry soil}}^{-1}$ ) to ensure optimum conditions for soil respiration (Rodrigo et al., 1997). The humidity of the head-space of the incubation jars was maintained using small pots of water placed next to the soil in order to prevent excessive evaporation and drying of the soil.

Jars containing soil were destructively sampled (four at a time from each modality) after 0, 7, 14, 42, and 98 days. Approximately 20-25g of the soil sampled from each sampled jar was dried at 40°C for 24h, and then stored. The samples were removed from storage and again dried at 40°C for 24h before soil aggregate stability analysis as per Le Bissonnais (1996), using all three tests. Soil moisture content was measured at each sample date by weighing a small soil subsample (2-4 g) before and after drying at 105°C for 24h.

#### 2.4.2.3 Incubation of fresh digestate

As a follow up to the dry digestate incubation trial, a small trial involving the fresh digestate of DCS and a control soil was devised. It was not possible to apply the same protocol to DPS as the moisture content was too high. This incubation was carried out over a shorter time scale of two weeks to assess the feasibility of a larger incubation trial with fresh digestate. The idea was to apply approximately the same protocol as for the dried digestate incubation while taking into account the considerable rise in soil moisture content resulting from the addition of wet digestate at high doses ( $4 \text{ g}_c/\text{kg dry soil}$ ). Soil was allowed to dry to around 20% SMC, before pre-incubation over 1 week at 20°C. Digestate was applied gently by pipette with minimal mixing of the soil. As a result, soil moisture increased to around 30%. The control jars were treated in a similar manner with an equivalent addition of demineralized water applied to bring the soil moisture content to the same level in order to eliminate any disparity between the two modalities. Jars were destructively sampled, in a similar manner as for the dry digestate incubation jars, at day 0 and day 14.

#### 2.4.2.4 Monitoring and calculating C mineralisation

Pots containing 20 ml 1 M NaOH were placed within four jars from each of the above modalities in order to monitor carbon dioxide evolved from the soil over the course of the incubation (by reaction with the NaOH). The pots were replaced at frequent intervals daily during the first weeks, then less frequently further on as C mineralisation naturally decreases, and the CO<sub>2</sub> captured by the removed pots was determined by titration method. Briefly, 1ml of solution was transferred from each pot into a flask along with 2.5 ml 30% BaCl solution, and one drop of thymolphthalein (acid-base indicator) which colours alkali solutions deep blue. The mixture was then titrated using 0.1 M HCl solution until the colouration disappeared and the liquid became clear, and the volume of HCl decanted was recorded. The quantity of NaOH remaining in the solution from the pots could be calculated and, by subtracting from the quantity in the original solution, the quantity reacted to form NaHCO<sub>3</sub> could be estimated. Finally the C-CO<sub>2</sub> reacted into the solution was calculated from the stoichiometry of reaction between NaOH and CO<sub>2</sub>.

#### 2.4.3 Results and discussion

##### 2.4.3.1 Impact of drying on digestate characteristics

The overall characteristics of the two digestates, DPS and DCS, before and after drying are given in *Table. 2.6*. Drying led to several changes in the digestate, including loss of volatile material – such as ammonium (N-NH<sub>4</sub>) and a small amount of organic matter (VS). Losses of TC were difficult to quantify, as the uncertainties of measurement were high. However, the losses of TKN were much larger, as nearly all N-NH<sub>4</sub> was lost from both digestates. As a result, the TC/TKN ratio was higher for the dried digestates, though the TC/Norg ratio remained approximately the same.

Comparison of the TC in the fractions obtained from sequential extraction for the two fresh digestates, given in *Fig. 2.4*, revealed small differences in the most readily extractible (SPOM) and non-extracted (NEOM) fractions. There were no significant differences between the two dried digestates when considered in terms of the TC in each fraction from sequential extraction. The fresh pig slurry digestate

DPS\_wet appeared to contain more highly complex compounds such as lignin and cellulose, making up the NEOM and PEOM fractions, while the fresh cow slurry digestate DCS\_wet contained more soluble organic matter (SPOM). Comparing between the fractions from sequential extraction of digestates before and after drying, some loss of C in the SPOM fraction was potentially observable. However, the uncertainties around these values were elevated due to error propagation (error bars not shown), and the differences relatively small in comparison. A reorganisation of the other fractions may have occurred, with DCS\_wet in particular showing more TOC in the SEOM fraction and less in the NEOM fraction compared to DCS\_dry. In another study of a solid digestate using the “Van Soest” sequential extraction method, the OM in the soluble fractions decreased by around 10 % after drying, while the more recalcitrant organic matter increased by 7% (Askri et al., 2016).

For the TN in each fraction from sequential extraction, the differences between fresh and dried digestates were much more significant (see Fig. 2.5b)). The TN of the SPOM fraction for fresh digestate, which contained the majority of the ammonium N for the fresh digestate, was much smaller in the dried digestates due to the loss of the ammonium N upon drying. Changes in the NEOM fraction were also observed, with less TN concentrated in this fraction for the dried digestates, however uncertainties in these measurements were high due to the method of calculating C and N contents of this fraction. The concentration of TN in the SPOM of DPS\_wet was considerably lower than the measured  $\text{N-NH}_4^+$  of the raw samples, which may be because some of the ammonium N volatilised from the samples before and during analysis.

Table. 2.6 Characteristics of fresh (wet) and oven-dried (dry) digestates

Characteristic		DCS_wet	DCS_dry	DPS_wet	DPS_dry
TS	g kg <sub>FM</sub> <sup>-1</sup>	103 ± 0	935 ± 1	40 ± 1	947 ± 0
VS	g kg <sub>TS</sub> <sup>-1</sup>	616 ± 5	604 ± 1	697 ± 17	687 ± 0
TC	g kg <sub>TS</sub> <sup>-1</sup>	337 ± 26	321 ± 1	392 ± 3	360 ± 5
TOC	g kg <sub>TS</sub> <sup>-1</sup>	330 ± 26	320 ± 1	390 ± 3	360 ± 5
TKN	g kg <sub>TS</sub> <sup>-1</sup>	67 ± 0.6	23.5 ± 0.5	123 ± 3.7	29.6 ± 0.2
N-NH <sub>4</sub>	g kg <sub>TS</sub> <sup>-1</sup>	42.9 ± 0.2	0.7 ± 0.4	88.8 ± 1.4	-
Norg*	g kg <sub>TS</sub> <sup>-1</sup>	24.2 ± 0.6	22.8 ± 0.6	34.3 ± 3.9	29.6 ± 0.2
TC/TKN		5	13.7	3.2	12.1
TOC/Norg		13.6	14	11.4	12.1
pH		8.4	-	8.4	-

\*Norg = TKN – N-NH<sub>4</sub>, Diff = difference between lab digestate and full-scale digestate, e = standard deviation.

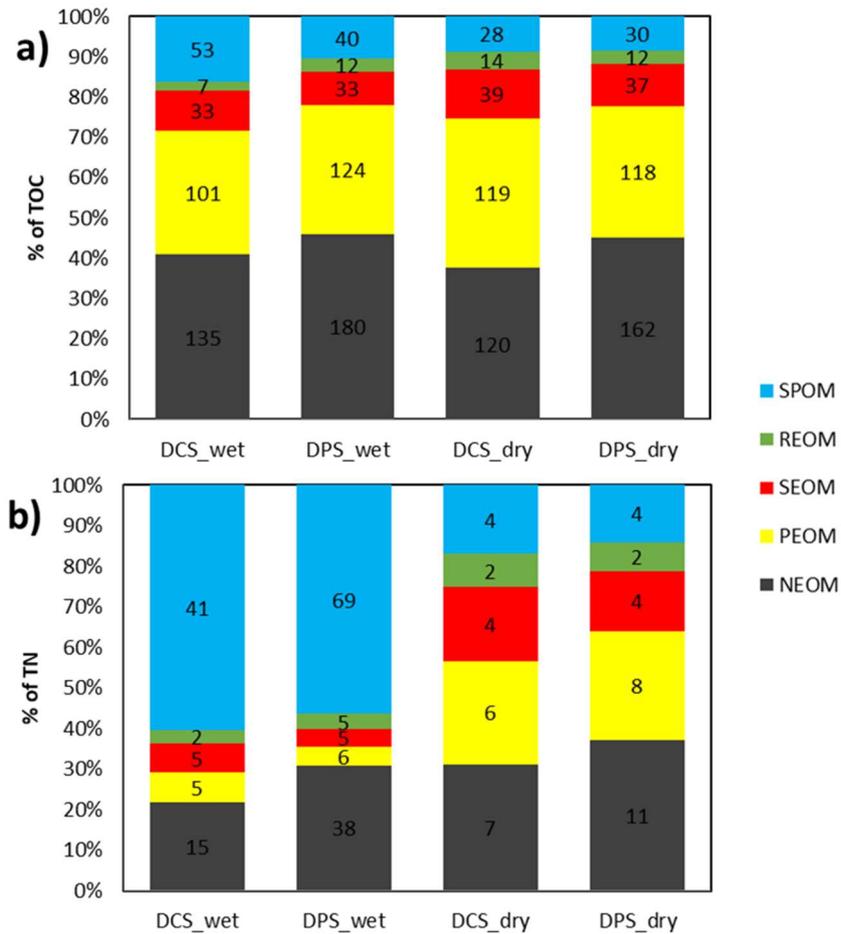
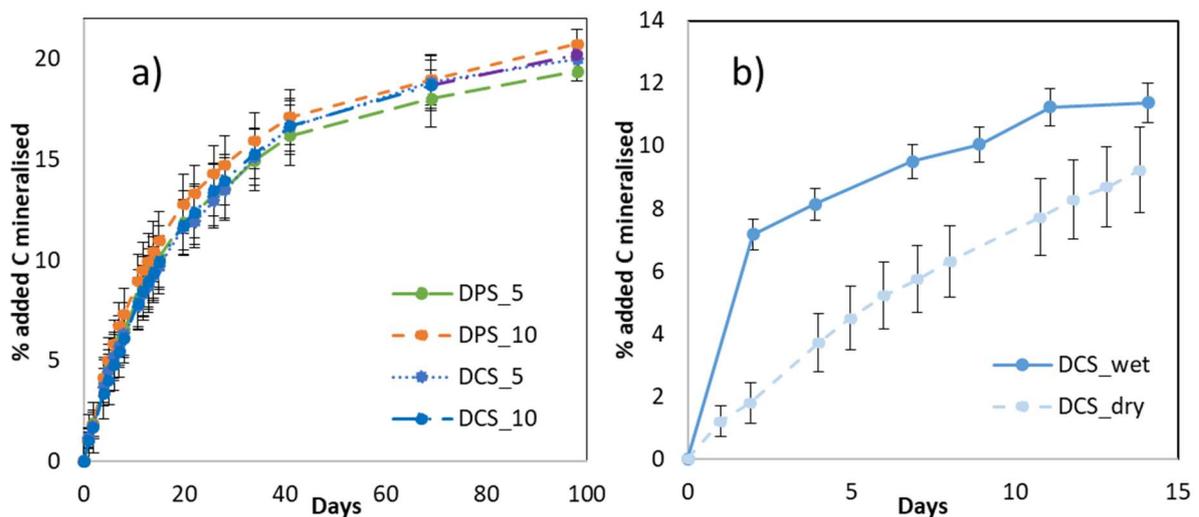


Fig. 2.5. The percentage of a) TOC and b) TN in the OM fractions of fresh (wet) and oven-dried (dry) digestates obtained by sequential extraction using the “EPS” method (Jimenez et al., 2014)

### 2.4.3.2 Mineralisation of CO<sub>2</sub> during incubation

The calculated percentage of added carbon mineralised was very similar across all modalities, regardless of the type of digestate or the dose of C added (*Fig. 2.6a*). The similarity in the rate and amount of C mineralisation for both digestates would corroborate the conclusion from the sequential extraction analysis that the two dried digestates had very similar OM properties. After 98 days of incubation, the total C mineralisation reached approximately 20% of added carbon, which is slightly lower than the mineralisation of digestates observed in certain other trials (Askri et al., 2016; Béghin-Tanneau et al., 2019), though was comparable to results obtained by (Häfner et al., 2022) for a wide range of fresh raw digestates.



*Fig. 2.6. Carbon mineralisation curves for a) the incubated wet digestates (DCS and DPS) at two doses (5 and 10 g<sub>C</sub> kg<sup>-1</sup> dry soil) and b) the incubated wet (DCS\_wet) and dry (DCS\_dry) digestates compared*

### 2.4.3.3 Comparison of carbon mineralised from dry and wet digestate in soil

Comparing the cumulative carbon mineralisation from the incubation of fresh digestate (DCS\_wet) and the dried digestate (DCS\_dry) (shown in *Fig. 2.6b*), it can be seen that C mineralisation was initially much faster for the fresh digestate, with a loss of approximately 7% of added C during the first two days of incubation. However, the rate of C mineralisation rapidly decreased and, by day 14, the slower but steadier mineralisation of the dry digestate was almost at a similar percentage of the added C. Preliminary experiments carried out by (Béghin-Tanneau et al., 2019) indicated little difference

between dried or fresh samples in terms of results from respirometry trials. Conversely, (Askri et al., 2016) found carbon mineralisation of a dried digestate was more than halved when compared to the same raw digestate. It is likely that the OM in wet digestates is more readily mixed into the soil and more bioaccessible to soil microorganisms than dried digestate.

#### 2.4.3.4 Aggregate stability dynamics following addition of dried digestates to soil

The results of soil AS analysis are shown in Fig. 2.7. Results from the analysis of structural stability using the “fast wetting” ( $MWD_{FW}$ ) and “ethanol” ( $MWD_{ETH}$ ) tests showed little significant difference between modalities and no significant evolution over time. On the other hand, results from the “slow wetting” ( $MWD_{SW}$ ) test showed a significant increase in MWD due to the addition of digestate. Of the three tests, the slow wetting test appeared to be the most sensible for observation of the aggregate stability dynamics resulting from digestate application, and closely resembled the mean curve for the three tests.

There were few differences in soil AS dynamics between the two digestates and the two doses applied up to day 42 of incubation. It may be that whatever factors were responsible for the AS dynamics were common to both digestates and did not bring a relative increase in impact at a higher dose (i.e. saturation of effect). Investigation of the effect of different doses of maize straw on soil AS by Cosentino (2006) revealed that, beyond a certain limit of added OC the additional effects on soil AS were minimal. At T0, soils to which digestate had been added were markedly more stable than the control. This effect diminished gradually for T1 (day 7) and T2 (day 14), but appeared to level off by T3 (day 42) to an  $MWD_{sw}$  slightly higher than the control. At T4 (day 98), soils having received the higher doses of the two digestates showed an increased  $MWD_{sw}$ , while the soils having received lower doses of digestates remained at a similar  $MWD_{sw}$  to T3.

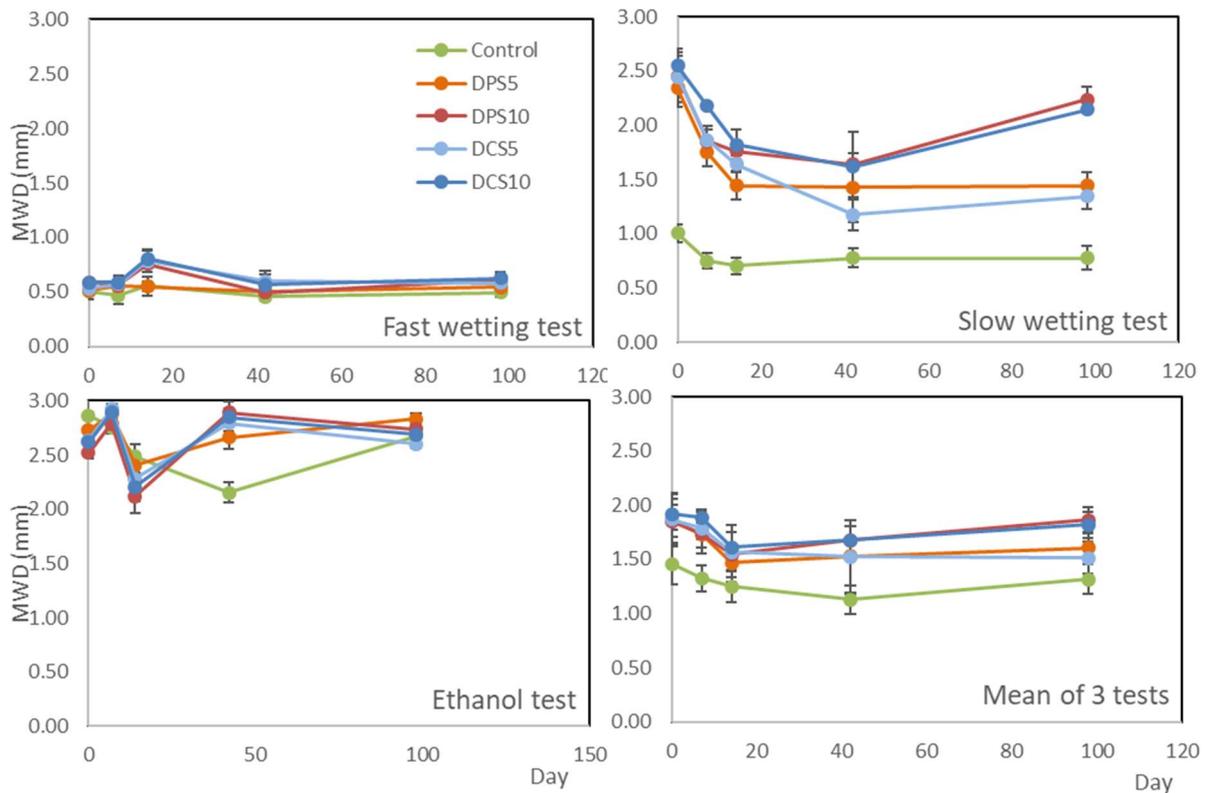


Fig. 2.7 Aggregate stability (MWD) dynamics of soil incubated with two digestates (DPS and DCS) at two application rates (5 and 10  $g_C kg_{dry\ soil}^{-1}$ ). Error bars represent standard deviation ( $n=4$ )

The immediate increase in  $MWD_{sw}$  suggests that there was an immediate physico-chemical stabilisation of aggregates by the added digestates at T0, while the downward trend in  $MWD_{sw}$  after this date would suggest a rapid breakdown of some of the component(s) responsible for this effect. This result has not been observed in other trials monitoring impacts of digestates on soil aggregate stability (Dénès et al., 2014; Sarker et al., 2018), and it is possible that this effect was due to the fact that digestates were dried prior to incubation. However, a study involving incubation of composts also noted an immediate increase in soil AS following amendment, attributing it to hydrophobic molecules coating aggregates (Annabi et al., 2007). Coating of the soil aggregates with hydrophobic molecules, or else a modification of aggregate porosity by fine particles of dry digestate, would explain the significant increase in  $MWD_{sw}$  relative to the control soil. These effects would have acted to impede water entry into the pore structure of the aggregate, and reduce the effects of slaking.

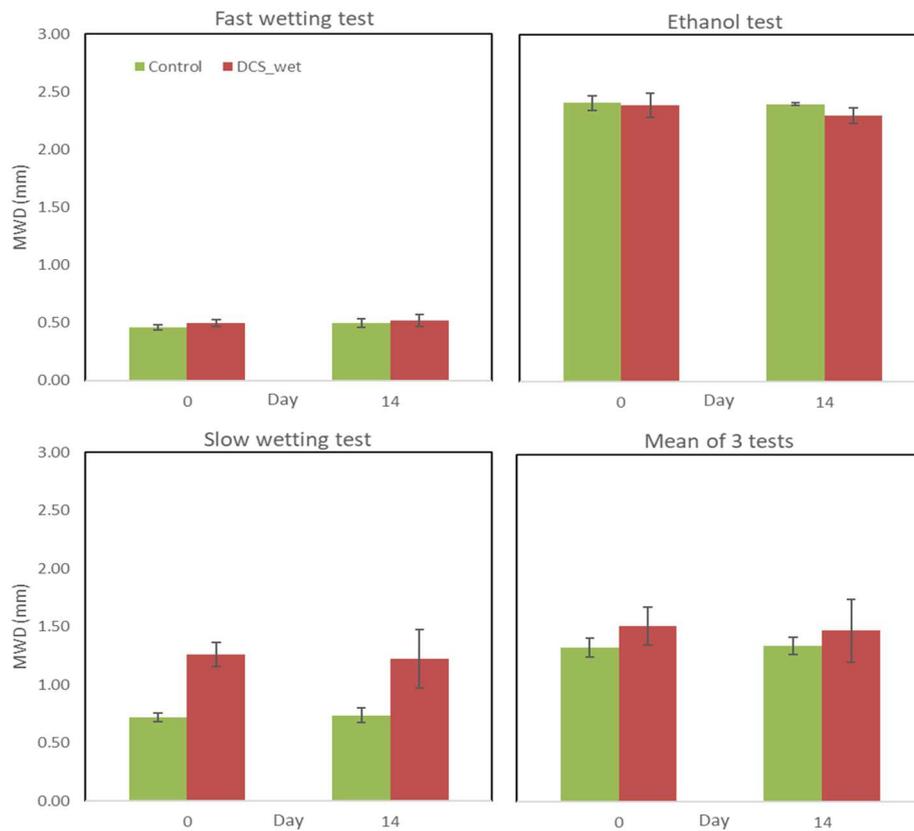
Some issues were encountered during drying of aggregate samples for testing, which led to aggregates being slightly humid at testing. This led to a slight increase in  $MWD_{FW}$  (less slaking effect as some pores still contained water) at T2, and a slight decrease observed for  $MWD_{ETH}$  (the ethanol is unable to displace completely the residual water in the aggregate pores). An excessively long drying time or elevated oven temperature may also have been responsible for the elevated MWD (across all tests) of control soil at T0, though it was not possible to verify this. The moisture content of the soil can affect analysis of soil AS (Almajmaie et al., 2017). The effect of drying may result in a greater disparity in aggregate stability between aggregates containing high levels of OM and those containing lower levels of OM (Haynes and Swift, 1990). Therefore, greater care was taken over future trials to achieve precise control of soil moisture during incubation (monitoring at each sample date) and sample preparation (strict timing of oven drying interval).

#### 2.4.3.5 Aggregate stability dynamics following addition of wet digestates to soil

Analysis of the aggregate stability of soil following addition of wet digestate are shown in *Fig. 2.8*. Again, the slow wetting test proved to be the best for distinguishing between the unamended and amended soil, while differences between modalities and between sample dates were not significant for the fast wetting and ethanol tests. The  $MWD_{sw}$  of the aggregates amended with DCS\_wet improved relative to the control at day 0, and remained significant at day 14, though some samples showed signs of a decreasing AS (not significant).

It is possible that the impact of moisture reduced the overall observed effect of the binding agents contained in the digestates. When adding the fresh digestates, the soil moisture content was greatly increased and caused some soil dispersion. This dispersed material then bound smaller aggregates to create larger aggregates. However, these were removed before aggregate stability analysis as the soil was systematically sieved beforehand to ensure only 3.15-5 mm aggregates were tested. In the end, there was little observable difference in  $MWD_{sw}$  between the control soils of the dry digestate incubation (0.8 mm) and wet digestate incubation (0.73 mm) averaged over all sample dates. This

would suggest that the differences in soil moisture content dynamics between the two incubation protocols only had a minor effect on the AS of unamended aggregates remaining in the specified size range.



*Fig. 2.8 Aggregate stability (MWD) of soil incubated with a wet raw digestate (Red bars) and without digestate (Green bars) as per the three tests proposed by (Le Bissonnais, 1996). Error bars represent the standard deviation (n=4).*

#### 2.4.4 Conclusion

The characterisation and agronomic testing of two digestates (DPS and DCS) showed both to be very similar in terms of their OM composition as determined by sequential extraction, their C mineralisation in soil and their effect on soil AS dynamics. These digestates had been selected based on an expected contrast between digestates produced from different feed substrates. Many AD plants in France are based on farms (Chambres d’Agriculture France, 2022), and these installations are often fed with a

large proportion of indigenously produced manures or slurries (Kovačić et al., 2022), along with plant residues from cover crop and various agro-industrial wastes, and so feed mixtures may be fairly similar.

Comparisons between two doses of dried digestate applied to the soil (5 and 10 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup>), revealed little observable difference in terms of both C mineralisation and AS dynamics. It was decided to remain with a dose closer to the lower value for future experiments (6 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup>), which is similar to that used in another thesis project testing the impacts of digestate on soil physical and biological properties (Béghin-Tanneau, 2020).

An incubation experiment using raw wet digestate, while moderately successful, presented a number of issues due to the additional moisture introduced to the soil that brought into question the validity of the results obtained. It is likely that some differences will always result from the method of digestate preparation and the parameters used in respirometry trials. However, the benefits of drying with respect to the greater precision achieved and the potential for adapting to digestates of widely varying moisture contents meant that this protocol was selected for use in all subsequent incubation experiments. It was considered that this method still provided useful information with regards to the impact of digestate OM on the soil AS, and could be compared to a number of other studies where organic amendments were also dried before incubation (Abiven et al., 2007; Annabi et al., 2007; Béghin-Tanneau, 2020; Sarker et al., 2018).

### 3 Characterising the effect of raw and post-treated digestates on soil aggregate stability

Based on the lessons learnt over the course of the experiments described in the previous chapter, it was decided to proceed by conducting a series of trials using an optimised incubation protocol and incorporating a larger number of digestates sampled from full-scale agricultural anaerobic digesters. It appeared that the difference in the mixture of feed materials tested did not necessarily result in significant differences in digestate OM quality or impact on soil AS. Consequently, the criteria for selection of the sampled digestates were broadened to cover a wider range of plant configurations and AD operating parameters. The following chapter is a copy of the published article:

Cooke, J., Girault, R., Busnot, S., Morvan, T., Menasseri-Aubry, 2023. Characterising the Effect of Raw and Post-Treated Digestates on Soil Aggregate Stability. Waste Biomass Valorization.

<https://doi.org/10.1007/s12649-023-02045-3>

# Characterising the effect of raw and post-treated digestates on soil aggregate stability

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## ○ Abstract

**Purpose.** Digestates from anaerobic digestion are increasingly used in agriculture. There is thus a need for better understanding of digestates' value as organic soil amendments, and how this varies depending on the process parameters and post-treatments applied. **Methods.** A range of fifteen digestates (raw, solid, phase separated, and composted digestates), originating from farm and centralised anaerobic digestion sites were characterised. Carbon mineralisation and soil aggregate stability (AS) dynamics following the addition of the digestates to soil were monitored over a 182-day incubation period at 20°C. **Results.** The mineralisation of carbon varied significantly between digestates, with the lowest percentage of carbon lost at 11.45% for composted digestate, and the highest at 49.27% for a solid batch digestate. Using hierarchical clustering of soil AS analysis at several sample dates, digestates were classified into four groups: those inducing 1) rapid but transient improvement of AS, 2) slower, temporary improvement of AS, 3) immediate improvement followed by a longer-lasting increase in AS, 4) minor or non-significant improvement in AS. **Conclusion.** Phase separation and composting of digestates were found to have more impact than other process parameters in determining the rate of mineralisation and dynamics in AS. **Impact.** Characterising and

classifying digestates by their potential impact on soil structure would help to improve decision making for digestate production and sustainable use in agriculture.

**Keywords.** Digestate, Anaerobic Digestion, Soil Aggregate Stability, Organic Amendment, Carbon Mineralisation

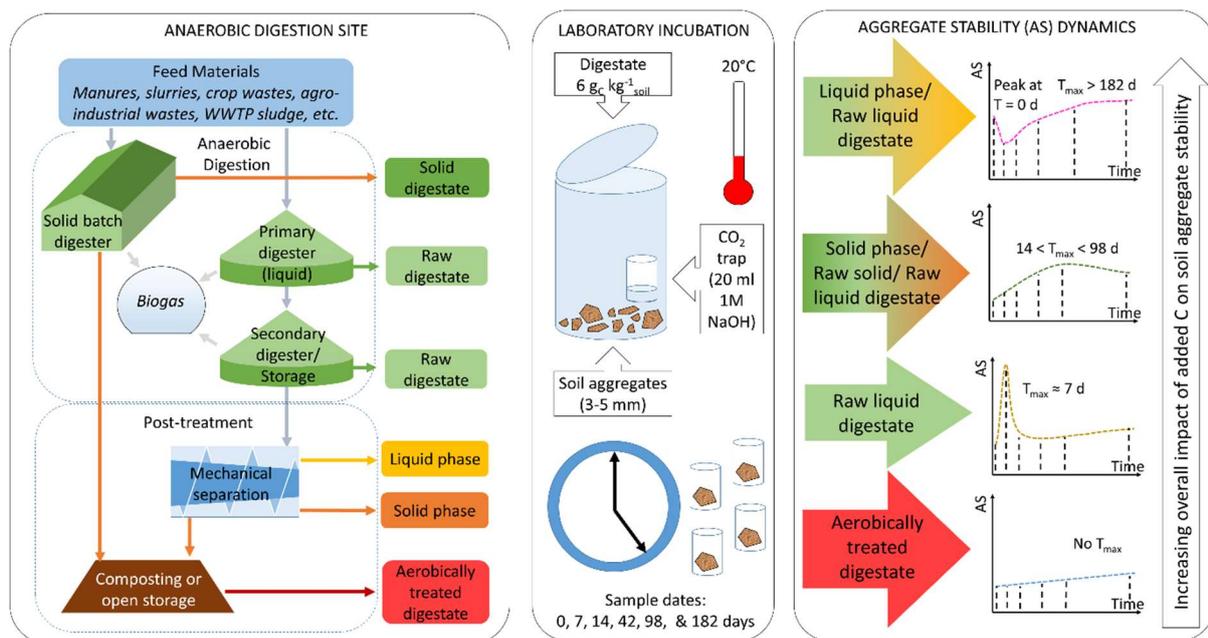


Fig. 3.1 Visual abstract of article: “Characterising the effect of raw and post-treated digestates on soil aggregate stability”

- Statement of Novelty

Despite the increasing use of digestates in agriculture, their short to medium-term impact on soil structure has rarely been characterised. This is the first study to include a wide range of digestates produced under different process conditions and to characterise them by measuring the stability of soil aggregates. We observed several aggregate stability (AS) dynamics following digestate amendment, and developed a typology based on our findings. We linked the typology to the anaerobic digestion process parameters, in particular phase separation and aerobic post-treatment. This study is

a step towards optimising the design of anaerobic digestion as a function of the impact of digestate dynamics on soil properties.

- Highlights
  - Digestate carbon mineralisation rates in soil varied between 11.45% and 49.27%
  - Digestate impact on soil aggregate stability was either positive or not significant
  - Differing dynamics in aggregate stability were observed after digestate amendment
  - Phase separation strongly affected the impact of digestates on aggregate stability
  - Increasing degree of stability of digestate reduced impact on aggregate stability
  
- Statements and declarations

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**Competing Interests.** The authors have no relevant financial or non-financial interests to disclose.

**Author contributions.** This article is part of Joshua Cooke's PhD thesis, supervised by Safya Menasseri and Romain Girault. All the authors contributed to the conception and design of the study. Joshua Cooke, Sylvain Busnot and Thierry Morvan prepared the material, collected and analysed the data. Joshua Cooke wrote the first draft of the manuscript. Safya Menasseri and Romain Girault commented on previous versions of the manuscript. All the authors gave their consent to submit the manuscript.

**Data availability.** The datasets generated during and/or analysed during the current study are not publicly available but can be obtained from the corresponding author upon reasonable request.

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team (part of the SOERE PRO network), the staff and students of the French Higher Education Institution in Agriculture, Food, Horticultural and Landscape Sciences (Institut Agro) Rennes who assisted in this research, and the staff of the anaerobic digestion sites from which samples were collected.

### 3.1 Introduction

In the context of increasing European policies focussing on a circular and green economy, anaerobic digestion of wastes for biogas production is a promising livestock waste management solution that enables both renewable energy production and nutrient recycling (Albuquerque et al., 2012b; Panuccio et al., 2021). The sludge remaining after anaerobic digestion (AD), known as digestate, is frequently used as a viable alternative to mineral fertiliser (Möller and Müller, 2012). Recently, the focus has been on its value as an organic amendment through its potential ability to increase soil carbon and improve soil physical and biological properties (Häfner et al., 2022; Pastorelli et al., 2021; Tambone et al., 2019).

When an anaerobic digester is installed on a farm, the organic wastes (manures, crop residues, etc.) generated in-situ, often mixed with exogenous waste streams such as those from the agro-industrial sector, are fermented in order to produce biogas (Albuquerque et al., 2012a). Anaerobic digesters may differ in their design and operational parameters including volume, temperature and the time spent in the digester (Weiland, 2010). Monitoring of changes in digestate composition over the course of AD has shown accumulation and degradation of various carbon and nitrogen fractions depending on the length of time spent in the digester (Bareha et al., 2019). Post-treatment of the resulting digestate is common, including mechanical separation into a liquid phase rich in nutrients and a solid phase rich in carbon, with subsequent storage or composting of the solid phase (Al Seadi et al., 2013). While, following phase separation, the solid phase of digestate is generally richer in organic carbon, the efficiency of the separation process used determines which fractions remain in the liquid phase

(Aguirre-Villegas et al., 2019; Akhlar et al., 2021), and hence, the liquid phase may still contain large quantities of organic matter (Tambone et al., 2019). These different processing methods produce digestates with a wide range of characteristics that can significantly affect their use as a soil amendment.

The accumulation of stable organic matter in digestates during treatment means they are often of potential use to increase the soil carbon pool (Béghin-Tanneau et al., 2019; Chen et al., 2012; Greenberg et al., 2019). However, the diversity of digestates produced by operational AD plants results in different carbon mineralisation rates (Levavasseur et al., 2022a), and there is still a need to identify and characterise the conditions under which digestates are produced and what effect they have as a result. Abubaker et al. (2015) reported that digestates from AD operating at different temperatures and with different feed mixtures displayed very different carbon mineralisation dynamics. In a study of the impact of digestate post-treatments including phase separation and composting on digestate carbon mineralisation, Askri et al. (2016) concluded that solid digestate contains much higher levels of labile carbon than raw or liquid digestate, but that this carbon mineralises more slowly in the soil. Conversely, Cattin et al. (2021) found that, in the short term, the organic carbon in the solid phase of digestate mineralised faster in soil than that in the raw digestate. Composting of digestates is reported to produce a highly stable amendment suitable for increasing soil carbon (de la Fuente et al., 2013; Teglia et al., 2011), although in turn, this could limit its positive impacts on soil biology and on other soil properties.

Digestates are widely considered to have many physical benefits for the soil, including reducing bulk density, increasing water retention (Garg et al., 2005), altering pore structure (Skic et al., 2020), increasing mechanical strength and improving overall fertility (Beni et al., 2012). Several recent field studies have demonstrated a positive (Albuquerque et al., 2012b; Badagliacca et al., 2020; Erhart et al., 2014; Frøseth et al., 2014) though occasionally non-significant (Manasa et al., 2020; Pastorelli et al., 2021) impact of applying digestate on soil aggregate stability (AS). Soil AS is a key indicator of the

soil's structural resistance to the formation of surface crusts and to soil losses through erosion (Barthès and Roose, 2002; Bronick and Lal, 2005).

The scale and the duration of the effects on soil AS following organic amendment depend on many different characteristics of both the soil and the organic materials used (Abiven et al., 2009; Sarker et al., 2018). Among the main mechanisms through which organic amendment may modify the soil AS are stimulation of the soil microbes that produce mucilaginous compounds that bind with soil particles (Cosentino et al., 2006), and of fungal hyphae that form a protective “net” around soil aggregates (Grosbellet et al., 2011; Oades and Waters, 1991). Likewise, coating of soil aggregates by lipids (Paré et al., 1999) or by more complex hydrophobic organic material (Dal Ferro et al., 2012; Piccolo and Mbagwu, 1999) in the organic amendment may directly affect soil AS. The long established conceptual model developed by Monnier (1965), which was more recently verified and applied to the POULOU model (Abiven et al., 2008), predicts that the dynamics in soil AS over time resulting from biological activity following organic amendment will depend on the quantity and quality of labile organic matter (OM) added.

From the above, it may be predicted that soil amendment using digestates of varying quality depending on their production will result in varying dynamics in soil AS. However, the dynamics in soil AS occurring over the short- to medium- term following the application of digestate amendment remain relatively unexplored. Likewise, few studies investigating the impact of digestate on soil physical properties such as soil AS take into account the wide range of different digestates. The objectives of this study were thus to:

- Characterise the value of digestate amendment based on carbon mineralisation and the effect of adding the digestate on soil AS dynamics;
- Produce a typology of the digestates based on the observed AS dynamics
- Assess any possible link between AD process parameters and the measured amendment characteristics of the digestates.

## 3.2 Materials and Method

### 3.2.1 Digestate and undigested organic amendment sampling

Fifteen digestates were collected from six on-farm AD sites and one centralised site located in France (see Table. 3.1). The digesters operated as either continuous stirred tank reactors (CSTR) or solid batch reactors (SBR), all within the mesophilic temperature range (38-42 °C). The digestates were originally selected to investigate the parameters considered most likely to affect the value of digestate as an amendment, notably:

- the length of the anaerobic treatment, known as the hydraulic residence time ( $HRT_{AD}$ ), which is calculated as the volume of the primary digester and any secondary digesters divided by the average volume of material fed to the digesters per day. The  $HRT_{AD}$  of the digestates used in the present study ranged from 25 to 120 days;
- the type and quantity of organic materials treated (manures, slurries, crop wastes, vegetable wastes, etc.), though for most of the sampled digestates, the composition of the feed was found to be primarily ruminant manure or slurry with a varying mixture of other feed materials;
- the post-treatment following AD. In this case, wherever possible, digestates were sampled from before and after existing post-treatment steps including phase separation by screw-press or centrifuge, and also following composting or open-air storage.

Table. 3.1 Summary of the anaerobic digestion process parameters for the digestates characterised, and the resulting categorical variables used in the analysis

Digestate	Type of digester (Location #)	Composition of digester feed (% of mixture, if known)	HRT <sub>AD</sub> (days)	Phase separator	Digester feed mixture	Type of post-treatment	Degree of stabilisation <sup>a</sup>
RD1a	CSTR (Farm 1)	Cow/Pig manure (>25%), Maize silage (>25%), Cow/Pig slurry, Crop residues, Waste fats, Vegetable waste	25	-	2	Raw (Liquid)	Low
RD1b	CSTR (Farm 1)		50	-	2	Raw (Liquid)	Medium
RD2	CSTR (Farm 2)	Cow manure (>50%), Cow slurry, Turkey manure, Vegetable waste, Animal feed waste, Road verge clippings	120	-	2	Raw (Liquid)	High
LP2	CSTR (Farm 2)		120	Screw press	2	Liquid phase	High
SP2	CSTR (Farm 2)		120	Screw press	2	Solid phase	High
RD3	CSTR <sup>c</sup> (Farm 3)	Cow slurry, Cow manure, Milk and whey permeate, Waste fats, Cereal wastes, Crop residues, Vegetable Waste, Road verge clippings	45	-	4	Raw (Liquid)	Medium
SP3	CSTR <sup>c</sup> (Farm 3)		45	Screw press	4	Solid phase	Medium
RD4	CSTR (Farm 4)	Cow manure (>30%), Maize straw (>30%), Crop residues, Vegetable waste	50	-	1	Raw (Liquid)	Medium
LP4	CSTR (Farm 4)		50	Screw press	1	Liquid phase	Medium
CS4	CSTR (Farm 4)		50	Screw press	1	Compost (Solid phase)	Aerobic
RD5	Solid batch (Farm 5)	Cow manure (>90%), Cereal wastes, Crop residues, Vegetable waste	60	-	3	Raw (Solid)	Medium
CS5	Solid batch (Farm 5)		60	-	3	Stored (Solid)	Aerobic
CS6	Solid batch (Farm 6) <sup>b</sup>	Goat/Cow manure (>90%), Vegetable waste	40	-	3	Stored (Solid)	Aerobic
LP7	CSTR (Centralised 1)	Sludge from waste-water treatment (>30%), Animal fats/Slaughterhouse waste, Vegetable waste	40	Centrifuge	5	Liquid phase <sup>d</sup>	Low
CS7	CSTR (Centralised 1)		40	Centrifuge	5	Compost (Solid phase) <sup>e</sup>	Aerobic
Undigested amendment							
CM	N/A	N/A	N/A	N/A	0	Undigested	None
MS	N/A	N/A	N/A	N/A	0	Undigested	None

HRT<sub>AD</sub> = Estimated Hydraulic Residence Time in AD, RD = Raw digestate, CS = Composted or stored digestate, LP = Liquid phase of digestate, SP = Solid phase of digestate, CM = Cow manure, CSTR = Continuous stirred tank reactor. Footnotes: <sup>a</sup>For the Degree of stabilisation: None = raw substrates, Low = non-stored digestate with HRT<sub>AD</sub> < 50 days, Medium = non-stored digestate with HRT<sub>AD</sub> 50-100 days (or with substrate pre-treatment in the case of RD3 and SP3), High = unstored digestate either with HRT<sub>AD</sub> > 100 days, Aerobic = solid digestate or the solid phase of digestate that has undergone aerobic transformation after anaerobic digestion, <sup>b</sup> solid batch process with permeate recycling, <sup>c</sup> plant includes proprietary hydrolysis pre-treatment, <sup>d</sup> pasteurized liquid phase after centrifugation, <sup>e</sup> solid phase after centrifugation, composted with woody green waste and sampled during active composting.

Three synthetic descriptive variables were created based on the information on AD production parameters gathered about each digestate. The “Type of post-treatment” refers to the post-treatment(s) applied to the digestate: Raw = no post-treatment, Liquid phase = liquid fraction after phase separation, Solid phase = solid fraction after phase separation, Composted = compost produced from solid digestate or from the solid phase. The “Degree of stabilisation” depends on the length of time spent in AD (the hydraulic residence time or  $HRT_{AD}$ ) and aerobic treatment applied to solid digestates (storage or composting), as well as pre-treatments such as substrate hydrolysis: None = raw substrate, Low = digestate with  $HRT_{AD} < 50$  days, Medium = digestate with  $HRT_{AD}$  from 50-100 days, High = digestate with  $HRT_{AD} > 100$  days, Aerobic = solid digestate that has undergone aerobic transformation through storage or composting. In the case of digestates RD3 and SP3, as the process includes proprietary hydrolysis pre-treatment, we decided to include them in “medium” stability despite the relatively short  $HRT_{AD}$ . The variable “Digester feed mixture” was adapted from the first-fertilising typology described by Wallrich et al. (Wallrich et al., 2018), developed as a method for grouping digestates by commonly occurring feed mixtures, where: 0 = Undigested amendment, 1 = Mostly ruminant manure with crop residues, 2 = Mostly ruminant manure and slurry with crop residues, 3 = Mostly ruminant manure, 4 = Mostly ruminant slurry, 5 = Mostly non-ruminant slurry.

Table. 3.2 Characteristics of digestates and undigested organic amendments sampled for incubation

Digestate	TS (g kg <sub>FM</sub> <sup>-1</sup> )	±	VS (g kg <sub>FM</sub> <sup>-1</sup> )	±	TC (g kg <sub>TS</sub> <sup>-1</sup> )	±	TKN (g kg <sub>TS</sub> <sup>-1</sup> )	±	TP (g kg <sub>TS</sub> <sup>-1</sup> )	±	TK (g kg <sub>TS</sub> <sup>-1</sup> )	±	TC :TKN	Dose C (g kg <sub>DS</sub> <sup>-1</sup> )	Incubation
RD1a	131	2	83	2	347	3	46.1	0.9	19.1	0.3	51.1	0.1	7.5	5.5	Spring 2020
RD1b	97	0	57	0	339	5	57.4	0.3	19.4	0.3	58.9	0.6	5.9	5.8	Spring 2020
RD2	97	1	69	1	413	1	60.7	0.5	14.4	0.2	46.3	0.9	6.8	5.3	Spring 2020
LP2	76	1	49	1	369	3	74.8	1.8	15.4	0.2	59.8	0.4	4.9	6.1	Spring 2020
SP2	293	27	246	26	462	0	27.1	0.5	12.2	0.4	16.1	1.6	17.0	6.0	Spring 2020
RD3	78	0	47	0	312	8	74.4	1.4	30.5	0.3	67.1	0.8	4.2	6.0	Autumn 2020
SP3	224	3	189	2	437	5	22.6	0.2	16.0	0.1	22.9	0.2	19.3	6.0	Autumn 2020
RD4	130	0	81	1	331	6	64.0	1.5	14.3	0.1	33.0	0.4	5.2	6.0	Autumn 2020
LP4	95	0	48	0	285	1	84.5	2.2	16.3	0.1	51.6	0.2	3.4	6.0	Autumn 2020
CS4	240	3	183	3	372	5	29.5	0.7	13.1	0.1	20.3	0.3	12.6	6.0	Autumn 2020
RD5	236	35	169	21	355	3	35.5	0.2	10.5	0.2	43.2	0.9	10.0	6.0	Spring 2020
CS5	252	29	197	-	364	13	29.8	1.3	12.8	0.5	56.7	1.8	12.2	6.0	Spring 2020
CS6	216	12	160	10	393	14	31.2	0.1	7.8	0.1	56.6	0.3	12.6	6.0	Spring 2020
LP7	24	0	16	0	353	3	273	3.4	19.3	0.3	49.7	0.7	1.3	6.0	Autumn 2020
CS7	429	7	222	2	280	5	27.0	1.3	12.1	0.6	9.0	1.2	10.4	6.0	Autumn 2020
Undigested organic amendment															
CM	220	3	188	3	441	2	22.6	0.6	4.1	0.0	30.4	0.2	19.6	6.0	Autumn 2020
MS	954	0	919	-	461	2	8.8	0.2	1.7	0.1	10.5	0.1	52.7	6.0	Autumn 2020

TS = Total solids, FM = Fresh matter, VS = Volatile solids, TC = Total carbon, TKN = Total Kjeldahl nitrogen, TP = Total phosphorus, TK = Total potassium, Dose C = Dose of carbon added in the form of organic residue per weight of dry soil, DS = Dry soil, RD = Raw digestate, CS = Composted or stored digestate, LP = Liquid phase of digestate, SP = Solid phase of digestate, CM = Cow manure. The errors (±) shown are standard deviations.

### 3.2.2 Characterisation of organic amendments

All the organic amendments contained a wide range of organic matter with different chemical characteristics (see Table. 3.2). Total solids (*TS*) and volatile solids (*VS*) were analysed by weighing the samples before and after drying at 105 °C for 24-48 hours followed by combustion at 550 °C for 5 hours, respectively. Total carbon (*TC*) and nitrogen (*TN*) were analysed by combustion using a Thermo Scientific Flash® 2000 CN analyser, following the manufacturer's instructions. Total Kjeldhal nitrogen (*TKN*) was obtained by steam distillation after mineralisation in strong acid using standard methods (APHA, 2005). A solution was obtained by mineralising the ashes resulting from the above-described combustion in a mixture of HCl (37%) and HNO<sub>3</sub> (65%) followed by digestion in an Anton Paar® HVT50 Microwave Reactor. Total phosphorus (*TP*) and total potassium (*TK*) in the solution were determined by spectrophotometry using a ThermoFisher Gallery® Discrete Analyser, and by microwave plasma-atomic emission spectrometry using a Agilent® 4200 MPAES, both following the manufacturers' instructions.

### 3.2.3 Incubation protocol

Due to the number of organic amendment samples involved and the scale of the experiment, incubation trials had to be carried out on two separate occasions (Table 2 indicates which trial a given organic amendment was incubated in). Consequently, two separate batches of soil were collected from the top layer (first 20 cm) of the same parcel at the EFELE experimental site (Le Rheu, France, 48°06'07 N, 2°47'44 W) in the spring and autumn of 2020. The soil is classified as a Luvisol-Redoxisol derived from loess silt deposits with the following characteristics: 14.2% clay, 71.0% silt, 14.8% sand, 2.06% organic carbon, carbon-nitrogen ratio: 10, pH: 6.1 and CEC<sub>Metson</sub>: 6.34 cmol.kg<sup>-1</sup> (adapted from Cheviron et al. (Cheviron et al., 2021)). Since 2012, the site has been managed using a maize-wheat-green manure crop rotation, and the plot where soil was sampled had not previously received digestate as organic amendment. Soil moisture was found to be similar on the two sampling dates, 0.22±0.01 g g<sub>dry soil</sub><sup>-1</sup> in spring 2020 and 0.21±0.01 g g<sub>dry soil</sub><sup>-1</sup> in autumn 2020, i.e. equivalent to 85-87% of soil water

holding capacity ( $SWHC = 0.249 \text{ g g}_{\text{dry soil}}^{-1}$ ). The soil was kept moist and stored at 5 °C until required for preparation of aggregates between 3.15 and 5 mm in diameter. The aggregates were obtained by gently breaking clumps of soil along lines of least resistance followed by gentle sieving. The soil aggregates were then stored in unsealed containers at 14-20 °C for at least 2 weeks, or until needed, to allow microbial activity to stabilise.

All organic amendments were dried at 40 °C for 1 week and ground to  $\leq 2 \text{ mm}$ . One week before the beginning of incubation, 110 g of soil aggregates were placed in airtight jars and incubated at 20 °C to allow the soil microorganisms time to acclimatise to the change in conditions. After the incubation period, except for the control soil, organic amendments were added and mixed with the soil in each jar to reach the dose of total C listed in Table 2 (approximately  $6 \text{ g}_C \text{ kg}_{\text{dry soil}}^{-1}$ ). In addition to 24 control jars containing soil with no amendment, 24 jars for each type of organic amendment were filled in this way. Aggregate moisture content was adjusted by spraying water to reach approximately  $0.199 \text{ g g}_{\text{dry soil}}^{-1}$ , or 80% of the water holding capacity at the time the jars were sealed. As the ratio of carbon to nitrogen for MS was high ( $C/N = 53$ ), to avoid nitrogen limiting conditions (Cosentino et al., 2006), a solution of ammonium nitrate was added instead of water to adjust the ratio to 15. The jars were then sealed and placed in an incubator in the dark at 20 °C for 182 days. At days 0, 7, 14, 42, 98 and 182, four jars representing each treatment were destructively sampled for analysis. The soil used to analyse aggregate stability (AS) was dried at 40 °C for 24 hours and then stored at 4 °C in sealed containers until analysis (Le Bissonnais, 1996). We decided not to add water during incubation to avoid breaking down the aggregates in the process. At each sampling date, the soil moisture content of each sample was measured by weighing it before and after drying at 105 °C for 24 hours. Samples with a moisture content less than 60% of the  $SWHC$  were not used for further analysis.

### 3.2.4 Measurement of soil aggregate stability

Soil AS was analysed using the method established by Le Bissonais (1996), which is the basis of the European standard method (ISO 10930, 2012) and is widely used on an international level (Rabot et al., 2018). The “slow-wetting” test was selected as the most suitable for fragile silt soils with low organic matter content (Le Bissonais and Arrouays, 1997). Briefly, approximately 5 g of the prepared aggregates, dried for 24 hours prior to analysis at 40 °C, were capillary rewetted for one hour, transferred onto a 0.05 mm mesh sieve, then immersed in ethanol and shaken. The remaining fraction was dried at 40 °C for 24 hours before dry sieving using sieves with a mesh size of 2 mm, 1 mm, 0.5 mm, 0.2 mm, 0.1 mm and 0.05 mm. The mean weight diameter (*MWD*) was calculated by multiplying the sum of the mass of soil remaining on each sieve by the mean aperture of adjacent sieves, and dividing the result by the total mass of aggregates.

To compare the experimental data from the two incubations, the  $\Delta MWD$  was calculated as the difference between the *MWD* of a soil-organic amendment mixture ( $MWD_{Treatment, n=4}$ ) and the mean *MWD* of the control soil jars ( $MWD_{Control, n = 4}$ ) at a given date:

$$\Delta MWD(mm) = MWD_{Treatment} - \underline{MWD}_{Control} \quad (1)$$

To summarise the AS curve produced by each soil-organic amendment mixture, the area under the curve (*AUC*) for each treatment from plotting  $\Delta MWD$  over time was calculated using the trapezoidal rule:

$$AUC(mmd) = \int_0^t f(x)\Delta x \approx \sum_{t=0}^{t=182} \frac{(\Delta MWD_{t+1} + \Delta MWD_t)}{2} dt \quad (2)$$

where *dt* is the difference in days between sample date *t* and the following sample date *t+1*, similar to the method used by Annabi et al. (2007). The calculated *AUC* can be considered to represent the overall impact of a given organic amendment on soil AS over the course of incubation; amendments with only an intermittent measured effect on *MWD* will have a low *AUC*, while those with a long-lasting effect will have a higher value.

### 3.2.5 Measurement of carbon mineralisation in the soil

The carbon dioxide produced by the soil-organic amendment mixtures during incubation was captured in traps containing 1 M NaOH placed in incubation jars representing each of the above treatments and left in place until day 182. The traps were replaced at frequent intervals and the conductivity of the solution measured in a way similar to that used by Wollum and Gomez (1970). An empirical model was used to convert the conductivity readings, taking into account variations in temperature and concentration of the solution, and given by the equation:

$$C_{sol}(mgL^{-1}) = \kappa * (a * T + b) + g * T + h \quad (3)$$

where  $C_{sol}$  is the mineralised carbon that reacts with the NaOH solution ( $mgCL^{-1}$ ),  $\kappa$  is conductivity ( $mS\ cm^{-1}$ ), and  $T$  is the temperature ( $^{\circ}C$ ) of the solution. The constants  $a$  ( $= 8.84 \times 10^{-4}\ mg\ cm\ mS^{-1}\ L^{-1}\ T^{-1}$ ),  $b$  ( $= -6.71 \times 10^{-2}\ mg\ cm\ mS^{-1}\ L^{-1}$ ),  $g$  ( $= 1.70 \times 10^{-2}\ mg\ L^{-1}\ T^{-1}$ ), and  $h$  ( $= 7.66\ mg\ L^{-1}$ ) were determined by calibration using readings from back titration of a range of solutions. The percentage of carbon mineralised by the added organic amendment,  $C_m$ , was calculated by subtracting the mean carbon mineralised in the jars containing the control soil, and dividing it by the calculated carbon dose (given in Table 2).

### 3.2.6 Modelling of carbon mineralisation dynamics

The quantity of carbon mineralised by the organic amendment after it was added to the soil follows a curve that can be traced by the combined first order and second order equation:

$$C_m(\%) = C_0(1 - e^{-bt}) + At \quad (4)$$

where  $C_m$  is the percentage of carbon mineralised by the organic amendment,  $t$  is time,  $C_0$  is the estimated “labile” carbon in the organic amendment (%),  $b$  is the coefficient of the rate of decay of the added labile carbon ( $\% \text{ days}^{-1}$ ), and  $A$  is the coefficient for the rate of decay of the remaining “recalcitrant” carbon ( $\text{days}^{-1}$ ). The data was fitted to the model using the « nls2 » package in R (Vaissie et al., 2021) for the nonlinear (weighted) least-squares estimation of the parameters of a nonlinear model using the brute force method.

### 3.2.7 Statistical analysis

All statistical analyses were performed in “R” (R Core Team, 2021). Depending on whether the data sets were balanced and passed the normality test, either parametric analysis of variance (ANOVA) or non-parametric Kruskal-Wallis test by ranks were used to determine significant differences ( $p \leq 0.05$ ) between group means, while the parametric Tukey honestly significant differences (HSD) test or non-parametric Dunn’s test was used for pairwise comparison of multiple group means. The “psych” package (Revelle, 2022), was used for the construction of the correlation matrix, Pearson’s correlation coefficients were estimated from pairwise comparison of variables, with  $p$ -values adjusted using Holm’s method. The correlation matrix was visualised using the “corrplot” package (Wei and Simko, 2021). Hierarchical clustering analysis was performed with scaled and centred data of the  $\Delta MWD$  for each organic amendment at each sample date using Ward’s (minimum variance) method.

Comparison by ANOVA of the mean values of  $MWD$  for the control soils in both incubations showed that, apart from on day 42, most of the AS of the two soils did not differ significantly. However, when the variability in  $MWD$  for the control soils over all dates was taken into account, the  $MWD$  values at this date were not excessively high. This being the case, statistical analysis was performed using data from the two experiments combined.

## 3.3 Results & Discussion

### 3.3.1 Characterisation of the effect of organic amendments on aggregate stability dynamics

Aggregate stability (AS) dynamics in amended soil compared to in the control soil without amendment are shown in Fig. 3.2. Most of the digestates tested significantly improved the AS of the soil compared to the control soil at some point during the incubation period. No negative effect of digestate was observed except in the soil amended with RD3 at day 0, although this decrease in  $MWD$  was not significant compared to the  $MWD$  of the control soil sampled at the same date. When increases in

*MWD* were observed, the soil concerned was no longer classified as “highly unstable” ( $MWD < 0.4$  mm) but simply as “unstable” ( $0.4 \text{ mm} < MWD < 0.8 \text{ mm}$ ), representing a reduction in the risk of crusting for this soil as according to the classification proposed by Le Bissonais (Le Bissonais, 1996). For most of the digestate-soil mixtures, the time to observed maximum *MWD* was 98 or 182 days, although several mixtures displayed a peak at day 7. The dynamics of *MWD* for mixtures of soil and undigested organic amendments, maize straw (MS) and cow manure (CM), was comparable to the dynamics observed in other studies (Abiven et al., 2007; Sarker et al., 2018). Soil amended with MS had a high *MWD* ( $> 0.7$  mm) between day 7 and day 182 of incubation, whereas many soil-digestate mixtures underwent only a small change in *MWD* over the same period. This would be expected in the case of more transformed (and therefore more stabilised) organic amendments (Abiven et al., 2009).

Soil mixtures containing the organic fraction of the liquid phase of digestates (LP2, LP4, LP7) showed a significant ( $p < 0.05$ ) improvement in *MWD* at day 0, as did two raw digestates (RD2, RD5) and two composted digestates (CS4, CS5). While most of the soil AS dynamics observed were very likely due to biological breakdown of the added digestates over time, the elevated *MWD* at day 0 was more likely a result of direct physical or chemical interaction between the digestate and the soil. Immediate increases in *MWD* following soil amendment have already been reported for both digestate (Manasa et al., 2020) and compost (Annabi et al., 2007), and were attributed by the authors to hydrophobic components in the amendments. Similarly, Voelkner et al. (2019) were able to directly measure changes in the wettability of soil amended with digestate and compost. For the two undigested organic amendments, MS and CM, no significant difference in the *MWD* was observed relative to the *MWD* of the control soil at day 0. This confirms the fact that the effect is not due to the experimental methods used for the preparation and application of the organic amendments.

The increased *MWD* found at 98 and 182 days in many of the incubated soil-digestate mixtures in the present study corroborates the results obtained by Sarker et al. (Sarker et al., 2018), who incubated a silty soil amended with a solid digestate. The persistent aggregate stabilisation following amendment

of the raw solid batch digestate RD5 is also in agreement with the results of two field trials (Alburquerque et al., 2012b; Badagliacca et al., 2020), where transient improvements in AS were observed several months after amendment with low  $HRT_{AD}$  raw digestates similar to RD1a. In our experiment, the organic fraction of the liquid phase (LP) of the digestates had a relatively large and significant effect on AS across most sample dates, and the improvement continued to increase until day 182. To the authors' knowledge, there are no published studies of the effect of the liquid phase of digestate on AS.

### 3.3.2 Characterisation of the effect of organic amendments on carbon mineralisation dynamics

The mean cumulative carbon mineralisation rates of each organic amendment in the soil are plotted individually in Fig. 3.3 along with the curve predicted by the selected model. As expected, the highest carbon mineralisation was found in soil amended with MS, which reached 62.2% added C after 182 days. This is in line with the results of other experiments (Béghin-Tanneau et al., 2019; Cosentino et al., 2006), although the results vary somewhat very likely due to differences in the quality of the residues. The lowest mineralisation rate was found for soil amended with a digestate co-composted with woody green waste (CS7) with only 11.45% added C, followed by the highly stable raw digestate (RD2) with 17.7% added TC. Only digestate RD5 from the solid batch AD reached over 50% of added C mineralised after 182 days, like CM, with C losses from the remaining digestates ranging from 19 to 41%.

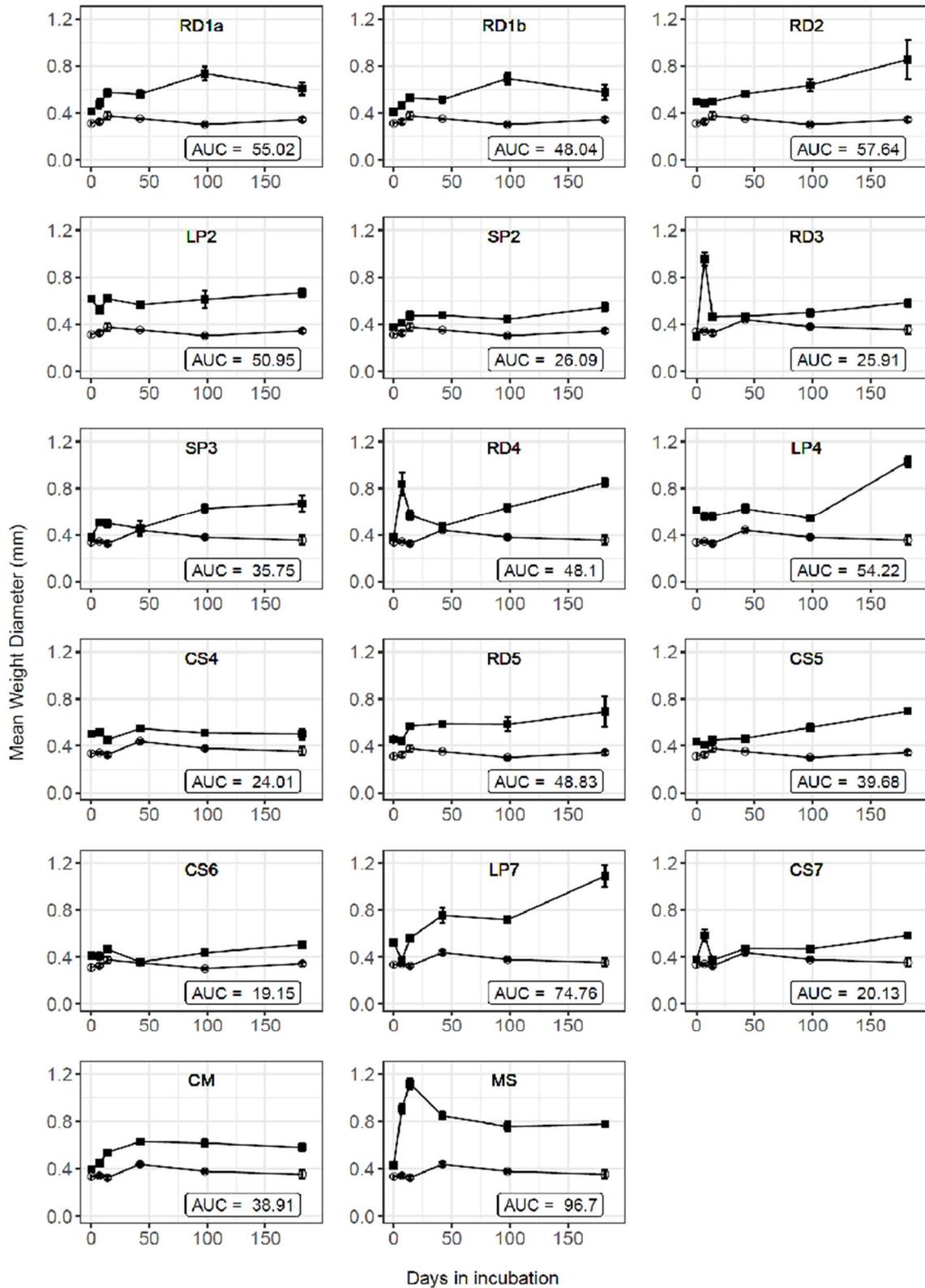


Fig. 3.2 Aggregate stability dynamics expressed as mean weight diameter (MWD) for soil-organic amendment mixtures (squares) and control soil (circles) incubated for 182 days. MS = Maize straw, CM = Cow manure, RD = Raw digestate, LP = Liquid phase of digestate, SP = solid phase of digestate, CS = Composted or stored digestate. Error bars denote standard error (n=4). Mean values for the calculated area between the two curves (AUC (mm day)) are given in the boxes at the bottom right of each plot.

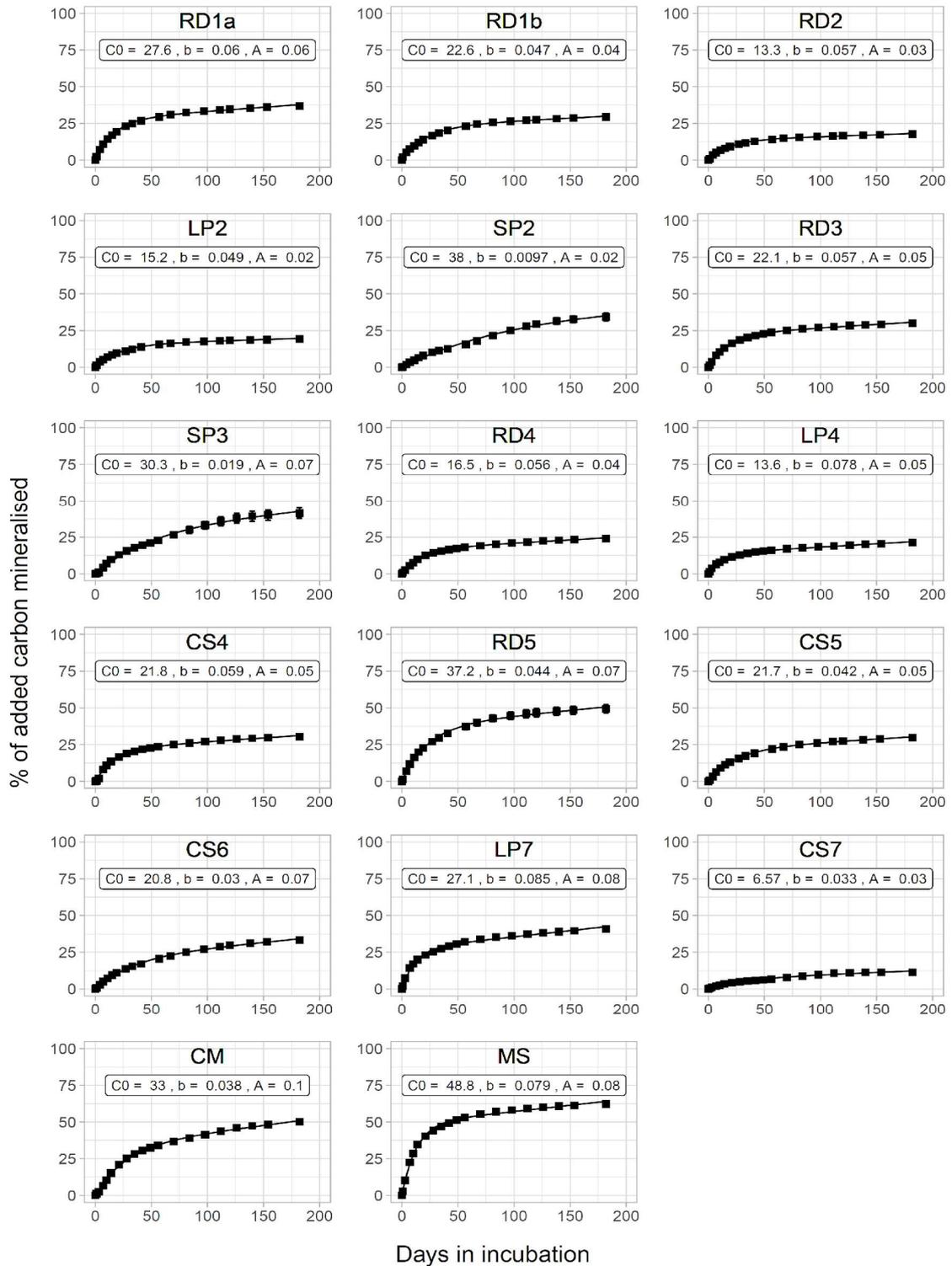


Fig. 3.3 Cumulative carbon mineralisation (expressed as percentage of total carbon added) in soil-organic residue mixtures during incubation. MS = Maize straw, CM = Cow manure, RD = Raw digestate, LP = Liquid phase of digestate, SP = Solid phase of digestate, CS = Composted or stored digestate. A model (represented by the solid line) was fitted to the data, described by equation:  $C_0 \cdot (1 - e^{-bt}) + A \cdot t$ . The constants estimated for each soil-digestate mixture are given in the box at the top of each plot, where  $C_0$  = labile carbon pool (% of added carbon),  $b$  = first-order rate constant ( $\text{days}^{-1}$ ),  $A$  = zero-order rate constant ( $\text{days}^{-1}$ ). Error bars denote standard errors ( $n=4$ ).

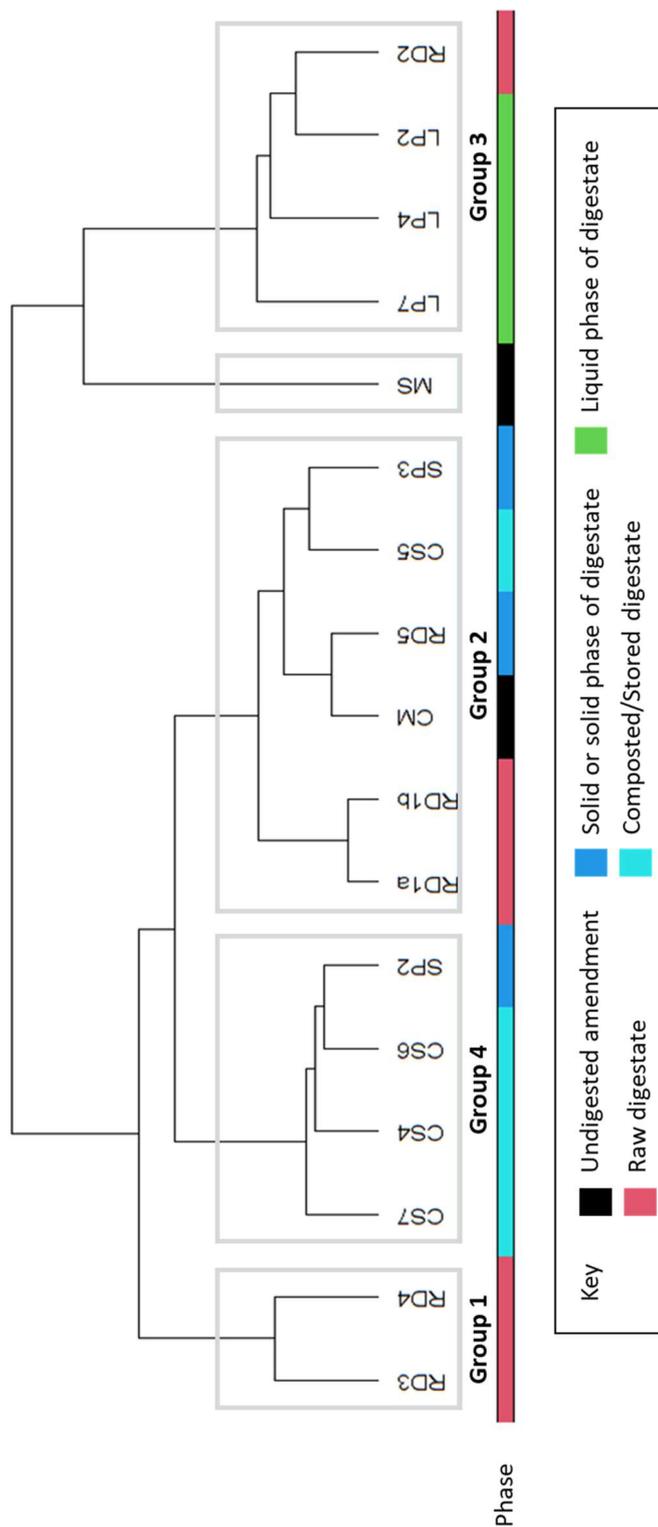


Fig. 3.4 Hierarchical cluster analysis (HCA) of organic amendments placed in incubation grouped by the measured mean  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) at six sampling dates (day = 0, 7, 14, 42, 98, and 182). MS = Maize straw, CM = Cow manure, RD = Raw digestate, LP = Liquid phase of digestate, SP = Solid phase of digestate, CS = Composted or stored digestate. Boxes define the five clusters revealed by the HCA, and used to establish the typology of digestate comprising four groups (plus one non-digestate cluster containing only maize straw MS).

The predicted coefficients for the combined first-order and zero-order model used, (see the box in each plot), indicate further differences between the dynamics of carbon mineralisation of digestates. While MS had the highest labile carbon content ( $C_0 = 47.78\%$ ) predicted by the model, the highest first order rate coefficient ( $b = 0.085$ ) was estimated for LP7. The estimated  $b$  for MS, LP7 and LP4 are comparable, suggesting that the liquid phases of digestates also contained highly bioavailable C and N like for MS. The raw solid batch digestate (RD5) and the two solid phases of digestate (SP2 and SP3) showed similar or higher predicted  $C_0$  than that of CM, i.e. around 32.96%, although the solid phases were estimated to have much lower  $b$  values corresponding to slower rates of mineralisation. Häfner et al. (2022) predicted similar figures for raw digestate using a slightly different mineralisation model, in which  $C_0$  ranged from 16.5-60.5% and  $b$  ranged from 0.01-0.1 days<sup>-1</sup>. Likewise, Askri et al. (2016) and de la Fuente et al. (2013) predicted  $C_0$  ranging from 11.5-28.8% and  $b$  from 0.04-0.69 day<sup>-1</sup> for several liquid phases of digestate.

### 3.3.3 First typology of the impact of digestate on soil aggregate stability

Hierarchical cluster analysis using the Ward method (see Fig. 3.4), was performed on the  $\Delta MWD$  calculated for soil-organic amendment mixtures at the six sample dates. From this analysis, the organic amendments were divided into five distinct groups. Four of the groups comprising digestate-soil mixtures displayed characteristic dynamic in soil AS: 1) rapid but transient stabilisation of soil aggregates at day 7, followed by gradual improvement until day 182, 2) slower, temporary improvement of soil AS, reaching maximum around day 98, 3) immediate stabilisation of soil aggregates at day 0, followed by a gradual increase in AS until day 182, 4) minor or non-significant improvement of soil AS on all sampling dates. The graphs in Fig. 3.5 are based on the average  $\Delta MWD$  for the digestates in each group on the six sampling dates and take the characteristic shape of the soil AS dynamics for each of the four groups in the typology. The maize straw (MS) amendment did not resemble any of the digestates we studied and hence formed a separate cluster in the hierarchy, while CM clustered in Group 2, along with digestates with a similar soil AS dynamics.

The groups formed by our typology could be related to the type of the digestate post-treatment, as can be seen from the colour bar at the bottom of Fig. 3.4, although with some exceptions. The liquid phases of digestates all clustered in Group 3, i.e. a prolonged impact on soil AS, including a strong direct impact at day 0. Similarly, most of the composted or stored solid phases of digestate clustered in Group 4, i.e. a very low relative impact on soil AS, even though Group 2 also contained one stored digestate from a solid batch process (CS5). The two solid phases of digestate clustered in two separate groups. These solid digestates had been stored briefly in an open storage bay prior to sampling. Rapid transformation and loss of carbon can occur during open-air storage of solid organic materials (Hansen et al., 2006), although this depends on conditions such as temperature and levels of aeration, leading to uneven levels of aerobic and anaerobic activity in the heap (Perazzolo et al., 2015). The strong impact of phase separation and aerobic post-treatments on the typology we developed means that the influence of the degree of stabilisation of digestate or digestate feed materials was unclear. Raw digestates with a low and medium degree of stabilisation were distributed across two groups in the typology, while the stable digestate RD2 clustered in Group 3 along with the liquid phases of digestate.

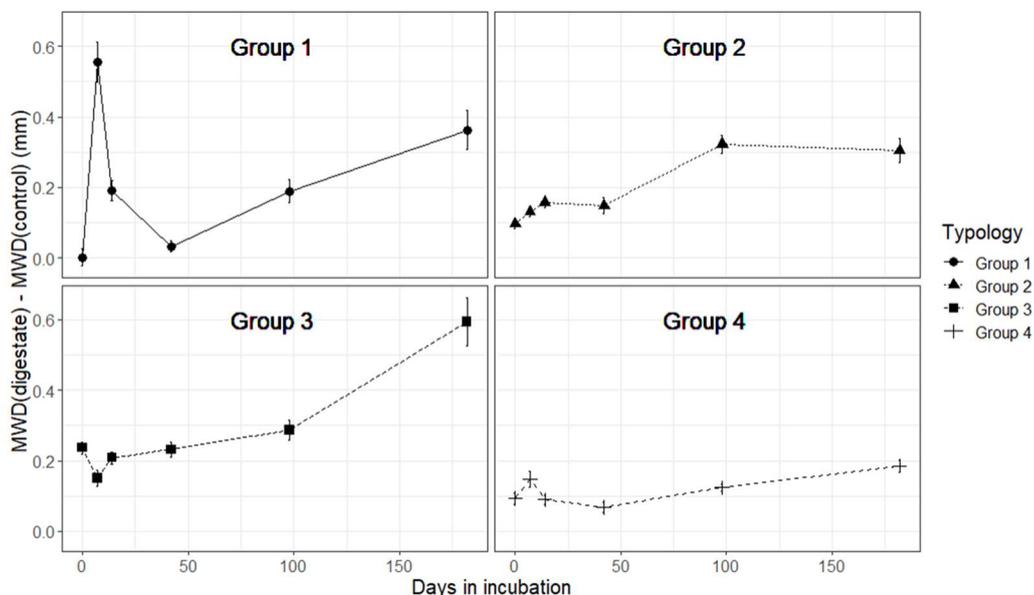


Fig. 3.5 Changes in the mean  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) of incubated soil-digestate mixtures grouped using the established typology into those demonstrating: 1) rapid but transient improvement of MWD at day 7 (n=2), 2) slower, temporary improvement of MWD (n=6), 3) immediate and longer lasting improvement of MWD (n=4), or 4) minor or non-significant improvement of MWD (n=4). The error bars denote standard errors.

### 3.3.4 Relationships between aggregate stability and carbon mineralisation

Fig. 3.6 shows correlations between the measured variables and the calculated coefficients for soil digestate mixtures at a significance level of  $p < 0.05$ . Neither the predicted labile carbon ( $C_0$ ) for digestates, nor the zero-order rate constant for the slowly degradable carbon ( $A$ ), was correlated with changes in soil AS. However, the  $\Delta MWD$  at days 14, 42, and 182, as well as the calculated  $AUC$ , were positively correlated with the first order rate constant ( $b$ ) for modelled C mineralisation, which represents the rate of uptake of labile carbon during the early stages of incubation. While carbon uptake by soil microorganisms can be rapid, a longer interval may be needed for microbial exudates to accumulate and influence the soil AS, as noted by Degens and Sparling (1996) for soil amended with glucose.

### 3.3.5 Impact of anaerobic digestion process parameters on carbon mineralisation

As mentioned above, the C mineralisation model predicted that the solid digestates and solid phases of digestate contained higher estimated  $C_0$  than the other types, while being on a par with CM. However, this fraction of carbon in solid digestate was broken down more slowly ( $b < 0.02 \text{ day}^{-1}$ ). The results obtained here corroborate the findings of Askri et al. (2016) who observed similar effects for C mineralisation of phase separated digestates. The two liquid phases of digestates made in screw press separators (LP2 and LP4) had similar mineralisation characteristics to the parent raw digestate (RD2 and RD4). Screw presses that treat digestate made from fibrous wastes such as animal manure may concentrate a high proportion of organic matter in the solid phase, but nevertheless leave considerable amounts of carbon in the liquid phase (Aguirre-Villegas et al., 2019; Guilayn et al., 2019b) including high concentrations of complex and recalcitrant molecules (Akhiar et al., 2017; Tambone et al., 2019).

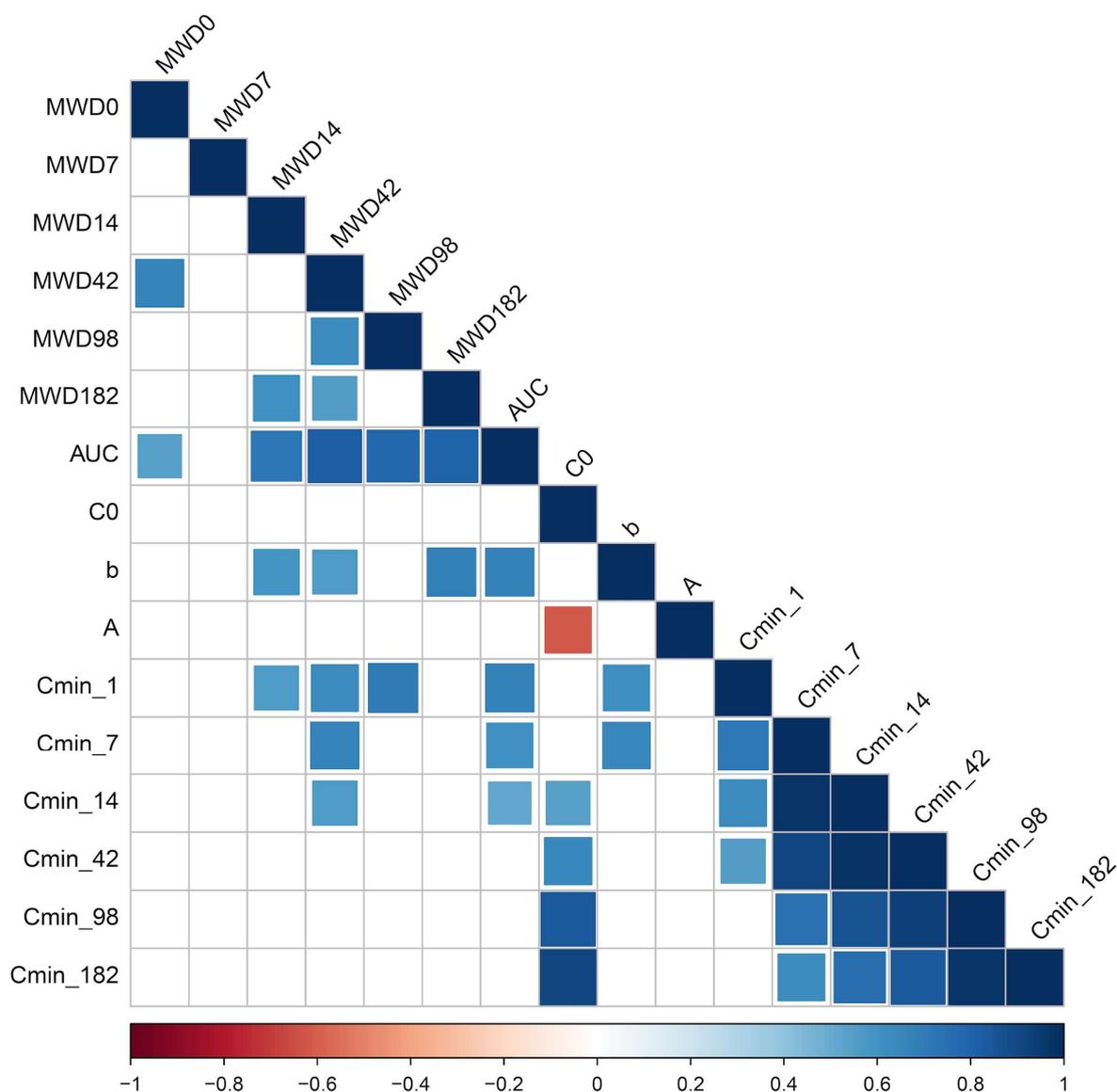


Fig. 3.6 Correlation matrix of the following calculated and measured variables used for incubated soil-digestate mixtures: the mean MWD measured at each sample date (MWD#), the calculated area under the curve of the AS dynamic (AUC), the cumulative % added C mineralised at each soil sampling date (Cmin\_#), and the coefficients of the C mineralisation model fit (C<sub>0</sub>, b, A). Pearson's correlation coefficients were estimated from pairwise comparison of variables. The size and the intensity of the colour of the square reflect the strength of the correlation; the blue squares represent positive correlations while the red squares represent negative correlations. Only correlations at a significance level of p < 0.05 following adjustment using Holm's method are shown.

Composted and stored solid digestates tended to have predicted C<sub>0</sub> values similar to those of raw and liquid digestates, suggesting that the labile carbon concentrated in the solid phase during separation was consumed during the aerobic treatment. Two separate studies (Maynaud et al., 2017; Teglia et al.,

2011) report that a considerable amount of organic carbon that is biodegradable under aerobic conditions remains in the digestate, but that this biodegradable matter is then lost during composting. In the case of CS5 and CS6, which were only stored in the open air and not fully composted, and also of CS4, which may not have been completely composted, the levels of labile organic carbon estimated by the model remained fairly elevated ( $20\% < C_0 < 22\%$ ). The level of stabilisation of the digestate also appeared to play a role in the levels of mineralisable carbon, though the differences were less contrasted than between post-treated digestates. In the case of the raw digestates only, the percentage C mineralisation after 182 days and the estimated  $C_0$  followed the order: low stabilisation (RD1a) > medium stabilisation (RD1b, RD3, RD4) > high stabilisation (RD2). Nyang'au et al. (2022) noted that C mineralisation in digestates after a secondary AD step was reduced compared to after only single-stage digestion, as the increased  $HRT_{AD}$  led to the concentration of more recalcitrant C fractions in the digestate.

### 3.3.6 Impact of anaerobic digestion process parameters on aggregate stability

Comparisons of the impact of soil-digestate mixtures on soil AS, as represented by the calculated values of  $AUC$  and grouped together according to the type of post-treatment, the degree of stabilisation and the main type of feed are given in the appropriate boxplots in Fig. 3.7. The raw digestates studied displayed considerable variation in  $AUC$  and, as already discussed, fall into several different groups in the typology we developed. The  $AUC$  values of the liquid phase of digestate were relatively higher than those of the solid phase (see Fig. 3.7(a)). Composted or stored solid and solid phases of digestates had significantly lower  $AUC$  values than raw digestate or the liquid phase such that, the order of impact, as measured by the  $AUC$ , digestate phases was as follows: Raw  $\approx$  Liquid phase > Solid phase  $\approx$  Composted/stored ( $p < 0.05$ ). These findings support the claim made by Tambone et al. (2019) that the liquid phase of digestates contains important OM components and thus may be of value as amendment.

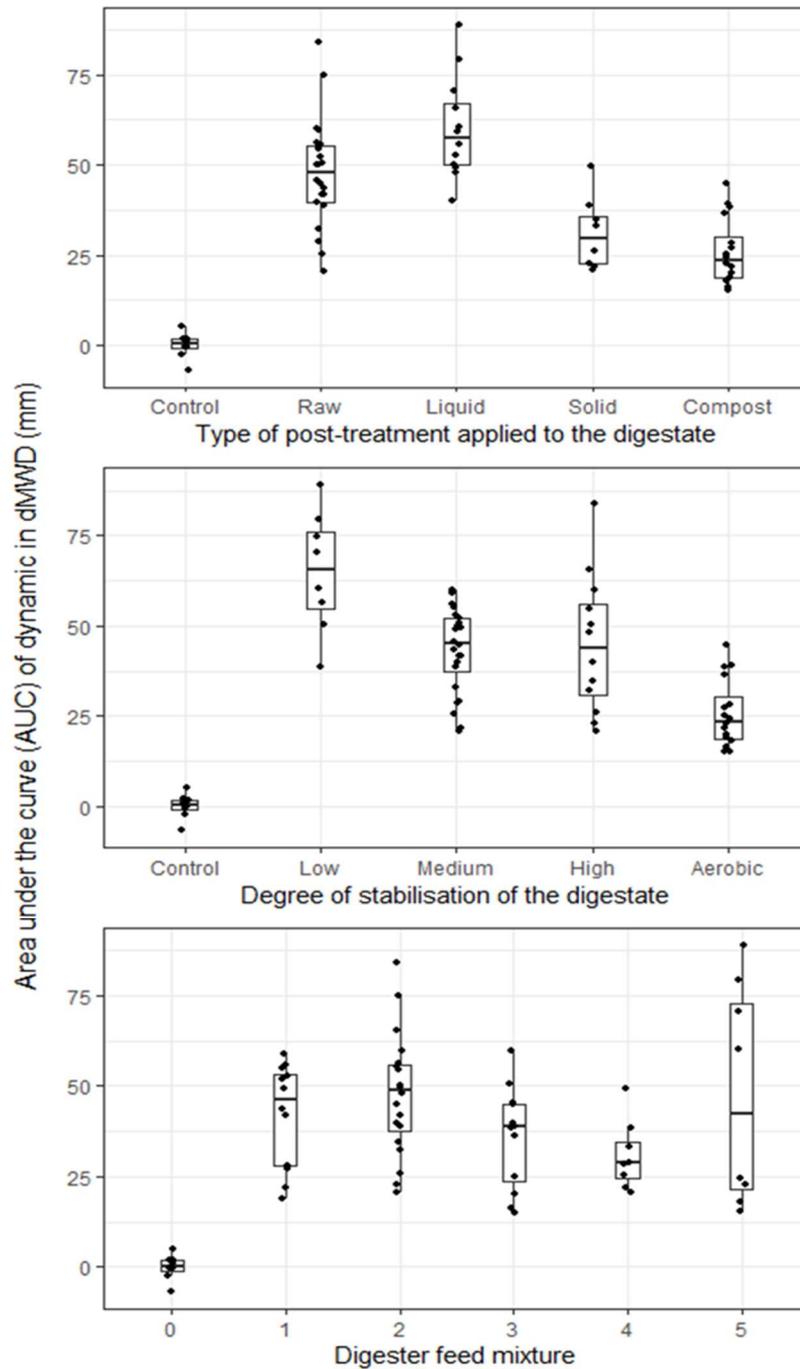


Fig. 3.7 Boxplots of the calculated area under the curve (AUC) of the dynamic in  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) for incubated soil-digestate mixtures, grouped according to (a) the type of post-treatment: Raw = Raw digestate (n=5), Liquid = Liquid phase of digestate (n=3), Solid = Solid phase of the digestate (n=3), Compost = Composted or stored solid phase of the digestate (n=4); (b) degree of stabilisation: Low = digestate with  $HRT_{AD} < 50$  days (n=2), Medium = digestate with  $HRT_{AD}$  50-100 days (n=4), High = digestate either with  $HRT_{AD} > 100$  days or with pre-treatment of the substrate (n=5), Aerobic = digestate that has undergone aerobic transformation through storage or composting (n=4); (c) type of digester feed mixture: 1 = Mostly ruminant manure with crop residues, 2 = Mostly ruminant manure and slurry with crop residues, 3 = Mostly ruminant manure, 4 = Mostly ruminant slurry, 5 = Mostly non-ruminant slurry. The black dots represent the values of each of the four replications for each soil-digestate mixture.

Digestates with a low degree of stabilisation had higher *AUC* values (see Fig. 3.7(b)), had a higher overall impact on soil AS than medium and highly stabilised digestate. Concerning the two raw digestates from the same AD site, RD1b ( $50 \leq HRT_{AD} < 100$  days) had less impact on soil *MWD* than RD1a ( $HRT_{AD} < 50$  days), however both were still classified in the same group by the typology (Group 2). Annabi et al. (2007) observed that increasing maturity of composts reduces their impact on soil AS, and concluded that this was a result of the reduction in labile carbon which drives soil biological activity. Increasing time in AD also reduces the concentration of biodegradable OM (Möller, 2015; Tambone et al., 2009), and leads to the accumulation of complex, aromatic substances (Akhiar et al., 2017). However, the highly stabilised digestates studied here displayed a wide range of calculated *AUC* values, as the effect of phase separation across the three digestates in this category (from the same AD site) appeared to be much more significant. Irrespective of the duration of anaerobic treatment, digestates that had been subject to aerobic treatment (either composted or stored), had lower calculated *AUC* values than low and medium stabilised digestates. In their review of the impact of different organic amendments on soil AS, Abiven et al. (2009) found that composted materials had much less impact on AS but that the effects lasted longer than for uncomposted materials. This means that the full impact of more stable organic amendments may not have been measured in the relatively short incubation time (182 days) used in this study.

No statistical difference was found between the calculated *AUC* for digestates grouped according to the digester feed mixture (see Fig. 3.7(c)). However, as already mentioned, the apparent strong determining effect of phase separation and aerobic treatment on the characteristics of the digestate amendment made it difficult to clearly distinguish the impact of other process parameters. More detailed analysis of digestates that had undergone similar treatment and levels of stabilisation provided some evidence that impacts differ depending on the composition of the feed. For example, RD1b, a digestate mainly made of manure and slurry, and RD4, a digestate mainly made of plant residues, were comparable in terms of their post-treatments and degree of stabilisation but were classified in different groups by our typology. Other groups of organic amendments had varying

impacts on soil AS depending on their origin or on the feed substrates used, such as straw (Martens, 2000), compost (Annabi et al., 2007; Tejada and Gonzalez, 2007) or manure (Li et al., 2011). Incubation of digestates produced under similar controlled conditions and based on pure feed materials, including different manures and slurries, water treatment waste, food waste or plant materials, would enable better differentiation between the composition of different feed mixtures.

### 3.3.7 Impact of anaerobic digestion process parameters on initial soil aggregate stability

Similar statistical analysis of the soil *MWD* at day 0 with digestates grouped according to the production parameters revealed significant differences between digestates based on the type of post-treatment of the digestate, as shown in the boxplot in Fig. 7. However, no significant differences were found between digestates at this date for the degree of stabilisation and digester feed mixture (data not shown).

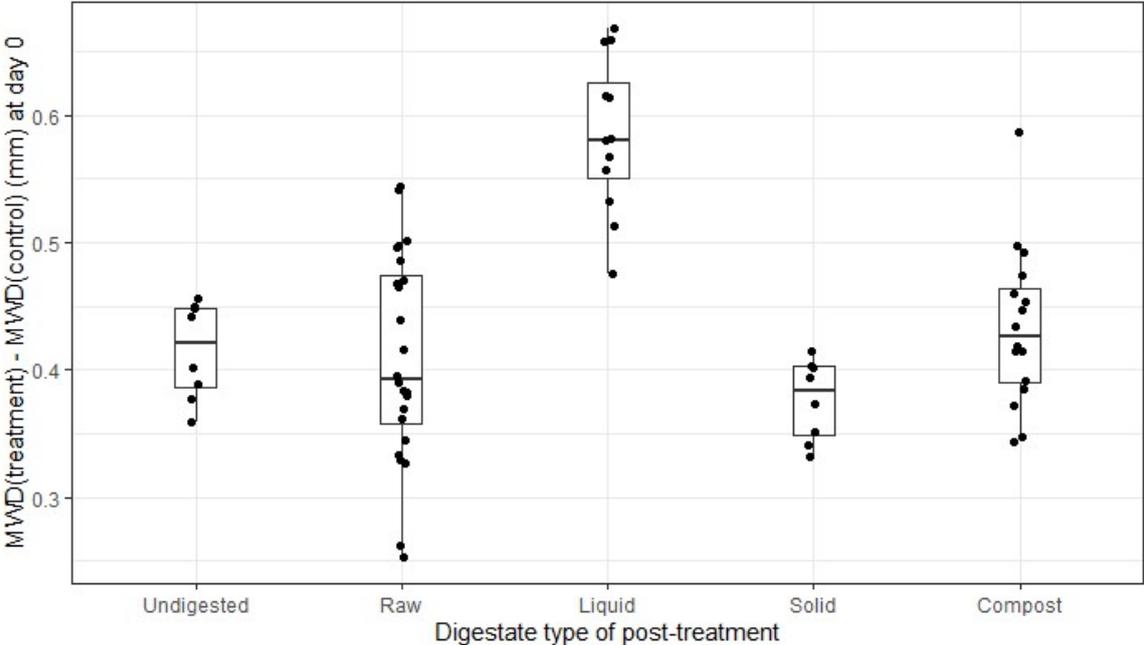


Fig. 3.8 Boxplot of the  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) at day 0 for incubated soil-organic amendment mixtures grouped according to the type of post-treatment: Undigested = Undigested organic amendment (n=2), Raw = Raw digestate (n=5), Liquid = Liquid phase of the digestate (n=3), Solid = Solid phase of the digestate (n=3), Compost = Composted or stored solid phase of the digestate (n=4).

At day 0, there was a clearer positive impact on *MWD* of the OM fraction of the liquid phase of digestate than of the other types of digestate. Raw digestate-soil mixtures displayed the widest range of *MWD* at day 0. As already mentioned, soil amended with digestate RD3 had a lower measured *MWD* than that of the control soil, although the difference was not significant ( $p > 0.05$ ). RD3 is a digestate containing permeate from filtration of milk and whey, and therefore rich in minerals, as demonstrated by the concentrations of TP and TK shown in Table 2. Mixtures containing solid digestate had little initial impact on *MWD*, while mixtures containing composted solid phase of digestate resulted in some improvement compared to the control soil. Annabi et al. (2007) observed a similar initial increase in *MWD* using other types of composted material, and attributed this effect to the presence of complex “humic-like” substances or lipids. Several authors who studied the effects of adding liquid digestate to soil, reported that it can increase water- or alkali- soluble phenols (Cardelli et al., 2018; Muscolo et al., 2017), while increases in such soluble phenolic substances have already been linked to improvement in soil aggregate stability following amendment with different types of straw (Martens, 2000). However, whether this applies equally to digestates remains to be proven.

### 3.3.8 Applicability of results to field conditions

Before applying the findings of this study at field scale, further verification is necessary using agronomic application rates and similar conditions, while taking into account the numerous other factors (climate, crop rotation, agricultural practices) which equally affect soil AS. It is nevertheless possible to hypothesise how different types of digestate will affect soil AS in field conditions.

The original idea behind applying relatively high rates of pre-dried digestate ( $6 \text{ g}_C \text{ kg}_{\text{dry soil}}^{-1}$ ) to soil in these incubation trials, was to simulate multiple repeated applications in subsequent years, which has been shown to cumulatively benefit long-term soil physical and biological properties, as already demonstrated for manures and slurries (Bhogal et al., 2018). The dose applied is also comparable to the doses used in other incubation studies of soil AS after organic amendments (Abiven et al., 2007; Annabi et al., 2007), which facilitates comparison of results between studies. Due to their often low

carbon-nitrogen ratios, the carbon dose of the dried digestates added to soil in this study is many times higher (on average 25 times higher) than the dose that would be applied annually per hectare in agriculture. For other types of organic amendment, increasing the dose of organic matter has been shown to increase its effect on soil AS (Özdemir et al., 2007). As such, in-field increases in soil *MWD* resulting from the application of digestate are expected to be less noticeable than in the soil incubated in this study. For liquid phases of digestate, the dose of  $6 \text{ g}_C \text{ kg}_{\text{dry soil}}^{-1}$  is more than 28 times greater than the typical annual agronomic application rate considering a nitrogen fertilisation rate at approximately  $160 \text{ kg}_N \text{ Ha}^{-1}$ , though only eight times greater for the solid phase of digestate. Thus, the likely impact of raw and of the liquid phase of the digestate on soil AS under field conditions would be even lower when compared to spreading of a solid phase at the same application rate, as their higher carbon-nitrogen ratio would result in higher amounts of organic carbon being incorporated into the soil. When comparing this incubation study with agricultural practices used to improve soil using digestates, another important aspect is the method of application and the resulting uneven distribution of the digestate throughout the soil profile. In the controlled laboratory conditions used for our incubation trials, digestate was carefully mixed with soil aggregates to obtain relatively homogenous contact between the two, whereas Hansen et al. (2016) pointed out that field application using spreading and ploughing equipment would very likely generate “hotspots” of concentrated material mixed with soil, and that laboratory trials with high doses of digestate may reproduce these highly localised conditions.

As mentioned above, digestates were also dried prior to incubation to avoid the high moisture content in fresh digestates “flooding” the incubation jars thereby creating suboptimal soil conditions for microbial activity. As pointed out by Rodrigo et al. (1997), optimal C and N mineralisation in the soil occur at close to soil water holding capacity, which is why aggregates were held at around 80% *SWHC* in our incubation trials. Rapid wetting of soil, which would be the case when highly diluted manures are applied, may also cause disruptive “slaking” of soil aggregates, where water entering the aggregate pores causes trapped air to rupture the aggregate structure (Le Bissonnais, 1996). However, in field

conditions, application rates of liquid digestates would be insufficient to damage the soil structure, and additional moisture would eventually infiltrate deeper layers. On the other hand, in some cases, wetting and drying cycles have been shown to increase the positive effect of organic amendment on soil AS (Cosentino et al., 2006), consequently dry weather following digestate spreading may improve soil structural stability.

Furthermore, drying the digestate may have partially modified the composition of the organic matter, and subsequently how this OM interacts with the soil, as well as how it is taken up by soil microorganisms. Askri et al. (2016) characterised a solid phase of digestate before and after drying by Van Soest fractionation, and observed that the OM in the soluble fractions (*SOL*) decreased by around 10%, while the recalcitrant (*LIC*) organic matter increased by 7%. However, drying of organic amendments prior to incubation is a fairly common practice (Abiven et al., 2007; García-Sánchez et al., 2015). Fresh digestates, as they would be applied at field scale, also contain large quantities of ammonium nitrogen ( $\text{NH}_4^+$ ) which was eliminated by the drying process. While it is certain that nitrogen content and availability affects mineralisation dynamics during incubation, care was taken to ensure that none of the organic amendments were incubated in nitrogen limiting conditions in the present trial. The main objective of the incubations was to study the effect of the organic carbon contained in digestate on the soil, rather than the impact of nitrogen fertilisation, and so this was not explored in further detail. Mineral nitrogen may attenuate the impact of organic amendments on soil AS (Le Guillou et al., 2011), meaning that N rich digestates might have less impact on soil AS compared to that observed in the present study. However, this should be weighed against the benefits to be had from applying digestate, such as additional fertility and improved crop development (Alburquerque et al., 2012b), which in turn, have a positive impact on soil AS.

### 3.4 Conclusions

The addition of organic matter from digestates increased carbon mineralisation and either improved or had little effect on soil AS. Soil AS dynamics varied significantly between digestate amendments resulting in four distinct groups. Related dynamics were linked to the anaerobic digestion process parameters, in particular the type of post-treatment. Raw digestates generally had a stronger impact on soil AS but lower rates of C mineralisation than cow manure at the quantities applied in the present study. The liquid phases of digestate following phase separation contained a small but rapidly mineralised carbon fraction and had a strong impact on soil AS. Although a larger proportion of labile carbon was present in the solid phases of digestate, this carbon was only mineralised slowly and the solid phases had apparently less impact on soil AS. Longer anaerobic treatment times, aerobic storage, and composting all appear to have led to a concentration of stable carbon in the digestate, and a subsequently reduced short-term effect on soil AS. Furthermore, the dynamics in soil AS varied significantly between different digestate amendments, falling into four distinct groups. Further investigation with more detailed chemical characterisation would further our understanding of the relationship between the composition of the digestate and its effect on the soil AS. Finally, while it is important to account for other aspects such as nutrient concentrations (N, P, K) and environmental constraints (pathogens, pollutants) when considering digestate use in agriculture, correct assessment of the benefits as an organic amendment should be of equal importance when designing optimal production and valorisation strategies.

## 4 Identification of controlling factors in the effect of digestate on short-term soil aggregate stability dynamics

In the previous chapter, we measured the soil AS dynamics following amendment with a range of digestates, and established a first typology of impact connected to several AD operating parameters used in their production. It was clear, as hypothesised in Table. 1.2 of chapter 1, that the differences between the dynamics observed were linked to differences in the digestate characteristics and their subsequent impact on various soil processes leading to aggregate stabilisation. The following chapter is a copy of an article intended for submission to the journal “Soil Use & Management”, where we used the results from the previous chapter, coupled with the methodology of characterisation of digestates as established in chapter 2, to explore the link between the two.

# Identification of controlling factors in the effect of digestate on short-term soil aggregate stability dynamics

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## ○ Abstract

The group of organic biomasses known as digestates, by-products of the anaerobic digestion (AD) process, may exhibit a wide range of characteristics that influence their impact on soil properties when used in agriculture. A study was devised to determine indicators that could be useful in prediction of the short-term impact of agricultural digestates on soil aggregate stability (AS), an important measure of soil resistance to crust formation and erosion. Detailed characterisation was carried out on 15 digestates sampled from full-scale AD plants, including sequential extraction of organic matter (OM), fluorescence analysis of the soluble and particulate (SPOM) fraction, and elemental analysis of whole digestate (Na, Ca, Mg, Fe, K, Al, Zn, and Cu). This data was compiled with soil analyses from samples incubated with the same digestates over 182 days, including measured soil pH and electrical conductivity, water droplet penetration time (*WDPT*), and aggregate mean weight diameter (MWD) following wet sieving. A partial least squares regression (PLS-R) model was generated to attempt to account for the maximum amount of variation in the “*AUC*”, a variable devised to simplify and summarise the complex dynamics of MWD observed over the course of soil incubation. The total organic carbon (TOC) and total nitrogen (TN) contents in SPOM and readily extracted (REOM) fractions were positively correlated with *AUC*, and contributed significantly to the PLS-R model. The TOC and TN in non-extracted (NEOM) fractions also contributed to the model through an inverse relationship. No

significant correlations were found between *AUC* and digestate total elemental concentrations, or with fluorescence analysis of the *SPOM* fractions. While various PLS-R models were generated that achieved a reasonable “goodness of fit” ( $R^2 = 0.62-0.85$ ), not all variation in soil AS dynamics could be explained by the dataset, suggesting a refinement of digestate characterisation may be needed. This research presents a first step towards identification of the principal mechanisms by which digestate addition may stabilise soil aggregates, and the physico-chemical characteristics directly or indirectly responsible for this effect. Monitoring of certain characteristics in various agricultural digestates should allow easier assessment of their potential value as an organic amendment for improvement of soil structure.

**Keywords.** Anaerobic digestion, Fluorescence spectrometry, Organic amendment, Sequential extraction of organic matter, Soil structure, Soil wettability

- Declaration of Interests

**Data availability.** Other than the data already provided in the article tables and in the supplementary information, datasets generated during and/or analysed during the current study are not publicly available, but can be obtained from the corresponding author upon reasonable request.

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**Conflicts of Interest.** The authors have no relevant financial or non-financial interests to disclose.

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Horticultural and Landscape Sciences (Institut Agro) Rennes who assisted in this research, and the staff of the anaerobic digestion sites from which samples were collected.

## 4.1 Introduction

The anaerobic digestion (AD) sector has grown continuously over recent years, generating increasing quantities of by-product “digestates” that are frequently destined for use in agriculture. Digestates can be valuable as a fertiliser, due to their rich content in nitrogen and other nutrients, but equally considered as an organic soil amendment, due to their content in organic carbon (Nkoa, 2014).

In AD, feed substrates, frequently mixtures of several or more organic residues such as slurries, manures, sludges, crop residues, food industry wastes, as well as dedicated energy crops, are placed under anaerobic conditions to allow fermentation and production of biogas for renewable energy (Insam et al., 2015). A range of possible process configurations and operation parameters are possible in AD, including different reactor configurations, residence times, operational temperatures, storage methods and post-treatments (Weiland, 2010). Different forms of post-treatment are frequently applied to digestates following anaerobic treatment, with the intention of creating a more stable or easily transportable product (Askri et al., 2016). More widely occurring post-treatments include phase separation using equipment such as screw presses, belt presses or decanting centrifuges (Guilayn et al., 2019b), and subsequent composting of the obtained solid phase (Maynaud et al., 2017). This range of operational configurations leads in turn to various forms of digestate with a range of potential physical and chemical characteristics (Guilayn et al., 2019a; Teglia et al., 2011).

Much of recent research work has investigated the different fertilising potential of digestates, in terms of their content in nitrogen and other plant available nutrients (Albuquerque et al., 2012b; Guilayn et al., 2019a; Jimenez et al., 2020b), and their potential for improving soil organic carbon stocks (Häfner et al., 2022; Levavasseur et al., 2022b). Digestates may contain components potentially interesting for their use as soil amendments (Muscolo et al., 2017; Tambone et al., 2019), though direct observations

of changes in soil physical properties following amendment are needed to verify this. Reviews discussing digestate impact on soil physical and biological health to date have been inconclusive on the matter, and highlight the need for further research (Insam et al., 2015; Möller, 2015; Nkoa, 2014). Several more recent studies have found that application of digestates can, under certain conditions, lead to improvements in soil aggregate stability (AS) (Badagliacca et al., 2020; Cooke et al., 2023; Pastorelli et al., 2021; Rezacova et al., 2021), a key indicator of the resistance of soil to surface crust formation and erosion.

Crusting and soil erosion are a particular risk in soils with a low clay content, where soil structural stability is considered to be principally governed by organic matter (OM) and interactions with soil biology (Amézketa, 1999). Specific fractions of the soil OM play a greater role than others in soil structure, though understanding and identification of these OM forms most linked to aggregate stabilisation is still a subject of much investigation (Jensen et al., 2019; Redmile-Gordon et al., 2020; Rezacova et al., 2021). Previous studies have linked dynamics in soil AS to changes in soil OM brought about by organic amendment. Martens (2000) was able to demonstrate through characterisation and incubation of different plant materials that readily biodegradable carbohydrates supported microbiological stabilisation of soil aggregates over the short-term, while more stable polyphenolic compounds appeared to be correlated to higher soil AS over the long-term. Similarly, Sarker et al. (2018) used  $^{13}\text{C}$  NMR of various substrates to show that spectral peaks indicative of higher content in carbohydrates were associated with positive medium to long-term impacts on soil AS, while peaks denoting high concentration in aromatic groups were negatively associated with soil AS. Abiven et al. (2008), using the “Van Soest” fractionation method, established a model linking cellulose and hemicellulose content in several contrasting organic amendments to the duration of their effect on soil AS, while lignin content was negatively correlated to the amplitude of this effect. However, these studies are based on the comparison of a panel of various organic amendments, and do not take into account the potentially high variability in OM quality within a given group, as is the case for digestates.

In fact, there are few published studies to date characterising two or more different digestates with a view to understanding their particular impact on soil AS dynamics.

Specifically adapted sequential extraction techniques, coupled with fluorescence spectrometry, have been successfully used in prediction of the anaerobic and aerobic biodegradability of OM in digestates (Fernández-Domínguez et al., 2021) and sewage sludges (Jimenez et al., 2014). Sequential extraction has also been used to model changes in organic carbon and nitrogen forms during anaerobic digestion (Bareha et al., 2018). Given the important role that stimulation of soil biological activity by organic amendment may play in aggregate stabilisation (Abiven et al., 2007), characterisation of the OM in digestate could also be used to predict the potential short-term impact of digestate on soil AS. Following an initial review of the literature, we hypothesised a number of potential characteristics that a digestate may possess, as well as the mechanisms via which they may directly or indirectly affect soil AS (see Table. 4.1).

The objective of this study was therefore to identify the principal digestate characteristics that may be responsible for both direct abiotic and indirect biotic alteration of soil AS through:

- A detailed characterisation of the digestate, as well as soil properties related to the proposed mechanisms of aggregate stabilisation as laid out in Table. 4.1,
- Analysis of the correlations between these digestate characteristics, soil properties, and dynamics in soil AS following amendment in order to identify potentially useful indicators,
- Development and testing of a statistical model for prediction of digestate impact on soil AS using selected indicators,

Table. 4.1 List of mechanisms by which typical components of digestates may potentially affect soil aggregate stability over the short-term

Component / proposed mechanism of action on soil aggregate stability	Scale and duration of impact*	Supporting sources
<b>Components inducing biotic response</b>		
Readily biodegradable OM (e.g. simple carbohydrates, amino acids, soluble proteins) are rapidly consumed by soil microorganisms that deposit exudates	Positive - rapid (but transient as exudates may also be rapidly consumed)	(Abiven et al., 2007; Chenu and Cosentino, 2011; Martens, 2000; Sarker et al., 2018)
Slowly biodegradable OM (e.g. ligno-cellulose, complex proteins) slowly broken down by soil microorganisms, leading to slow production and turnover of exudates and development of mycelial networks	Positive – but slower than above	(Abiven et al., 2007; Annabi et al., 2007; Martens, 2000; Sarker et al., 2018)
High available N (organic or mineral) relative to labile C content accelerates OM decomposition by soil microorganisms that may deposit aggregate binding agents, or conversely increases consumption of same binding agents and native SOC	Positive/Negative – rapid transient effect, with exhaustion of OM over long-term	(Haynes and Naidu, 1998; Le Guillou et al., 2011; Vandevivere et al., 1990)
Toxins or heavy metals accumulated in high concentrations may suppress soil microbiological activity	Negative – long-term	(Nkoa, 2014; Nwuche and Ugoji, 2008)
<b>Components inducing abiotic response</b>		
Hydrophobic organic molecules (e.g. lipids, waxes, microbial residues, “humic” substances, etc.) coat soil aggregates (or line soil pores), reducing water entry into soil aggregates and subsequent destruction by slaking	Positive – immediate (but transient if biodegradable material)	(Paré et al., 1999; Piccolo and Mbagwu, 1999; Voelkner et al., 2019)
Large/High $M_r$ organic molecules (e.g. microbial residues, aromatic compounds, “humic” substances) interact with soil particles (clay) and minerals to increase aggregate cohesion (formation of OM-mineral complexes)	Positive – immediate (persistent)	(Mbagwu et al., 1991; Oades and Waters, 1991; Rezacova et al., 2021)
(Minerals forming) poly-cations (e.g. $Ca^{2+}$ , $Mg^{2+}$ , $Fe^{3+}$ , $Al^{3+}$ ) reinforce bonding between soil particles (clays) and OM	Positive – immediate (persistent?)	(García-Orenes et al., 2005; Haynes and Naidu, 1998; Tejada and Gonzalez, 2007)
(Minerals forming) mono-cations (e.g. $Na^+$ , $K^+$ or $NH_4^+$ ) interfere with bonding between soil particles (clays) and OM	Negative – immediate (persistent?)	(Jin et al., 2015; Voelkner et al., 2015b; Whalen and Chang, 2002)
Basic/Acid amendment may cause a shift in soil pH, changing the availability and/or absorption of minerals (affecting above)	Positive/Negative – immediate (persistent?)	(Chorom et al., 1994; Haynes and Naidu, 1998)

\*As hypothesised by the author based on the studies cited

## 4.2 Materials and method

### 4.2.1 Digestate characterisation

The sample set consisted of fifteen digestates, previously reported in Cooke et al. (2023), sampled from six agricultural AD plants and one territorial AD plant located in France. These digestates are the products of a range of different feed materials, AD process parameters, and post-treatments (see Table. 4.2). All digestates were dried at 40°C until a stable moisture content was reached and ground to <2 mm before characterisation.

Total solids (TS) and volatile solids (VS) content of samples were determined by weighing before and after drying at 105°C for 48 hours, and weighing after combustion at 550°C for 5 hours, respectively. Total Carbon (TC) was determined through combustion method using a Thermo Scientific Flash® 2000 CN analyser, as per the manufacturer's instructions. Inorganic Carbon (IC) was similarly determined by analysis of the dry ash following combustion at 480°C. Total Organic Carbon (TOC) was subsequently calculated by subtracting the IC from TC. Total Kjeldahl Nitrogen (TKN) was determined through steam distillation after mineralisation in strong acid as per standard methods (APHA, 2005). For elemental analysis, a solution was obtained from mineralisation and microwave digestion of the dry ash from combustion at 550°C in a solution of HCl (37%) and HNO<sub>3</sub> (65%), and using an Anton Paar® HVT50 Microwave Reactor. Total Potassium (K), Calcium (Ca), Sodium (Na), Iron (Fe), Aluminium (Al), Magnesium (Mg), copper (Cu) and zinc (Zn) contents of the solution were obtained by microwave plasma-atomic emission spectrometry using an Agilent® 4200 MPAES, as per manufacturer's instructions. The TOC and TKN measured for each digestate are shown in Table. 4.2, while the remaining digestate characteristics are provided in a larger table in the supplementary information (see Appendix 2).

#### 4.2.1.1 Sequential extraction of digestate organic matter

Sequential extraction as per the method established by Jimenez et al. (2015) was used to characterise the organic matter contained in each digestate. Briefly, 0.5 g *TS* of digestate was extracted using increasingly aggressive solutions to obtain the following:

- Extractible Solute from Particulate Organic Matter (*SPOM*) (2x 15 minutes agitation at 300 rpm and 30°C in 10 mM CaCl<sub>2</sub>), essentially containing sugars and water-soluble proteins,
- Readily Extractible Organic Matter (*REOM*) (4x 15 minutes agitation at 300 rpm and 30°C in 10 mM NaOH + 10 mM NaCl), containing mostly proteins and lipids,
- Slowly Extractible Organic Matter (*SEOM*) (4x 60 minutes agitation at 300 rpm and 30°C in 0.1 M NaOH), containing humic- and fulvic- like substances, complex proteins, and some remaining lipid compounds,
- Poorly Extractible Organic Matter (*PEOM*) (4x 180 minutes agitation at 300 rpm and 30°C in 72% H<sub>2</sub>SO<sub>4</sub>), containing mainly hemicellulose and cellulose
- Non-Extractable Organic Matter (*NEOM*), the pellet remaining after sequential extraction, composed of mainly lignin and highly inert ligno-cellulosic compounds.

All solutions were centrifuged at 13100 rpm and 4°C for 20 minutes, with the pellet passed to the next extraction and the supernatant filtered at 0.45 µm before analysis.

The TOC and TN content in the first four fractions were determined using a Shimadzu® TOC-L analyser, as per the manufacturer's instructions. The TOC in the *NEOM* was calculated as the TOC of the whole digestate measured by combustion method minus the sum of the TOC of all extracted fractions. The TN in the *NEOM* was calculated as the TKN of the whole digestate minus the sum of the TN of all extracted fractions.

Table. 4.2 Summary of the anaerobic digestion process parameters and digestate carbon and nitrogen content (adapted from Cooke et al. 2022)

Digester Type (Location #)	Digester Feed Composition (% of mixture, if known)	HRT <sub>AD</sub> (days)	Phase Separator	Digestate Type	TS <sup>e</sup> g kg <sup>-1</sup> <sub>FM</sub>	TOC <sup>f</sup> g kg <sup>-1</sup> <sub>TS</sub>	TKN <sup>f</sup> g kg <sup>-1</sup> <sub>TS</sub>
Digestate							
RD1a	CSTR (Farm 1) Cow/Pig manure (>25%), Maize silage (>25%), Cow/Pig slurry, Crop residues, Waste fats, Vegetable waste	25	-	Raw (Liquid)	131 ± 2	343 ± 1	24.4 ± 0.5
RD1b		50	-	Raw (Liquid)	97 ± 0	335 ± 0	25.1 ± 0.8
RD2	CSTR (Farm 2) Cow manure (>50%), Cow slurry, Turkey manure, Vegetable waste, Animal feed waste, Road verge clippings	120	-	Raw (Liquid)	97 ± 1	407 ± 2	31.5 ± 1.5
LP2		120	Screw Press	Liquid Phase	76 ± 1	363 ± 1	35.5 ± 0.3
SP2		120	Screw Press	Solid Phase	293 ± 27	457 ± 1	24.3 ± 0.3
RD3	CSTR (Farm 3) <sup>a</sup> Cow slurry, Cow manure, Dairy permeate, Waste fats, Cereal wastes, Crop residues, Vegetable Waste, Road verge clippings	45	-	Raw (Liquid)	78 ± 0	311 ± 2	32.0 ± 1.1
SP3		45	Screw Press	Solid Phase	224 ± 3	439 ± 3	18.5 ± 0.8
RD4	CSTR (Farm 4) Cow manure (>30%), Maize cane (>30%), Crop residues, Vegetable waste	50	-	Raw (Liquid)	130 ± 0	329 ± 2	28.9 ± 0.2
LP4		50	Screw Press	Liquid Phase	95 ± 0	283 ± 2	29.3 ± 0.3
CS4		50	Screw Press	Compost	240 ± 3	370 ± 2	19.9 ± 0.5
RD5	Solid Batch (Farm 5) Cow manure (>90%), Cereal wastes, Crop residues, Vegetable waste	60	-	Raw (Solid)	236 ± 35	350 ± 1	25.3 ± 0.8
CS5		60	-	Stored (Solid)	252 ± 29	358 ± 2	26.6 ± 0.1
CS6	Solid Batch (Farm 6) <sup>b</sup> Goat/Cow manure (>90%), Vegetable waste	40	-	Stored (Solid)	216 ± 12	388 ± 0	22.2 ± 0.4
LP7	CSTR (Centralized) Sludge from waste-water treatment (>30%), Animal fats/Slaughterhouse waste, Vegetable waste	40	Centrifuge	Liquid Phase <sup>c</sup>	24 ± 0	359 ± 6	57.7 ± 1.2
CS7		40	Centrifuge	Compost <sup>d</sup>	429 ± 7	280 ± 1	25.7 ± 0.4

HRT<sub>AD</sub> = Estimated Hydraulic Residence Time in AD, RD = Raw digestate, CS = Composted or stored digestate, LP = Liquid phase of digestate, SP = Solid phase of digestate, CSTR = Continuous Stirred Tank Reactor, TS = Total Solids, FM = Fresh Matter, TOC = Total Organic Carbon, TKN = Total Kjeldahl Nitrogen. <sup>a</sup> plant includes proprietary hydrolysis pre-treatment, <sup>b</sup> solid batch process with permeate recycling, <sup>c</sup> pasteurized liquid phase after centrifugation, <sup>d</sup> solid phase after centrifugation, composted with woody green waste and sampled during active phase of composting, <sup>e</sup> TS of fresh digestate before drying and preparation, <sup>f</sup> TOC and TKN after drying and preparation

#### 4.2.1.2 EEM Fluorescence of the *SPOM* fraction from sequential extraction

Fluorescence analysis of digestate was used to characterise aromatic groups in the organic matter of the *SPOM* fraction of digestate obtained by sequential extraction. Filtered extracts of were systematically diluted to  $5 \text{ mg}_{\text{TOC}} \text{ L}^{-1}$  to avoid fluorescence quenching effects before placing in a 1 cm path length quartz cuvette for analysis by 3D-LPF spectroscopy with a Perkin Elmer LS55. As per Jimenez et al. (2014) and Muller et al. (2014), excitation wavelengths were varied at 10 nm increments between 200 and 600 nm, excitation and emission monochromator slit widths were set to 10 nm, and fluorescence emission values were recorded at 0.5 nm increments from 200 to 600 nm. The spectra obtained were decomposed into seven zones (I-VII) according to the method of Muller et al. (2014), with Zones I-III designating simple proteins and amino acids (e.g. Tryptophan, Tyrosine) and Zones IV-VII designating more complex molecules (e.g. proteins, lignin, humic- and fulvic- like substances). The proportion of fluorescence in a given zone “*i*”  $P_f(i)$  was calculated by estimation of the fluorescence zone volume  $V_f(i)$ , by the equations:

$$V_f(i) (\text{U.A. mg}_{\text{TOC}}^{-1} \text{L}) = \frac{V_{f, \text{raw}}(i)}{\text{TOC}_{\text{sample}}} \times \frac{\sum_{i=1}^7 S(i)}{S(i)} \quad (4)$$

$$P_f(i) (\%) = \frac{V_f(i)}{\sum_{i=1}^7 V_f(i)} \times 100 \quad (5)$$

Where  $\text{TOC}_{\text{sample}} (\text{mg}_{\text{L}}^{-1})$  is the TOC of the sample analysed,  $S(i) (\text{nm}^2)$  is the calculated area of the fluorescence zone *i*. Subsequently, the “complexity index” ( $CI_{\text{SPOM}}$ ) was calculated as the ratio of the sum of the fluorescence volumes of the most complex fluorescence zones (IV–VII) over the sum of the fluorescence volumes of the simple molecule zones (I–III).

#### 4.2.2 Incubation protocol

The precise protocol used for aerobic incubation of the digestates is detailed in Cooke et al. (2023). Briefly, digestates were mixed at a dose equivalent to  $6 \text{ g}_{\text{TC}} \text{ kg}_{\text{dry soil}}^{-1}$  with 110 g of prepared aggregates (3.15 – 5 mm diameter) in hermetically sealed jars and incubated over 182 days in the dark and at

20°C. At days 0, 7, 14, 42, 98 and 182, four of the jars from each modality were destructively sampled for analyses. Soil for most analyses was dried at 40 °C for 24 hours and then stored at 4 °C in sealed containers until needed. Soil for qPCR analysis was placed in airtight containers and stored at -80°C until needed.

#### 4.2.3 Soil physical and chemical analysis

The soil used is classified as a Luvisol-Redoxisol derived from loess silt deposits, with the following characteristics: Clay = 14.2 %, Silt = 71.0 %, Sand = 14.8 %, TOC = 2.06 %, TOC-TN ratio = 10, and  $CEC_{Metson} = 6.34 \text{ cmol.kg}^{-1}$ . The soil had not previously been in contact with digestate or other organic amendments. The mean soil moisture content at the beginning of incubation was  $0.22 \text{ g g}_{\text{dry soil}}^{-1}$  or approximately 88 % of water holding capacity ( $SWHC = 0.249 \text{ g g}_{\text{dry soil}}^{-1}$ ). Soil electrical conductivity (EC) and pH were analysed on air-dried samples collected at day 0 of incubation just after digestate incorporation, by suspension in ultrapure water (1:5 dry soil to water ratio).

Soil aggregate stability analysis was performed using the method established by Le Bissonais (1996), which forms part of the European standard method (ISO 10930, 2012). The “slow-wetting” test was selected for use, as it is considered the most adapted to analysis of fragile silt soils (Le Bissonais and Arrouays, 1997). Briefly, 5 g of 3.15-5 mm aggregates were pre-dried for 24 hours at 40 °C, capillary rewetted for 1 hour on a water saturated foam sponge, transferred on a sieve of 0.05 mm and immersed five times in ethanol using a Hénin or Yoder apparatus. The remaining fraction was then dried at 40 °C for 24 hours before passing through a nest of sieves of mesh sizes 2 mm, 1 mm, 0.5 mm, 0.2 mm, 0.1 mm and 0.05 mm. The mean weight diameter ( $MWD$ ) was calculated by taking the sum of the mass of soil remaining on each sieve multiplied by the mean aperture of adjacent sieves and divided by the total mass of aggregates. The  $\Delta MWD$  was then calculated as the difference between the  $MWD$  of a soil-digestate mixture ( $MWD_{Treatment}$ ,  $n = 4$ ) and the mean  $MWD$  of the control soil jars ( $MWD_{Control}$ ,  $n = 4$ ) at a given date:

$$\Delta MWD(mm) = MWD_{Treatment} - MWD_{Control} \text{ (SEEquationARABIC5)}$$

Finally, the area under the curve (*AUC*) plotted by  $\Delta MWD$  over time was estimated for each modality using the trapezoidal rule:

$$AUC(mmd) = \int_0^t f(\Delta MWD) dt \approx \sum_{i=1}^n \frac{(\Delta MWD_{i-1} + \Delta MWD_i)}{2} \Delta t_i \quad (2)$$

Where  $\Delta MWD_i$  is the mean difference in mean weight diameter measured at a sample date  $i$ , while  $\Delta t_i$  is the difference in days between a sample date  $i$  and the previous sample date  $i - 1$ . This is equivalent to the Riemann sum as previously used by Annabi et al. (2007a) for comparison of AS dynamics following compost amendment. The *AUC* can be taken as an indicative value that summarises the overall impact of a given organic amendment on AS over the course of the incubation. As such, amended soil with a low and/or transitory measured effect on  $\Delta MWD$  relative to the control soil will have a low *AUC*, while those with an elevated and/or persistently higher  $\Delta MWD$  will have a high *AUC*. The standard deviation ( $\sigma_{AUC}$ ) for the *AUC* estimated by trapezoidal method was calculated by the formula:

$$\sigma_{AUC}(mmd) = \frac{1}{4} \left( \sigma_{\Delta MWD,0}^2 \Delta t_1^2 + \sigma_{\Delta MWD,n}^2 \Delta t_n^2 + \sum_{i=1}^{n-1} \sigma_{\Delta MWD,i}^2 (\Delta t_i + \Delta t_{i+1})^2 \right) \quad (3)$$

Where  $\sigma_{MWD,i}$  is the standard deviation in the  $\Delta MWD$  at a sample date  $i$ . The mean  $\Delta MWD$  at each sample date and calculated *AUC* for digestate-soil mixtures are given in Table. 4.3.

The water droplet penetration time (*WDPT*) of soil aggregates was analysed in as per the method of Chenu et al. (2000). Briefly, a 3  $\mu$ l droplet of deionised water was placed on the surface of a 3.15-5 mm aggregate using a micro-syringe (Gilson Microman®) and the time taken for the droplet to be completely absorbed into the aggregate was recorded. If absorption occurred in less than one second, the *WDPT* was recorded as equal to one second. At least five aggregates from each incubation jar were tested in this manner, for a minimum of 20 aggregates per modality and per sample date (5 x 4 incubation jars).

Table 4.3 Summary of the  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) and  $WDPT$  (Water Droplet Penetration Time) measured on soil aggregates amended with digestate and sampled at different

Day	WDPT (s)			$\Delta MWD$ (mm)						AUC (mm d)	max $\Delta MWD$ (mm)
	0	14	98	0	7	14	42	98	182		
<b>RD1a</b>	3.8	6.2	8.1	0.10	0.15	0.20	0.21	0.43	0.26	55.0	0.43
<b>RD1b</b>	4.2	6.7	5.7	0.10	0.14	0.15	0.16	0.39	0.23	48.0	0.39
<b>RD2</b>	3.2	4.4	5.3	0.19	0.16	0.12	0.21	0.34	0.51	57.6	0.57
<b>RD3</b>	2.2	2.6	3.7	-0.04	0.61	0.14	0.03	0.12	0.23	25.9	0.61
<b>RD4</b>	2.8	4.2	3.3	0.04	0.50	0.24	0.03	0.26	0.50	48.1	0.54
<b>RD5</b>	3.0	3.2	3.8	0.14	0.11	0.19	0.24	0.28	0.35	48.9	0.45
<b>LP2</b>	3.4	3.0	3.8	0.30	0.19	0.24	0.21	0.31	0.32	51.0	0.38
<b>LP4</b>	3.1	3.7	3.5	0.28	0.22	0.23	0.19	0.16	0.68	54.2	0.68
<b>LP7</b>	4.3	3.7	6.8	0.19	0.03	0.24	0.32	0.34	0.78	74.8	0.78
<b>SP2</b>	2.3	2.9	5.0	0.06	0.09	0.09	0.12	0.14	0.20	26.1	0.21
<b>SP3</b>	1.8	1.8	2.7	0.04	0.16	0.17	0.02	0.25	0.32	35.8	0.32
<b>CS4</b>	1.9	2.2	2.2	0.16	0.17	0.13	0.11	0.13	0.15	24.0	0.21
<b>CS5</b>	4.6	4.6	5.7	0.13	0.09	0.08	0.11	0.26	0.35	39.7	0.35
<b>CS6</b>	2.0	3.7	4.0	0.10	0.08	0.09	0.01	0.14	0.16	19.2	0.16
<b>CS7</b>	1.4	2.1	2.3	0.05	0.24	0.05	0.03	0.09	0.23	20.1	0.28
<b>MAX</b>	4.6	6.7	8.1	0.30	0.61	0.24	0.32	0.43	0.78	74.8	0.78
<b>MIN</b>	1.4	1.8	2.2	-0.04	0.03	0.05	0.01	0.09	0.15	19.2	0.16

dates over the course of incubation and the calculated value for AUC, the estimated area under the curve traced by MWD plotted over time. Values represent the mean of data for each digestate (data originally from (Cooke et al., 2023))

During analysis, many aggregates in amended soil showed little or no modified wettability ( $WDPT < 2$  s) compared to the control soil, due to the heterogeneity of mixing and coating of aggregates with digestate. Thus, the median  $WDPT$  was calculated instead of the mean (as was done by Cosentino et al. (2006)). While analysis of a large number of aggregates (>100) showed the mean and the median to be converging, the median value reached a stable value faster (at approximately 20), thus allowing fewer aggregates to be tested while still obtaining a representative measurement.

## 4.2.4 Data treatment and statistical analysis

### 4.2.4.1 Compilation of dataset

Sequential extraction of digestate was carried out in triplicate, so that three sets of measurements of TOC and TN were obtained per OM fraction and per digestate. Before analysis by statistical modelling, all concentrations of TOC, TN, or other elements, were used to calculate the equivalent in mass (g) of each component that had been added to the incubation soil. This was done in order to guard proportionality between the different measured variables (soil and digestate). Fluorescence spectrometry analysis was also carried out separately on the three triplicate *SPOM* fractions for each digestate.

In the case of analyses of soil samples, as all had been carried out in quadruplicate (for the four incubation jars per modality per sample date), this needed to be reduced to three values to match with the digestate characteristics dataset. This was done using the mean value, and the mean plus or minus the standard deviation. For example, three *AUC* values were calculated based on the four *AUC* values for four repetitions:  $\underline{AUC}$ ,  $\underline{AUC} + \sigma_{AUC}$ , and  $\underline{AUC} - \sigma_{AUC}$ . These data were then attributed at random to one of the triplicate dataset of explanatory variables (TOC, TN of the 5 fractions from sequential extraction, elements, and complexity index  $CI_{SPOM}$ ), in order to avoid any bias generated from placing all maximum and minimum values together. The above process allowed the heterogeneity of the datasets to be taken into account, whilst also augmenting the power of subsequent statistical analyses.

Statistical tests were carried out using the “R” programming environment (R Core Team, 2021). Significant differences ( $p \leq 0.05$ ) between means of analysed variables were tested by one-way analysis of variance (ANOVA), while post-hoc pairwise comparison between means was performed by Tukey least significant differences test. For analysis of correlations between  $\Delta MWD$  and *AUC*, Principal Component Analysis (PCA) was performed using the “Factoshiny” package (Vaissie et al., 2021).

#### 4.2.4.2 Partial Least Squares Regression (PLS-R)

Analysis by PLS-R was chosen over other multivariate statistical methods for analysis of digestate OM fractions obtained by sequential extraction and their contribution to soil AS because the dataset involves a large amount of correlated independent variables that risk effects from collinearity and makes techniques such as ordinary least squares (OLS) regression unreliable. PLS-R modelling was performed using XLStat® software, with a single “predicted” (Y) variable (in most cases the AUC) being selected along with a varying number of different “explanatory” (X) variables, depending on the model. Approximately 80% of individuals in the dataset were used to train the model, while the remaining 20% were set aside for model testing. Cross-validation was carried out by jack-knife method (LOO - Leave one out), where one sample at a time was removed from the training dataset to test model stability.

Two parameters were considered when assessing the results of PLS-R analysis:

$$R^2 = 1 - \frac{RSS}{TSS} \quad (4)$$

Where, *RSS* is the residual sum of squares and *TSS* is the total sum of squares. The  $R^2$  provides a metric of the “goodness of fit”, or explained variation, of the manifest variable (Y) and varies between 0 and 1 ( $R^2 = 1$  being a perfect model fit).

$$Q^2 = 1 - \frac{PRESS}{TSS} \quad (4)$$

Where *PRESS* is the predicted residual error sum of squares. The  $Q^2$  is a measure of the “goodness of prediction” or predicted variation as determined from cross-validation, and may vary between  $-\infty$  and 1 (a  $Q^2$  above 0.5 indicates a good predictability of the model (Jimenez et al., 2014)). Optimisation of each model was performed by manually reducing the number of dimensions in the model to obtain a  $Q^2 \geq 0.5$  wherever possible, while maintaining enough dimensions for a high  $R^2$ . This method allowed

selection of models that were both a good fit to the existing data, while also providing robust prediction for the test dataset.

## 4.3 Results and discussion

Initially, the characteristics of the digestates determined by sequential extraction, fluorescence spectrometry, and elemental analysis, were compared and discussed, followed by analysis of soil sampled during incubation trials. The calculated *AUC* values were subjected to a PCA analysis to determine how well they represent the observed dynamics in soil AS. The variables to be used in the generation of a PLS-R model were thus selected and a preliminary analysis carried out between digestate characteristics and soil properties. Finally, a number of PLS-R models were constructed using combinations of variables in order to determine the quantity of variation in *AUC* and measures of soil AS that could be explained by the gathered dataset. An assessment of the predictive power of the best models, and analysis of the contribution of different variables in each case, was used to determine the principal indicators of digestate impact on soil AS, and investigate the potential soil stabilisation mechanisms at work.

### 4.3.1 Characterisation of digestates

#### 4.3.1.1 Sequential extraction of organic matter fractions

Proportions of TOC in the fractions obtained by sequential extraction for each digestate are presented in the bar chart in Fig. 4.1. For all digestates, a relatively small proportion of TOC was found in the more bio-accessible fractions, with *SPOM* at between 1.4% and 11.6%, and *REOM* at between 1.7% and 8.7%. Muller et al. (2014), in development of an earlier version of a similar procedure for sequential extraction, reported comparable proportions of *SPOM* (in their case, DOM + S-EPS) for undigested and digested sludges, while proportions of *REOM* (in their case, RE-EPS) were higher in their study at up to 15%. Subsequently, the *SEOM* represents between 11.0% and 25.6% of TOC in the digestates. This fraction may contain proteins and accumulated microbial residues, as well as humic-like substances

(Bareha et al., 2019; Muller et al., 2014). High proportions of TOC were found in the *PEOM*, between 49.5% and 14.9%, said to be primarily extracted hemicellulose and cellulose. In particular, RD5 a digestate of cow manure and bedding from solid batch AD shows the highest proportion of TOC in the *PEOM* (49.5%), which may be explained by the reduced efficiency of this process in breaking down OM into biogas (Rocamora et al., 2020). On average, the largest proportion of TOC, between 24.2% and 57.57%, remained in the fraction not extracted during the procedure and therefore was classified as *NEOM*. This remaining matter is said to be mainly composed of lignin and other high weight aromatic molecules (Jimenez et al., 2015), though recent study using NMR spectroscopy for *NEOM* extracted from digestate found it to be a mixture of aliphatic and aromatic-CH groups resembling proteins and biomass residues (Laera et al., 2019). Our results are coherent with other published data of sequential extraction of digestate (Fernández-Domínguez et al., 2021; Maynaud et al., 2017), though extraction methods and analysis techniques differ somewhat from those used in this study, making comparison difficult.

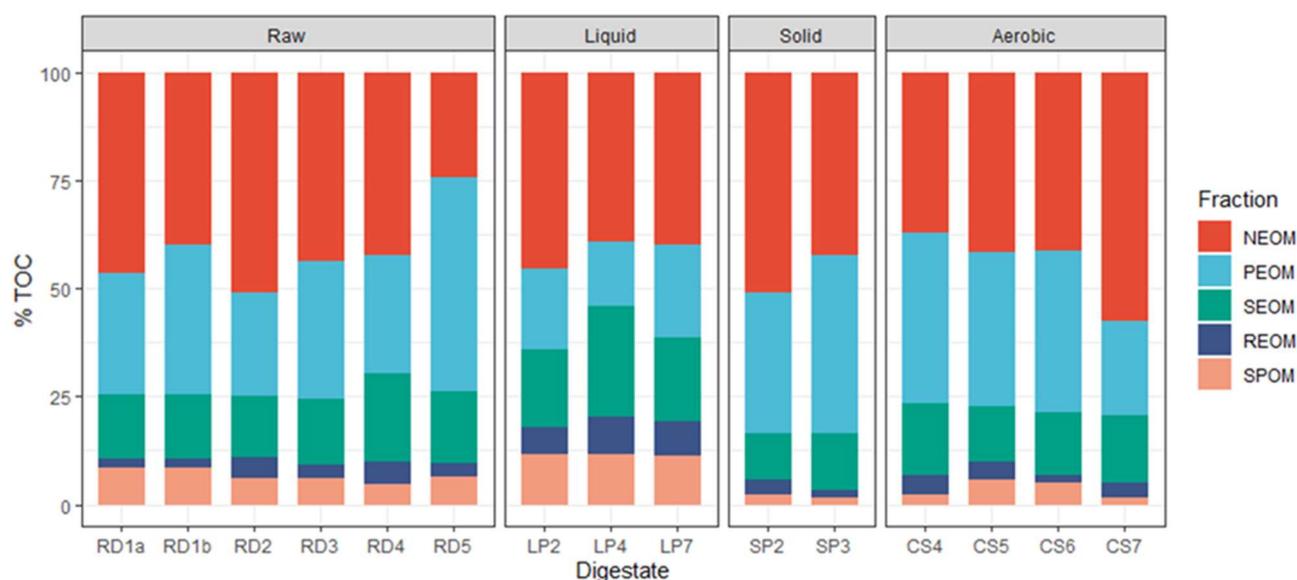


Fig. 4.1 Proportion of TOC measured in the five fractions obtained from the sequential extraction technique for 15 different digestates. Error bars represent the cumulative standard deviation for the *NEOM*.

Phase separation of digestate appears to have a relatively significant effect on the TOC distribution in digestate OM fractions. The liquid phases of digestate show a profile significantly different ( $p < 0.01$ ) to raw or solid digestate for all fractions except *NEOM*. Solid phases of digestate have a significantly lower proportion of *SPOM* than raw or liquid phase of digestate and a higher proportion of *PEOM* than the latter. This would correspond with the fact that, during phase separation, soluble and small particulate OM may pass through the separator and be concentrated into the liquid phase while more fibrous plant matter (hemicellulose/cellulose) is retained in the solid phase. Subsequent post-treatment of solid digestate by open-air storage or composting appears to result in a slight increase in the proportion of more readily extracted fractions (*REOM+SPOM*), although the composted digestates (CS4 and CS7) show lower (but not significantly different) concentrations of *SPOM* than the aerobically stored digestates (CS5 and CS6).

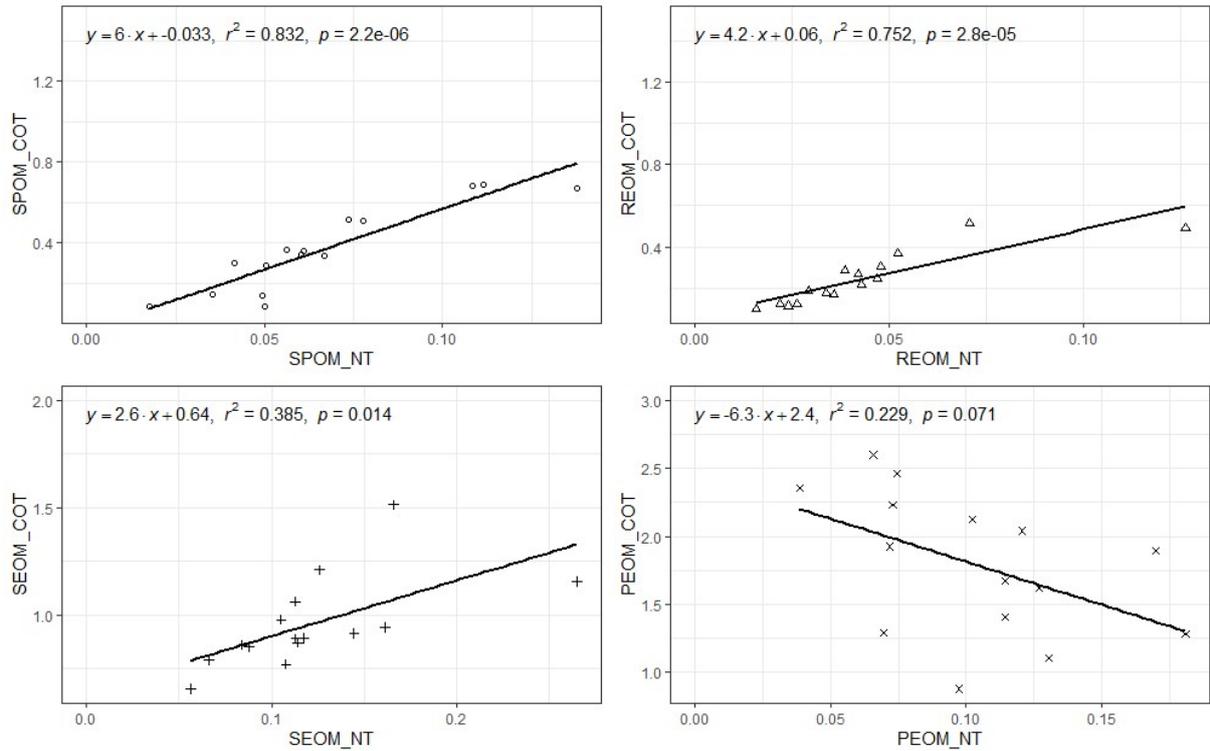


Fig. 4.2 Total Organic Carbon (TOC) plotted versus Total Nitrogen (TN) in each of the four fractions obtained by sequential extraction from 15 different digestates. Values shown are the estimated quantity of each fraction applied to the soil during incubation trials ( $\text{g kg}_{\text{dry soil}}$ )

Quantities of extracted TN in each fraction were also analysed and found to be comparable to the TOC, with generally more TN in the less extractable fractions. For the *SPOM* and *REOM*, and to a lesser extent the *SEOM* fraction, the TOC and TN contents were highly correlated, as can be seen from Fig. 4.2. This suggests that the composition of these fractions may have been similar across most of the digestates, mainly differing in terms of the concentration of these compounds. The TN extracted into the *SEOM* varied from 17.82% to 32.93% of digestate TN, which would confirm that this fraction extracted a large quantity of proteins in the digestate (Jimenez et al., 2015), though high proportions of TN were also subsequently extracted in *PEOM* (12.09% to 29.52%) or else remained in the non-extracted *NEOM* (23.02% to 40.93%). The *PEOM* fraction is supposed to be composed of cellulose and hemicellulose compounds that do not contain nitrogen. Thus, the presence of higher levels of nitrogen in this fraction has been suggested by Bareha et al. (2018) to be due to less extractable proteins trapped in these complex structures of plant-derived materials

#### 4.3.1.2 Fluorescence analysis of the *SPOM* fraction from sequential extraction

The complexity index (*CI*) is the estimated ratio of complex fluorescing organic compounds (e.g. humic- and fulvic- like substances, complex proteins, lignin) and simple fluorescing organic compounds (e.g. tryptophan, tyrosine, simple proteins, etc.) (Muller et al., 2014). The *CI* of the extracted *SPOM* fraction ( $CI_{SPOM}$ ) for each digestate is given in Fig. 4.3, varying between 0.309 (lower complexity) and 0.838 (higher complexity), with values not significantly different denoted by the same superscript letter. These values are largely comparable to results from analysis of a larger range of digestates (Fernández-Domínguez et al., 2021), where the fluorescence complexity index covered a slightly larger range between 0.3 and 1.5. High errors for values of  $CI_{SPOM}$  resulting from relatively large uncertainties in estimation meant that differences in fluorescence volumes for each zone were mostly insignificant between digestates. There would appear to be similarities in the  $CI_{SPOM}$  for digestates sampled from the same AD plant, for example with digestates RD2, SP2 and LP2, that are representative of the same digestate before and after phase separation. This is coherent with observations that digestates

“inherit” aspects of their spectral characteristics from the parent organic material (Provenzano et al., 2011), and as such digestates from the same feedstock may retain similarities despite undergoing varying treatment (Fernández-Domínguez et al., 2021). While the *SPOM* is believed to be mainly composed of readily extractable sugars and amino acids, there can also be a significant amount of complex OM present in this fraction for digestate, as is confirmed by the presence of fluorescing zones at higher emission-excitation wavelengths for all digestates.

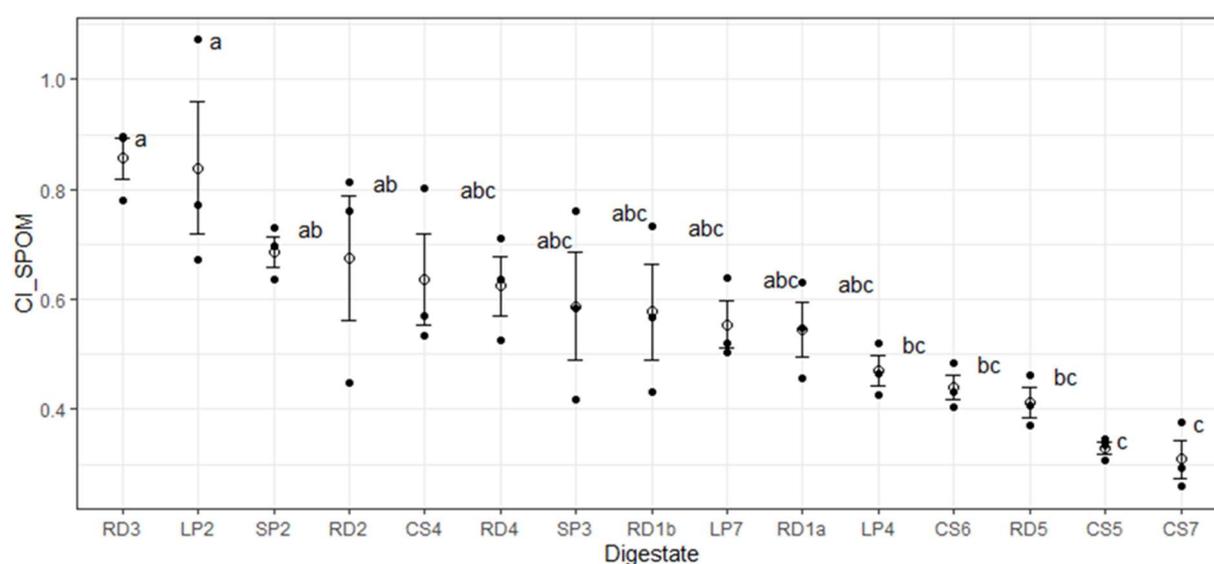


Fig. 4.3 Calculated complexity index for the SPOM fraction ( $CI_{SPOM}$ ) of extracted OM from digestates. Black dots represent calculated values for each repetition ( $n=3$ ), while hollow circles represent the mean. Error bars denote standard deviations. Same letters to the right of the points denote values that are not significantly different ( $p>0.05$ )

#### 4.3.1.3 Total elemental composition

A full table of digestate characteristics, including total elemental composition (TK, Na, Ca, Fe, Mg, Al, Cu and Zn) is provided in the supplementary information (Appendix 2). In general, the raw and liquid phases of digestate contained higher levels of minerals than solid or composted digestates. For many of the digestates studied here, after drying and preparation for analysis their content in potassium (K) and phosphor (TP) was found to be similar or even higher than the TKN. Eich-Greatorex et al. (2018)

also found very high mineral concentrations in their study of digestates, stating that care should be taken not to exceed recommended doses of these minerals when spreading digestate in field conditions.

The hypothesis behind analysing the elemental composition of the digestates, along with the pH and EC of the soil after amendment, was that significant changes to soil chemistry following amendment may equally impact soil structure (Bronick and Lal, 2005; Haynes and Naidu, 1998). Application of digestates and biosolids rich in monovalent cations,  $\text{Na}^+$  and  $\text{K}^+$ , has been known to cause disruption of soil aggregates (Jin et al., 2015; Voelkner et al., 2015b) or conversely helped to improve soil AS in heavily saline soils by reducing exchangeable sodium (García-Orenes et al., 2005; Manasa et al., 2020). For other mineral rich organic amendments, improvements in soil AS have been attributed to higher concentrations in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations associated with humic acids (Tejada and Gonzalez, 2007).

#### 4.3.2 Analysis of soil incubated with digestate

##### 4.3.2.1 Assessment of *AUC* as a reduced variable for summarising aggregate stability dynamics

The detailed results of soil aggregate stability (AS) dynamics for digestate-soil mixtures results and the calculated *AUC* were first presented in Cooke et al. (2023) and the values are given in Table. 4.3. Although it may have been possible to evaluate the link between digestate characteristics and measured  $\Delta MWD$  at each sample date, this was considered to be both bulky and time consuming. Therefore, a method was determined to summarise the many measurements into one reduced variable. The *AUC* was calculated (as described in the material and method), summarising the  $\Delta MWD$  values measured at each date over the course of incubation, i.e. the amplitude and persistence of impact that amendment with digestate has relative to the unamended control soil. This method was first proposed by Annabi et al. (2007) as a way of comparing between complex dynamics in soil AS following amendment with different composts. To evaluate how well the calculated *AUC* represented the relatively diverse dynamics in soil AS following digestate amendment, a PCA analysis was

performed on all  $\Delta MWD$  data along with the *AUC* as a supplementary variable, and the bi-plot is shown in Fig. 4.4. The first principal component, which explains 52.52% of variation was found to be well correlated with the majority of  $\Delta MWD$  at different sample dates as well as the *AUC*. Only the  $\Delta MWD$  at day 7 of incubation was found to be poorly represented across the first dimension of the PCA.

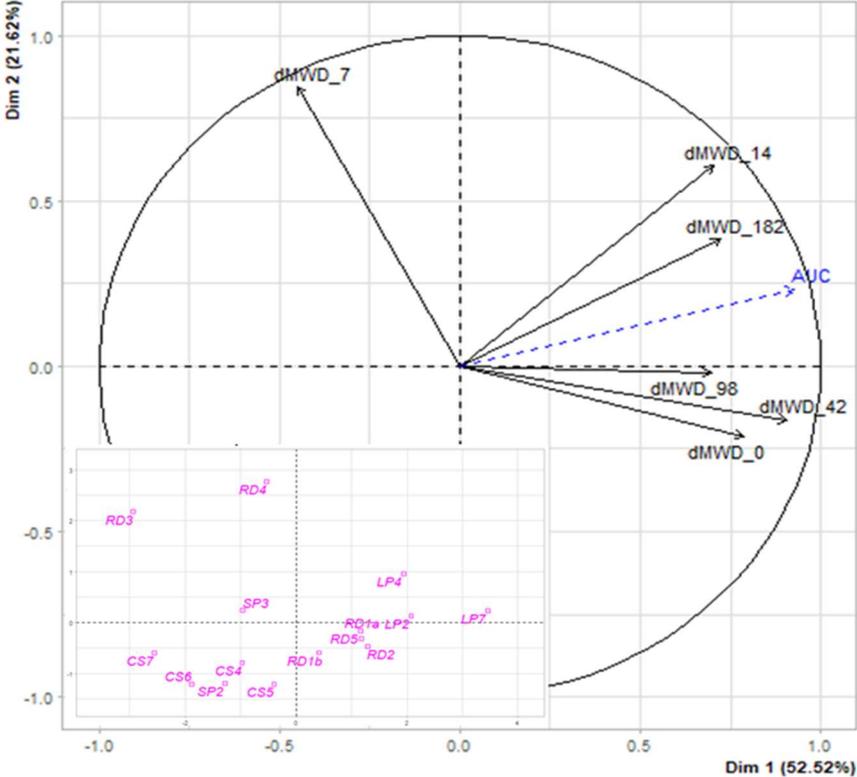


Fig. 4.4 Principal Component Analysis (PCA) biplot for  $\Delta MWD$  ( $MWD_{Treatment} - MWD_{Control}$ ) measured on soil amended with digestates after different lengths of time in incubation (0, 7, 14, 42, 98, and 182 days), with estimated *AUC* ( $\sum_{i=1}^n \frac{(\Delta MWD_{i-1} + \Delta MWD_i)}{2} \Delta t_i$ ) as a supplementary variable.

4.3.2.2 Dynamic in wettability of aggregates and link to soil aggregate stability

For many of the incubated digestates, the median *WDPT* was increased relative to the control soil (*WDPT* = 1 ± 0.3 s) for all sample dates (see Table. 4.3). A significant correlation was found between increased *WDPT* and an increase in measured  $\Delta MWD$  for amended soils across all dates (see Fig. 4.5). This would suggest that the two effects are linked and result from a modified wettability of the surface of the soil aggregates following coating with digestate. Indeed, Chenu et al. (2000) determined that

the predominant mechanism by which soil AS was increased with OM addition was increasing wetting times, thus slowing slaking and fracturing of aggregates. However, no linear correlation was found between *WDPT* and  $\Delta MWD$  at day 0 of incubation, though a number of soil-digestate mixtures showing high initial  $\Delta MWD$  did also show a reduced wettability which may indicate deposition of a hydrophobic coating of digestate. Other mechanisms may have contributed in the initial stabilisation of aggregates aside from the modification of their surface wettability by hydrophobic OM. The correlation between *WDPT* and  $\Delta MWD$  only became statistically significant ( $p = 0.002$ ) after 98 days of incubation. It could be that accumulation of microbial deposits increased both the structural stability and wettability of soil aggregates by modification of surface pore size, as has been suggested by Cosentino et al. (2006). It has been proposed that, in some cases pore modification has a greater impact on soil AS than hydrophobic coating (Dal Ferro et al., 2012).

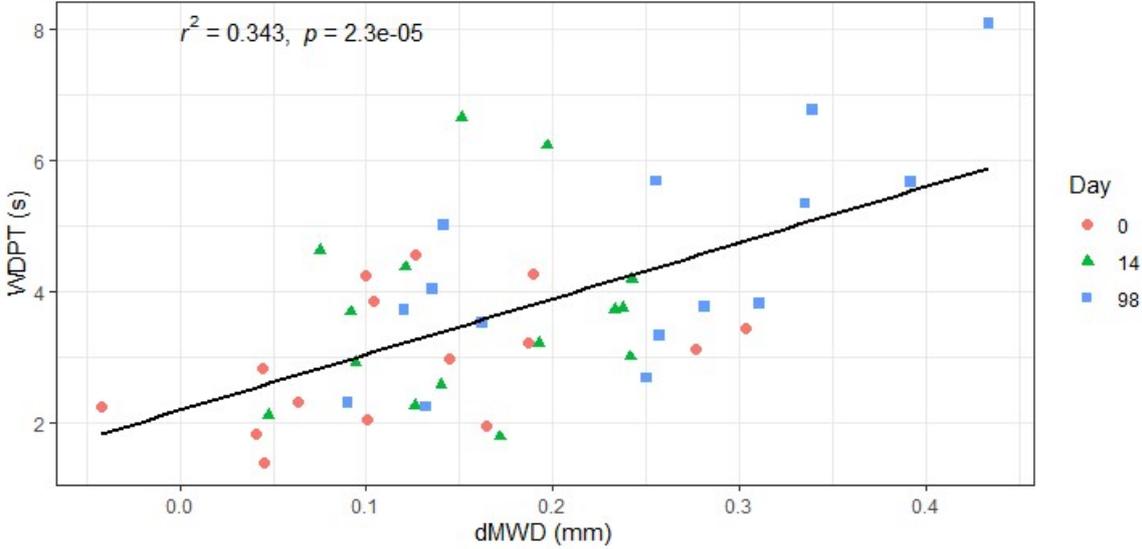


Fig. 4.5 Scatter plot of water droplet penetration time (*WDPT*) against the differential mean weight diameter ( $\Delta MWD = MWD_{Treatment} - MWD_{Control}$ ) for all modalities and sample dates (circles = 0 days, triangle = 14 days, squares = 98 days) The coefficient of correlation ( $r^2$ ) and significance  $p$ -value, given above the plot are for the linear model using all data points, represented by the solid line. The *WDPT* for the control soil at all times was approximately  $1 \pm 0.3$  s.

#### 4.3.2.3 Changes in soil electrical conductivity and pH

Soil without digestate amendment sampled at day 0 of the incubation trials was found to have a pH of  $6.7 \pm 0.4$  and an EC of  $105 \pm 53 \mu\text{S cm}^{-1}$ . Despite the high variability in measured values, there was no statistically significant differences between unamended soils from the two conducted incubation trials. Addition of digestate at the doses used for incubation led to high shifts in both soil pH and soil EC, as measured at day 0 of incubation (see supplementary information in Appendix 2). The highest shifts in EC were from amendment with liquid phase of digestate LP4 ( $535 \pm 152 \mu\text{S cm}^{-1}$ ) and notably raw digestate RD3 ( $924 \pm 99 \mu\text{S cm}^{-1}$ ) while a less pronounced effect on EC was observed for most solid phases ( $224 \pm 120 \mu\text{S cm}^{-1}$ ) and aerobically treated ( $262 \pm 58 \mu\text{S cm}^{-1}$ ) digestate. Strong changes in pH from acid to alkali conditions were also observed, with LP4 inducing the highest pH change up to 8.15. No significant linear correlation was found between the  $\Delta\text{MWD}$  and either pH or EC of soil at day 0 of incubation.

### 4.3.3 Comparison of digestate characteristics and their impact on soil AS dynamics

#### 4.3.3.1 The effect of organic matter fractions from sequential extraction

The more easily extracted fractions (*SPOM*, *REOM*, *SEOM*) were well correlated with variations in calculated *AUC*, with the strongest relationship between *SPOM* and *AUC* for both measured TOC and TN in this fraction (see Fig. 4.6). As such, the strength of correlation between *AUC* and TOC decreases with increasing difficulty of extraction of the fraction ( $\text{SPOM} < \text{REOM} < \text{SEOM} < \text{PEOM}$ ). While the TN for all fractions are significantly correlated to *AUC*, for *REOM* and *PEOM*, the strength of the correlation is dependent on an outlier digestate (LP7) for certain fractions. LP7 is a liquid phase of digestate produced from centrifugation and pasteurisation of digested sewage sludge and agro-industrial wastes, and showed high levels of TN in all fractions while also exhibiting a very high impact on soil AS.

A few studies have found that introduction of increased levels of readily available nitrogen along with an amendment of crop residues may reduce their positive effect on soil AS in the long-term (Le Guillou et al., 2011; Vandevivere et al., 1990), as organic matter is more rapidly broken down and exhausted. However, it was also proposed by the same authors that this depends on the OM quality of the organic amendment. In the case of several digestates incubated in this study, soil microbial activity may have been increased by readily biodegradable organic nitrogen forms in the easily extracted OM fractions (*SPOM* and *REOM*) and maintained by slower release of nitrogen from the poorly extracted OM fractions (*SEOM* and *PEOM*), generating a prolonged improvement in soil AS.

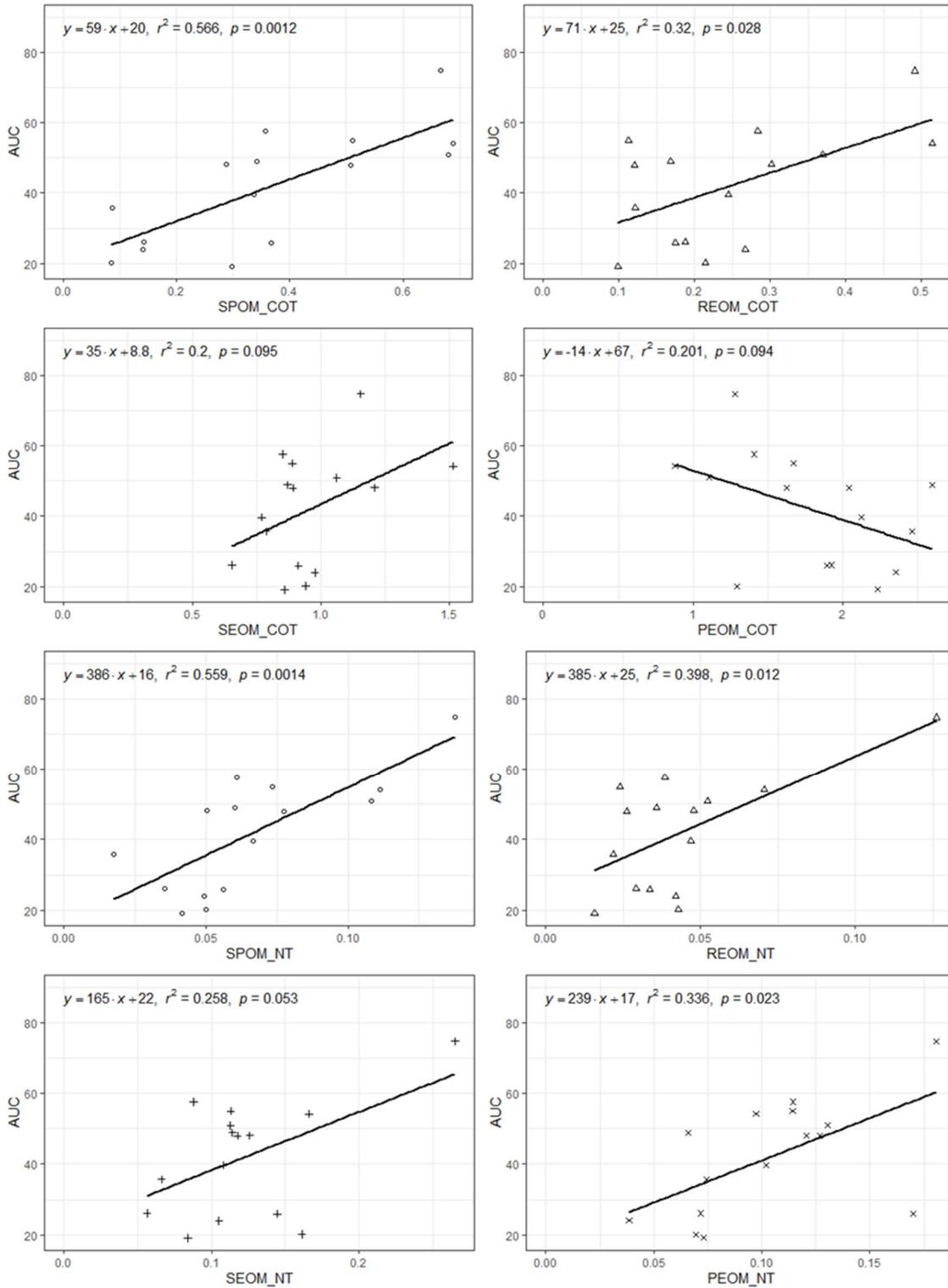


Fig. 4.6 Plots of TN and TOC in each extracted fraction from sequential extraction of digestates (as per Jimenez et al. (2014)) versus the AUC ( $\sum_{i=1}^n \frac{(\Delta MW D_{i-1} + \Delta MW D_i)}{2} \Delta t_i$ ). Points represent mean values for each digestate, while solid lines represent the line of best fit for the equation obtained by linear regression given in the top left corner of each plot.

It is probable that the *SPOM* and *REOM* fractions provided easily biodegradable material that stimulated microbial activity and led to stabilisation of soil aggregates through deposition of microbial “gels” or formation of mycelial networks (Bronick and Lal, 2005). Soil amended with readily decomposed organic residues will be rapidly stabilised, though the effect may not last a significant length of time (Abiven et al., 2007). The incubation trials studied in this paper were carried out over approximately 6 months, which is a relatively short period of time compared to field studies conducted over consecutive years (Badagliacca et al., 2020; Pastorelli et al., 2021). Therefore, the calculated *AUC* represents the cumulative effect of amendments on soil AS over a limited period, and may explain why this measure was less well correlated with the more slowly extracted fractions *SEOM* and *PEOM*.

#### 4.3.3.2 The effect of fluorescent OM and complexity index of the *SPOM* from sequential extraction

No significant linear correlation ( $p < 0.05$ ) was found between the proportion of fluorescence zones  $P_f$  or the calculated complexity index  $CI\_SPOM$  and the calculated *AUC* (data not shown). Given the very high variability between repetitions for the same digestate, this is unsurprising and limits the utility of the dataset in further analysis. However, this does not rule out a potential link between fluorescence groups and soil aggregation, and it is proposed that, given the clear correlation between digestate *SPOM*\_TOC and the impact on soil aggregate stability, further spectral analysis of this fraction could yield useful information on the amendment value of digestates. Indeed, Rezacova et al. (2021) suggest analysis of aromatic function groups by FTIR spectroscopy as a potential indicator of soil aggregation following compost and digestate amendment.

All digestate fractions contained some proportion of soluble complex fluorescent matter, including the liquid phases of digestate (LP2, LP4, and LP7), that were found to have a wide range of values for  $CI\_SPOM$  (0.47-0.83). This has previously been noted by Tambone et al. (2019), who point out that liquid fractions, containing high concentrations of stable OM, may be useful as effective soil

amendments. Soluble complex organic molecules that are mobile within the soil may be in part responsible for the direct stabilisation of soil aggregates through binding and overlapping across soil particles (Annabi et al., 2007; Slepetiene et al., 2022). Though to verify this mechanism would require isolation and quantification of this complex OM followed by testing alone with soil to extract this effect from the multitude of other interactions. In a similar manner, analysis of phenolic acids contained in different plant residues has already been proposed as a way of indicating their potential for improvement of soil AS over the medium term (Martens, 2000).

#### 4.3.3.3 The effect of digestate elemental composition

The hypothesis behind analysing the elemental composition of the digestates, along with the changes in pH and EC of the soil after amendment, was that alteration of the soil chemistry may impact soil structure as much as the added organic matter (Bronick and Lal, 2005; Haynes and Naidu, 1998). Application of digestates and biosolids rich in monovalent cations  $\text{Na}^+$ ,  $\text{K}^+$  has been known to cause disruption of soil aggregates (Jin et al., 2015; Voelkner et al., 2015b) or conversely helped to improve soil AS in heavily saline soils by reducing exchangeable sodium (García-Orenes et al., 2005; Manasa et al., 2020). For other mineral rich organic amendments, improvements in soil AS have been attributed to higher concentrations in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations associated with humic acids (Tejada and Gonzalez, 2007).

Despite the high doses of certain elements incorporated with digestates into the soil for this study, no correlation could be found between the concentrations of individual elements and the calculated *AUC*. There was a similar lack of correlation between observed changes in soil pH and EC at the start of incubation and *AUC*. Eich-Greatorex et al. (2018) also found no evidence of significantly negative effects from application of high mineral content digestates on soil physical properties. Nonetheless, it is possible that the addition of high quantities of monovalent ions such as  $\text{Na}^+$  and  $\text{K}^+$  counteracted the benefits to soil AS from incorporating high proportions readily available OM. For example, RD3 a

digestate containing mineral-rich permeate from ultrafiltration of dairy products (TP = 25.42 g kg<sup>-1</sup>, K = 55.98 g kg<sup>-1</sup>, Na = 31.79 g kg<sup>-1</sup>) also had a relatively high amount of TOC in the *SPOM* (6.18% of digestate TOC) but only had a rapid transient negative impact on soil AS following amendment with a very low calculated *AUC*. It may be of interest in future studies to analyse the mineral content of different fractions obtained by sequential extraction, in a similar manner to Laera et al. (2019), to see how these might be taken up into the soil and affect soil aggregates.

#### 4.3.4 PLS-R prediction of *AUC* from digestate characteristics

##### 4.3.4.1 Model testing and performance

Multiple PLS regression models were created using the *AUC* as the predicted variable (Y), with combinations of the digestate characteristics as the explanatory variables (X). Subsequently, optimal model fit was determined by reducing dimensionality to maximise R<sup>2</sup> while maintaining Q<sup>2</sup> above 0.5 where possible. Table 4.4 provides model parameters and the resulting values for Q<sup>2</sup> and R<sup>2</sup> of several of the PLS-R models tested. Among the groups of explanatory variables used are the TOC analysis of the 5 fractions obtained by sequential extraction (*EXT\_TOC*), the TN analysis of the 5 fractions obtained by sequential extraction (*EXT\_TN*), and the elemental composition analysed on the whole sample (Na, K, Ca, Mg, Fe, Al, Zn, Cu). Other explanatory variables used include *CI\_SPOM*, *EC\_0*, *pH\_0* and *WDPT\_0*, representing the complexity index for the *SPOM* fraction, the EC, the pH and WDPT at sample day 0 of incubation trials, respectively.

The first PLS-R model using 10 explanatory variables (TOC and TN analysis of the 5 extracted fractions), while reasonably stable (Q<sup>2</sup> = 0.57), explained only a moderate amount of the variation in *AUC* (R<sup>2</sup> = 0.71). No significant improvement was observed for a similar model using the same variables with *CI\_SPOM*. Indeed, the model was found to be marginally less stable (lower Q<sup>2</sup>) due to the increase in number of variables, suggesting that this data does not contribute significantly to explaining the remaining variation in *AUC*. Jimenez et al. (2017) found that inclusion of complexity indicators into PLS

modelling helped improve prediction of anaerobic and aerobic degradability of carbon in various organic residues, though Bareha et al. (2018) found that simple measurement of TN in fractions obtained by sequential extraction sufficed for modelling anaerobic degradability of N in their model set. It could be that, analysis of the complexity index for all fractions is needed to significantly improve model performance, though this would need to be investigated further.

Reduction of the number of explanatory variables to include only the *EXT\_TOC* resulted in a model that performed slightly worse ( $R^2 = 0.62$ ) than for the first model (*EXT\_TOC + EXT\_TN*), while the model using only *EXT\_TN* variables performed marginally better, with an  $R^2$  at 0.74. Therefore, the measured TN in the fractions was found to be a potentially more useful indicator for explaining variations in *AUC* between digestates than the TOC. It could be possible that, under the conditions of the incubation trials, the distribution and chemistry of nitrogen in the digestate OM played a more important role in governing the soil AS dynamics than the organic carbon. However, given the strength of correlation between these two characteristics for each fraction (as shown in Fig. 4.2), it is difficult to dissociate the effect of TOC and TN in any case.

*Table. 4.4 PLS-R model parameters (Y = predicted variable, X = explanatory variables, n.Dim – specified number of model dimensions) and results for performance indicators returned by XLStat for several of the tested models.*

Y	X	n. X	n. Dim	Q <sup>2</sup>	R <sup>2</sup> Y	R <sup>2</sup>
<i>AUC</i>	<i>EXT_TOC, EXT_TN</i>	10	2	0.57	0.69	0.71
	<i>EXT_TOC, EXT_TN, CI_SPOM</i>	11	2	0.54	0.69	0.71
	<i>EXT_TOC</i>	5	2	0.45	0.60	0.62
	<i>EXT_TN</i>	5	2	0.64	0.72	0.74
	<i>EXT_TN, Na, K, Ca, Mg, Fe, Al, Zn, Cu</i>	13	3	0.51	0.77	0.79
	<i>EXT_TN, K, Fe, Na</i>	8	4	0.52	0.82	0.85
	<i>EXT_TN, EC_0, pH_0, WDPT_0</i>	8	3	0.54	0.78	0.81

To attempt to find an improved model, the five variables in *EXT\_TN* were retained given their greater performance in the PLS model, and other variables added in turn. Introduction of the measured

elemental composition in the digestates as added to the soil further improved the model performance, while selection of just several elements (K, Na, Fe) led to an equally robust model with an even higher level of explained variation ( $R^2 = 0.85$ ). For digestate in particular, RD3, the *AUC* was overestimated in the previous models using only TOC or TN data from digestate OM fractions. In the model using the elemental composition data, the *AUC* for RD3 was more accurately predicted, and so heavily influenced the amount of explained variation for this model. This digestate was found to be particularly rich in a number of elements when compared to most of the other digestates (see supplementary information in Appendix 2), and so stands as an outlier in the dataset in terms of its characteristics. As proposed previously, extremely high levels of monovalent mineral salts in digestate may disrupt soil aggregate stability to the extent that this counteracts positive effects from the readily biodegradable organic C and N introduced. However, further study of other similarly mineral-rich digestates would be required to prove that this mechanism is the reason for the low overall impact of RD3 on soil AS in incubation.

As for the elemental concentrations in the digestate, addition of initial measurements of soil pH ( $pH_0$ ), EC ( $EC_0$ ) and hydrophobicity ( $WDPT_0$ ) following amendment did incrementally improve the amount of explained variation, but reduced the model robustness at the same time. As addition of other variables provides limited improvement to the model's explanation of variability in *AUC*, it was decided to focus on analysis of the models limited to only five explanatory variables, *EXT\_TOC* or *EXT\_TN*. The advantage of using a minimal number of variables in the model is the relative ease with which additional data may be acquired for other digestates in other situations.

#### 4.3.4.2 Contribution of digestate OM fractions to model prediction of *AUC*

From the two PLS-R models for the prediction of the *AUC* using the either *EXT\_TOC* or *EXT\_TN*, the contribution of each variable can be assessed by comparison of the estimated model coefficients after normalisation as shown in Fig. 4.7. The model equations with the estimated coefficients are given by:

$$AUC = 46,77 + 39,15 * TOC_{SPOM} + 26,68 * TOC_{REOM} + 2,79 * TOC_{SEOM} - 2,54 * TOC_{PEOM} - 9,82 * TOC_{NEOM} (6)$$

$$AUC = 19,81 + 323,97 * TN_{SPOM} + 168,31 * TN_{REOM} + 22,41 * TN_{SEOM} + 129,40 * TN_{PEOM} - 160,45 * TN_{NEOM} (7)$$

For the *EXT\_TOC* and *EXT\_TN* models, the *SPOM* and *REOM* fractions had a significant positive impact on the predicted *AUC*, as already demonstrated through the simple linear regression performed earlier on. In addition to this, the *NEOM* had a strong negative contribution to the model. As the *NEOM* was calculated as the remaining OM after subtraction of all extractable OM, this would confirm that most of the extractable TOC and TN may have contributed towards increasing *AUC* through stimulation of soil biological activity over the incubation period. In a similar manner, for the “Pouloud” lognormal function model developed by Abiven et al. (2008), a strong negative correlation was found to exist between the non-extractable lignin fraction, as determined by Van Soest fractionation, and the amplitude of impact of an organic soil amendment on soil AS. As opposed to models developed by Jimenez et al. (2017) for predicting the aerobic and anaerobic carbon mineralisation of sewage sludges and digestates, where the *SEOM* fraction appears to play an important role, the *SEOM* fraction had no significant contribution in the *EXT\_TOC* and *EXT\_TN* models. The *SEOM* fraction, obtained through extraction in an alkali solution of NaOH, is believed to contain proteins, lipids and some humic- and fulvic- like molecules. Thus, the fact that this fraction does not significantly contribute to the model, may suggest that these components in digestate had little effect on soil AS via the mechanisms proposed in Table 4.1.

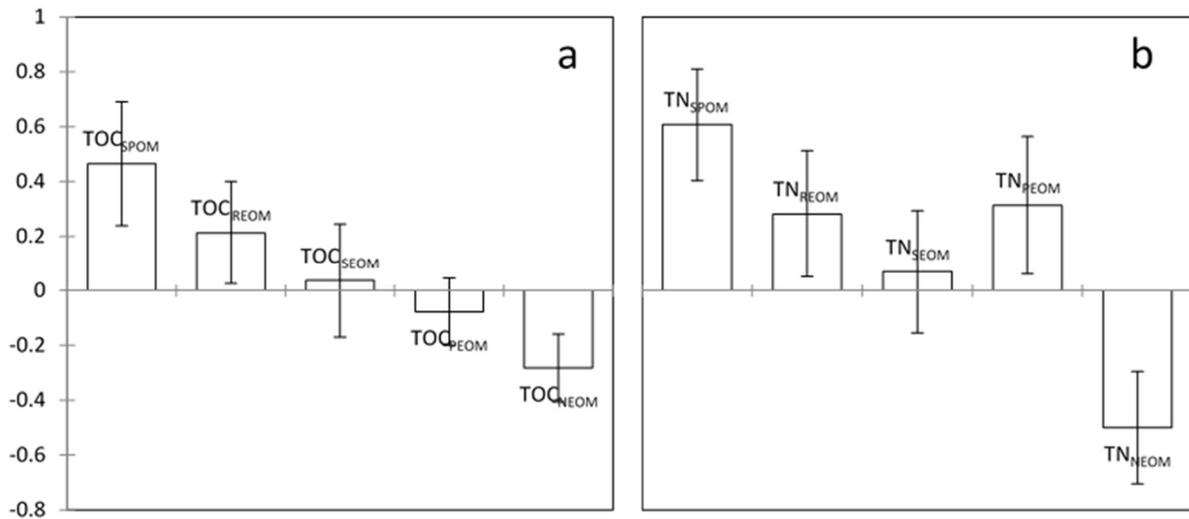
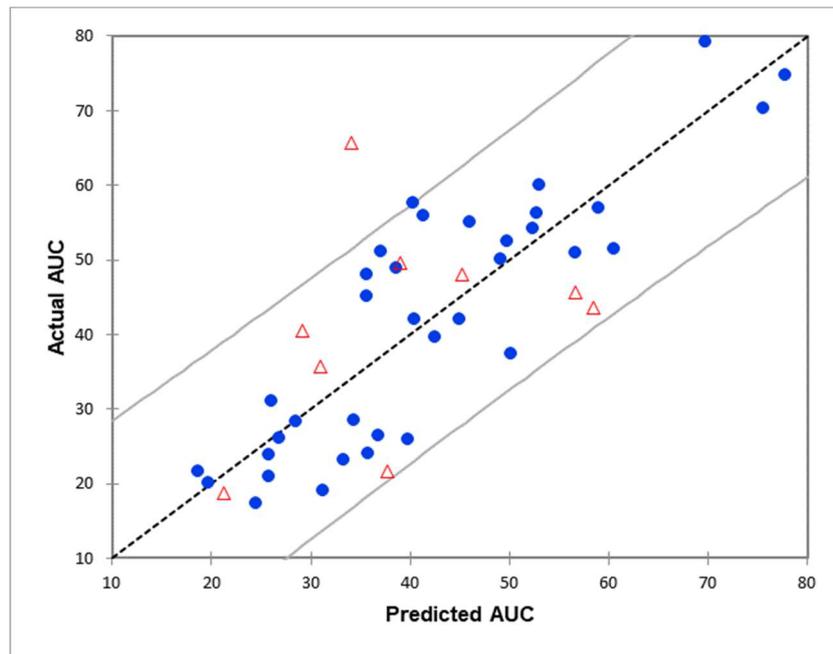


Fig. 4.7 Normalised coefficients for PLS-R model explaining variation in AUC as a function of a) TOC of fractions obtained by sequential extraction of digestates (*EXT\_TOC*), and b) TN of fractions obtained by sequential extraction of digestates (*EXT\_TN*). Error bars represent the 95% confidence interval.

For the *EXT\_TN* model, the normalised coefficients follow a similar pattern as for those of the *EXT\_TOC* model, with the exception that *PEOM\_TN* also contributes positively towards AUC. The *EXT\_TN* model performs reasonably well in predicting AUC for the test dataset, as can be seen from Fig. 4.8, though within a fairly large 95% confidence interval as traced by the grey lines ( $\pm 33$  mm day). As discussed earlier, the levels of TN within the *PEOM* fraction of digestates would indicate simple and complex proteins trapped within the less biodegradable OM structures. It could be that accessing this nitrogen while breaking down the remaining cellulose and hemicellulose in the digestate helps feed microbial activity and generates higher aggregate stabilisation.



*Fig. 4.8 X-Y plot of AUC vs. predicted AUC by the PLS-R model using TN content of fractions obtained by sequential extraction of digestate OM (EXT\_TN) as explanatory variables*

#### 4.3.5 Identifying the mechanisms of soil aggregate stabilisation following digestate amendment

While the PLS-R models analysed were able to explain around 70-80 % of variation in the *AUC*, a remaining 20-30% of variation was not explained by the variables measured on both the digestate and the soil. It is probable that this level of unexplained variation is due to the selectivity and accuracy of the methods used. While it would be time consuming, a more detailed characterisation of digestate using a broader range of techniques, especially those aimed at differentiating between recalcitrant and readily biodegradable components in the soluble OM and the availability of other nutrients in the soil electrolyte, would most likely improve model performance. Nevertheless, the findings made in this study do allow some insight into the possible mechanisms affecting soil aggregate stabilisation following digestate amendment.

In terms of the biotic mechanisms by which digestate may indirectly affect soil aggregate stability, as laid out in Table. 4.1, the findings of this study appear to verify that organic matter degradation by soil microbes is of key importance in stabilising soil aggregates. This validates research carried out on a wider range of organic amendments (Abiven et al., 2007; Sarker et al., 2018). Certain fractions of OM in digestate were strongly correlated to the overall impact on soil AS following amendment, notably those obtained from extraction by weak CaCl solution (*SPOM*) and weak alkali solution (*REOM*). There was no clear evidence that the TOC in more recalcitrant fractions (*SEOM* and *PEOM*) had an impact over the period covered by these soil incubations (182 days). However, more advanced statistical analysis by PLS-R showed a negative correlation between the non-extracted TOC and TN in the *NEOM* fraction and the *AUC*, suggesting that all extractible OM combined has a positive impact on soil AS through a regulated supply of C and N to soil microorganisms. As already mentioned, the incubation trials were limited to 6 months and so the measured changes in soil AS from amendment with digestate only cover the short to medium term and not the impact over several years. Longer-term changes in soil AS may be influenced by the more slowly biodegradable OM in digestate, which would remain a much longer time in the soil.

While inputs of organic carbon are clearly important for feeding soil biological activity, the extraction of nitrogen from different fractions of the digestate OM may be a governing factor in augmenting or attenuating aggregate stability dynamics, which would explain why the *EXT\_TN* model performs marginally better than the *EXT\_TOC* model. Nitrogen limiting conditions are considered to significantly affect soil AS dynamics (Abiven et al., 2007), though it is not always evident whether the effect will be negative or positive (Le Guillou et al., 2011). High levels of organic nitrogen in digestate are generally indicative of proteins (Bareha et al., 2018), and recent research has shown that certain soil microbial extracellular proteins are as important in soil aggregate stabilisation as extracellular polysaccharides (Redmile-Gordon et al., 2020). Furthermore, Yan et al. (2021) found that for the same digestate with lower C/N ratios (adjusted by ammonium sulphate addition) levels of humification in the soil were

increased, thus it is equally possible that nitrogen coupled with the organic carbon is essential in the formation of complex OM contributing to soil AS.

With regards to the abiotic mechanisms of aggregate stabilisation by digestate OM as proposed in Table. 4., the characterisation performed here was unable to find clear evidence to support that these play a significant role alongside biotic mechanisms, at least over the short time-scale studied. In particular, there was little indication from analysis of the data gathered that humic-like substances in digestate had an effect on soil AS. The complexity index for the *SPOM* fraction was intended to identify complex molecules corresponding to dissolved humic- or fulvic-like substances, while the *SEOM* fraction from sequential extraction was expected to contain less soluble “humic-like” substances. Alkali solutions, similar to that used in the *SEOM* extraction method, have been used to characterise (Slepetiene et al., 2022) or extract (Guilayn et al., 2020) humic substances from digestates. However, while humic substances are implicated in the formation and stabilisation of soil aggregates (Rezacova et al., 2021), the exact nature of “humified” organic matter has been put under question in recent years (Lehmann and Kleber, 2015). It is believed that alkali extracted OM may simply be a diverse mixture of organic molecules such as lignin, proteins and lipids (Kelleher and Simpson, 2006). In the case of digestates, it is possible that the alkali extracted *REOM* and *SEOM* fractions consist mainly of proteins and other microbial products accumulated from microbial growth over the course of anaerobic digestion (Bareha et al., 2019). Regardless, the TOC and TN extracted in *SEOM* appear not to be strongly linked to *AUC*, as would have been expected if either humic-like substances or lipids were playing a role in protection or binding of soil aggregates. Although, linear correlation between the above variables (see Fig.6) did show a small correlation ( $p < 0.1$ ), and perhaps a more detailed characterisation could help to further understand the nature of this OM fraction and the interactions with soil AS.

Although humic-like substances from digestate may not have had an effect on soil AS, there was a correlation found between soil AS and the aggregate wettability as measured by the *WDPT*, which indicates that a modification in the hydrophobicity or the pore structure of soil aggregates occurred upon amendment with digestate. This hydrophobic effect could be attributed to other amphiphilic components in the digestates (Voelkner et al., 2019), that have already been found to affect soil AS following amendment with manures (Paré et al., 1999).

Many of the digestates in this study, due to their high concentration in mineral matter, had a strong impact on soil pH and EC, but it was not possible to ascertain if this had a subsequent significant effect on soil structure. Certainly, the interaction between soil electrolyte and soil particles leading to formation of stable aggregates is a complex area of research beyond the scope of this study. It is the case that many mineral rich digestates contained equal amounts of elements forming polyvalent cations (Fe, Al, Ca, Mg) and those forming monovalent cations (Na, K), such that the opposing interactions of the two on the soil lead to effects being cancelled out. The benefits of the added organic matter could also have outweighed any possibly negative effect of mineral rich digestates such as RD3 on soil AS, but investigation of such complex opposing interactions would require a much larger dataset than had been obtained for this study.

#### 4.4 Conclusions

Detailed analysis of a number of digestates, coupled with incubation trials and monitoring of several key soil properties has shown that the variability in their impact on soil aggregate stability may well be due to differences in OM characteristics affecting different mechanisms of aggregate stabilisation. The advances made in this study suggest that further research could be focussed on characterisation of easily extracted OM fractions in order to establish a direct link between soil aggregate stabilisation and specific soluble organic components of digestate. This research also opens the possibility for developing indicators for rapid assessment of the amendment value of digestates produced under a

wide variety of process conditions prior to soil application. Consequently, it may be feasible to adapt agricultural anaerobic digestion processes to optimise the organic amendment value of the produced digestate, for example, by preferential production of higher concentrations of readily extractible OM fractions. Certainly, other considerations should be taken into account at the same time, such as the effects digestate optimisation would have on biogas production, the biological stability of the digestate, and the fate of increased soluble matter in digestate following field spreading in terms of pollution and phytotoxicity.

## 5 Short-term dynamics in aggregate stability following field injection of digestate on a temperate climate, silt loam soil

The incubation trials reported in the two previous chapters formed the core of our research into the impacts of digestates on soil AS as they could be observed under strictly controlled conditions and using high application rates to increase the measured response. However, one intention from the outset of the project had been to test how these effects translated to field conditions, and therefore validate their applicability in this context. With the participation of one of the farmers and AD plant managers from whom digestate had been previously obtained (the 'DCS' in chapter 2), a field trial was organised on a parcel of their land and using the digestate produced on site. The following chapter is a copy of an article intended for submission to the journal "Soil Science Society of America Journal".

# Short-term dynamics in aggregate stability following field injection of digestate on a temperate climate, silt loam soil

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## ○ Abstract

**Purpose.** The impact of digestates produced from anaerobic digestion (AD) on soil physical health may vary, depending on the agricultural practices used and the contexts in which they are incorporated. However, there is still insufficient published research on the short-term impacts of digestate application on soil structural stability under field conditions.

**Methods.** In a field trial using full-scale farming equipment, and a raw liquid digestate from agricultural AD, a complete randomised block experiment was devised with the following treatments: No digestate (D0), digestate injection at 25 m<sup>3</sup> Ha<sup>-1</sup> (D25) and digestate injection at 50 m<sup>3</sup> Ha<sup>-1</sup> (D50). Dynamics in soil aggregate stability (MWD), soil moisture content (SMC), total carbon (TC), dissolved organic carbon (DOC), mineral nitrogen (Nmin) and electrical conductivity (EC) were monitored at several dates over 8 months of cultivation of a winter oilseed rape crop.

**Results.** Significant differences ( $p < 0.05$ ) between treatments D50 and D0 were observed one day after digestate injection, though these effects rapidly disappeared and no differences were observed between treatments for the rest of the trial. Linear correlation between monitored variables showed that high soil DOC, EC and Nmin, indicative of the presence of high concentrations of digestate, were

linked to elevated MWD directly after injection. Seasonal dynamics in soil aggregate stability, probably due to varying SMC as a result of rainfall, were greater than the largest difference between treatments.

**Conclusion.** Well timed application of digestates in autumn to may help to protect soil structure over a period of deteriorating weather conditions. Since raw digestates are concentrated in available nitrogen, the small quantity of organic carbon introduced in an agronomic dose is quickly mineralised, meaning that positive effects on soil aggregate stability may be minimal and short-lived.

**Keywords.** Anaerobic digestion, N dynamics, Organic amendment, Soil structure, Soil organic carbon

- Statements and declarations

**Data availability.** Datasets generated during and/or analysed during the current study are not publicly available, but can be obtained from the corresponding author upon reasonable request.

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**Conflicts of Interest.** The authors have no relevant financial or non-financial interests to disclose.

## 5.1 Introduction

Digestate produced from anaerobic digestion has great potential for use as a rich fertilizer that can be produced on-farm from indigenous and imported waste streams, thus improving nutrient cycling and farm autonomy. Their content in organic carbon (OC) also promises benefits from their use in terms of augmenting soil organic carbon (SOC) stocks and improvement of soil physical quality, though their full impact on soil health is still in need of further research (Nkoa, 2014; Rezacova et al., 2021).

One useful method for assessment of soil physical health, in terms of its' resistance towards the formation of surface crusts and soil erosion, is the measurement of the aggregate stability (AS) (Barthès and Roose, 2002). The soil AS can be used in the assessment of the impact of changes in agricultural practices on the soil (Le Guillou et al., 2011; Plante and McGill, 2002; Rezacova et al., 2021). The changes in soil chemistry, organic matter and biology that occur following organic amendment may generate a dynamic response in soil AS (Amézqueta, 1999; Bronick and Lal, 2005), particularly over the course of a growing season (Abiven et al., 2007; Sarker et al., 2018). Despite this, few studies have investigated the effect of digestate on soil physical properties (Möller, 2015), and even fewer have monitored their effect on dynamics in soil AS over the short-term (Albuquerque et al., 2012b).

In-laboratory testing has shown that soil AS can follow relatively complex dynamics over six months following amendment with digestate (Cooke et al., 2023), albeit at an elevated dose ( $6 \text{ g}_C \text{ kg}_{\text{soil}}^{-1}$ ). Given the high nutrient content and low dry matter content of liquid digestates, the quantity of OC introduced to the soil at agronomic application rates is frequently low, and so observed effects on soil physical properties may be minimal (Bhogal et al., 2018). In the case of other types of organic amendment, a reduced dose will subsequently reduce the observed effect on soil AS (Özdemir et al., 2007), though not necessarily following a linear relationship. Addition of high concentrations of mineral nitrogen may further attenuate the impact on soil AS, at least in the case of high C-N ratio organic amendments (Le Guillou et al., 2011). The introduction of monovalent cations to the soil, including ammonium ( $\text{N-NH}_4^+$ ) may cause aggregate dispersion (Whalen and Chang, 2002). This could mean that  $\text{N-NH}_4^+$  rich digestates will induce very little change soil AS, or even have a negative effect, despite increasing SOC. However, this should be weighed against the benefits of digestate application such as enhanced soil fertility and improvement to crop development (Albuquerque et al., 2012b), which may in turn have a positive impact on soil AS. Furthermore, under field conditions, the effects resulting from changes in climate (Bottinelli et al., 2017), may be greater than the effects resulting from the agricultural practice being studied. Therefore, it remains to be seen if soil AS dynamics similar

to those observed under controlled conditions can be observed in-field following digestate amendment at agronomic application rates.

The objective of the field trial reported here was to study the short-term dynamics in AS of a cultivated silty loam soil following injection of anaerobic digestate at two application rates. We also aimed to take into account the spatial variability of the AS due to other uncontrolled factors such as soil disturbance, climatic variations, and in-field soil heterogeneity.

## 5.2 Materials and methods

### 5.2.1 Experimental setup

The study was carried out between the end of August 2021 and the end of March 2022 at a site located in the commune of Gévézé, Brittany (48°14'22.2"N 1°50'22.9"W). The soil is a brunisol-luvisol containing 15.2% clay, 24.1% fine silt, 52.9% coarse silt, 6.9% fine sand, 0.9% coarse sand, and 2.13% organic matter, with a pH of 6.15, a potential CEC of 7.03 cmol kg<sup>-1</sup>, and an average bulk density of 1.30±0.08 g cm<sup>-3</sup>. The clay mineralogy is dominated by illite-mica (muscovite), chlorite and kaolinite.

The field was planted with oilseed rape (*Brassica Napus*) that formed part of a maize-wheat-cover crop rotation that had been in place for at least 10 year previously. Prior to the trial, winter wheat planted in the autumn of 2020 was harvested in August 2021. Raw digestate was applied to the field by injection (depth 5 cm) followed by mouldboard ploughing (depth 20-25 cm) on the 2<sup>nd</sup> September 2021, and seed bed preparation plus sowing of the oilseed rape was performed a week later. An herbicidal treatment was applied a week after to control broad-leaf dock (*rumex obtusifolus*) development. The entire field received the same level of intervention (sowing density, ploughing, chemical treatment, etc.). There were no further interventions until the crop was harvested in June of the following year.

The experimental set up was of a complete randomised block design (CRBD) covering a surface 2700 m<sup>2</sup>, comprising nine strips (12 m wide by 25 m long) receiving one of three different treatments: D0 -

No digestate, D25 - Injection of 25 m<sup>3</sup> Ha<sup>-1</sup> digestate (= 165 kg<sub>N</sub> Ha<sup>-1</sup>), or D50 - Injection of 50 m<sup>3</sup> Ha<sup>-1</sup> digestate (= 330 kg<sub>N</sub> Ha<sup>-1</sup>). A CRBD was selected over a fully randomised plot design as a small gradient in soil characteristics (soil moisture content, SOC) perpendicular to the blocks was observed from analysis prior to the field trial. The orientation and size of the blocks was chosen to minimise any disruption of the farmer's interventions. The injection rig was passed over the strips receiving D0 treatment but without injecting any digestate, so that all parts of the field underwent a similar amount of soil disturbance.

### 5.2.2 Digestate sampling and characterisation

The digestate was produced at a nearby on-farm anaerobic digester, comprising a primary and secondary digester operating as a continuous stirred tank configuration at mesophilic temperatures (38°C), and an uncovered slurry pit at ambient temperature. The digester is fed daily with a mixture of cow slurry, manure, turkey manure, and various agro-industrial wastes, by hopper conveyor. The hydraulic retention time in anaerobic digestion, including storage, was at least 60 days at the time of sampling of the digestate.

The total solids (TS) content of digestate was determined by weighing before and after drying for 48 h at 105°C, and the volatile solids (VS) content by weighing before and after combustion at 550°C for 5 h. Total carbon (TC) and total nitrogen (TN) was analysed on air dried samples by combustion method using a Thermo Scientific Flash<sup>®</sup> 2000 CN analyser, as per manufacturer's instructions. The total kjeldahl nitrogen (TKN) was obtained by steam distillation of samples following mineralisation in strong acid, as per standard methods (APHA, 2005). A solution was obtained by mineralising the ashes resulting from the above-described combustion in a mixture of HCl (37%) and HNO<sub>3</sub> (65%) followed by digestion in an Anton Paar<sup>®</sup> HVT50 Microwave Reactor. Total phosphorus (TP) and potassium (K) in the solution were determined by spectrophotometry using a ThermoFisher Gallery<sup>®</sup> Discrete Analyser, and by microwave plasma-atomic emission spectrometry using an Agilent<sup>®</sup> 4200 MPAES, respectively, as

per manufacturers' instructions. The results of physico-chemical characterisation of the digestate are summarised in Table. 5.1. The digestate is typical of a wet raw digestate from animal effluents, with a low C/N ratio and a high percentage of N-NH<sub>4</sub><sup>+</sup> in the nitrogen fraction. While the application rates used were governed by the nitrogen content of the digestate, the estimated amount of carbon added to the soil was calculated and found to be equivalent to 0.7 and 1.4 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> for D25 and D50, respectively.

*Table. 5.1. Digestate characteristics and equivalent quantity of applied material at the two application rates used in the trial*

<b>Characteristic</b>	<b>Concentration (g kg<sub>FM</sub><sup>-1</sup>)</b>	<b>Application rate (kg Ha<sup>-1</sup>) at 25 m<sup>3</sup> Ha<sup>-1</sup> (D25)*</b>	<b>Application rate (kg Ha<sup>-1</sup>) at 50 m<sup>3</sup> Ha<sup>-1</sup> (D50)*</b>
TS	89.3 ± 0.1	2232	4464
VS	60.5 ± 0.2	1514	3027
TC	29.5 ± 0.3	737	1475
TKN	6.6 ± 0	165	330
N-NH <sub>4</sub>	4.2 ± 0	106	211
TP	1.7 ± 0.1	42	84
K	3.3 ± 0	83	167
C-N ratio**	4.5	-	-
pH	8.4	-	-

\*Application rate in kg Ha<sup>-1</sup> calculated based on soil bulk density = 1.3 kg m<sup>-3</sup> and tillage depth = 0.25 m, \*\*C-N ratio =TC/TKN

### 5.2.3 Soil sampling and analysis

Soil sampling was carried out on the 1<sup>st</sup> September 2021 (T0), the day before digestate amendment, then on 3<sup>rd</sup> September (T1), 15<sup>th</sup> September (T2), 7<sup>th</sup> October (T3), 26<sup>th</sup> October (T4), and 24<sup>th</sup> November (T5), that correspond approximately to the length of the vegetative phase of crop development. A final sample was taken on 28<sup>th</sup> March in 2022 (T6) following oilseed rape flowering. The centreline perpendicular to the long axis of each block was determined by GPS, and two samples taken from approximately 3 m either side of this centreline in order to avoid the tyre tracks left by the tractor passage (see supplementary information in Appendix 3 for detailed sampling plan). Samples were stored and analysed separately so that spatial variation in soil properties, regardless of the treatment or block, could be observed. Fresh sampled soil was either stored at 4°C (for up to one week)

before treatment, or else air-dried depending on the analysis. Soil for aggregate stability analysis was gently broken up along lines of weakness, left to air-dry, then sieved to obtain aggregates of 3-5 mm.

Soil moisture content ( $SMC = \frac{g_{\text{water}}}{g_{\text{dry soil}}}$ ) was analysed by weighing of samples before and after drying at 105°C for 48 hours. Soil mineral nitrogen (N<sub>min</sub>) in the form of nitrate (N-NO<sub>3</sub><sup>-</sup>), nitrite (N-NO<sub>2</sub><sup>-</sup>), and ammonium nitrogen (N-NH<sub>4</sub><sup>+</sup>) was determined on a solution, obtained by extraction of 25 g of fresh soil in 50 mL 2M KCl for 1 hour, using a Thermo Fisher Gallery® Discrete Analyser, as per manufacturer's instructions. Dissolved organic carbon (DOC) content of the same solution was obtained by combustion method using a Shimadzu TOC-L analyser, though data was only obtained for sample dates T0-T3 due to an equipment failure during the experiment.

Soil aggregate stability was analysed using the "slow-wetting" method established by (Le Bissonnais, 1996), selected as being the most adapted of the three tests for silt rich soils that are usually highly unstable (Algayer et al., 2014). Around 5 g of 3.15-5 mm aggregates, pre-dried for 24 hours at 40 °C, were gently rewetted by placing on a pre-soaked sponge until saturated with water. The aggregates were then briefly agitated on a fine 250 µm sieve, and the remaining soil was dried at 40 °C for 24 hours before dry sieving to various size fractions ranging from 0.05 to 2 mm. The mean weight diameter (MWD) was calculated by taking the sum of the mass of soil remaining on each sieve multiplied by the mean aperture of adjacent meshes, in a similar manner to Kemper and Rosenau (1986).

#### 5.2.4 Statistical analysis

All statistical analysis was carried out using "R" software (R Core Team, 2021). Normal distribution of data in different classes was tested using the Shapiro-Wilk normality test, and found to be significantly non-normal ( $p < 0.05$ ) at several dates across several treatments. Therefore, it was decided to use non-parametric analysis of variance using the R "stat" and "PMCMRplus" packages (Pohlert, 2022). Replicate data for each block and treatment for each date were combined to give a mean value in order to allow use of Quade's test for unreplicated blocked data to evaluate significant differences

between treatments, and Quade's post-hoc test for multiple comparisons of rank sums. Similarly, Friedman's non-parametric test was used to evaluate significance across sample dates, with post-hoc testing using Nemenyi's all-pairs comparison method. Correlation matrices were constructed using the "psych" package (Revelle, 2022), with Pearson's correlation coefficients estimated from pairwise comparison of variables, and  $p$ -values adjusted using Holm's method. The correlation matrix was visualised using the "corrplot" package (Wei and Simko, 2021).

## 5.3 Results

### 5.3.1 Dynamics in soil aggregate stability

In Fig. 5.1(a), a boxplot is shown giving the measured MWD for samples grouped by treatment and sample date. Prior to digestate application (T0), a high variability in soil AS was observed ( $0.6 < \text{MWD} < 1.2$  mm), though there was no significant difference between samples from the different treatment plots. Sampling one day after digestate spreading and ploughing (T1), the MWD of soil from the D50 plots was found to be significantly higher relative to the control D0 ( $p = 0.088$ ), while the MWD of soil from the D25 plots was also slightly higher but not significantly different from D0. This is similar to (Pagliai et al., 1981), who observed a significant rise in soil AS only for the higher of two application rates of anaerobically treated sewage sludge. Although the differences between the mean MWD of soil receiving different treatments at T0 and T1 were not significantly different, the MWD in the control plots (D0) at T1 was slightly lower than at T0. At T2 (mid-September), D0 was again significantly lower than measured at T0 ( $p < 0.05$ ). At the sample date, the soil MWD of D25 and D50 had also reduced, and no significant differences were found between treatments. At T3, only the soil MWD for D25 was significantly different to the other treatments, while the MWD for D0 was at its lowest level at this sample date (approx. 0.57 mm). There was a slight increase in the MWD of soil sampled from all treatments, including D0, at T4. However, at this date and at all dates afterwards, the MWD of soil sampled was not found to be significantly different ( $p > 0.05$ ) between treatments.

### 5.3.2 Dynamics in soil moisture content and mineral nitrogen concentration

Soil moisture content (SMC) for samples from each treatment and at each sample date have been plotted in Fig. 5.1(b). There were no significant differences in the SMC for the three treatments at any given date. Though not measured directly on the soil, the water holding capacity was estimated by applying soil physical properties to a suitable pedotransfer function (Pollacco, 2008) to be  $0.286 \pm 0.01 \text{ g g}_{\text{dry soil}}^{-1}$ . From T0 to T2 the SMC was relatively constant, and then rose over the winter to close to the estimated water holding capacity between T3 and T5, before dropping back down to a slightly lower SMC in spring at T6. This is consistent with the seasonal climate of Brittany region, where winters are wet, and soil is frequently close to saturation.

The dynamics in soil mineral nitrogen content  $N_{\text{min}}$  ( $=\text{N-NO}_3^- + \text{N-NO}_2^- + \text{N-NH}_4^+$ ) of the soil (0-20 cm) represented in Fig. 5.1(c), shows a strong peak at T1 for samples taken from D50. This is as expected given the high rate of application of mineral nitrogen rich digestate. A maximum  $N_{\text{min}}$  of  $0.145 \text{ mg g}_{\text{dry soil}}^{-1}$  was measured. For reference, an application rate of  $330 \text{ kg}_N \text{ Ha}^{-1}$  is equivalent to  $0.127 \text{ mg}_N \text{ g}_{\text{dry soil}}^{-1}$ .  $N_{\text{min}}$  concentrations reduced quickly in the 0-25 cm soil profile in the month after digestate application, particularly for the D50 plots. This was most likely due to leaching or volatilisation, as observed plant development was still minimal over this time. It was noticed upon ploughing the field (one day after T0), that some maize residues remaining in the lower soil profile (25cm) had been brought up by the plough. This may have contributed sufficient labile C to increase microbial activity and lead to some N immobilisation directly following digestate application (Johansen et al., 2013).

After T3, there were only small differences between the soil treated with digestate D50 and the D0 control. The  $N_{\text{min}}$  of the D0 control also increased slightly from T1 to T2, before gradually decreasing. This could have either been due to liberation of  $N_{\text{min}}$  from decomposing plant residues from the previous crops, or from N transport from the rest of the field, though this was not possible to prove.

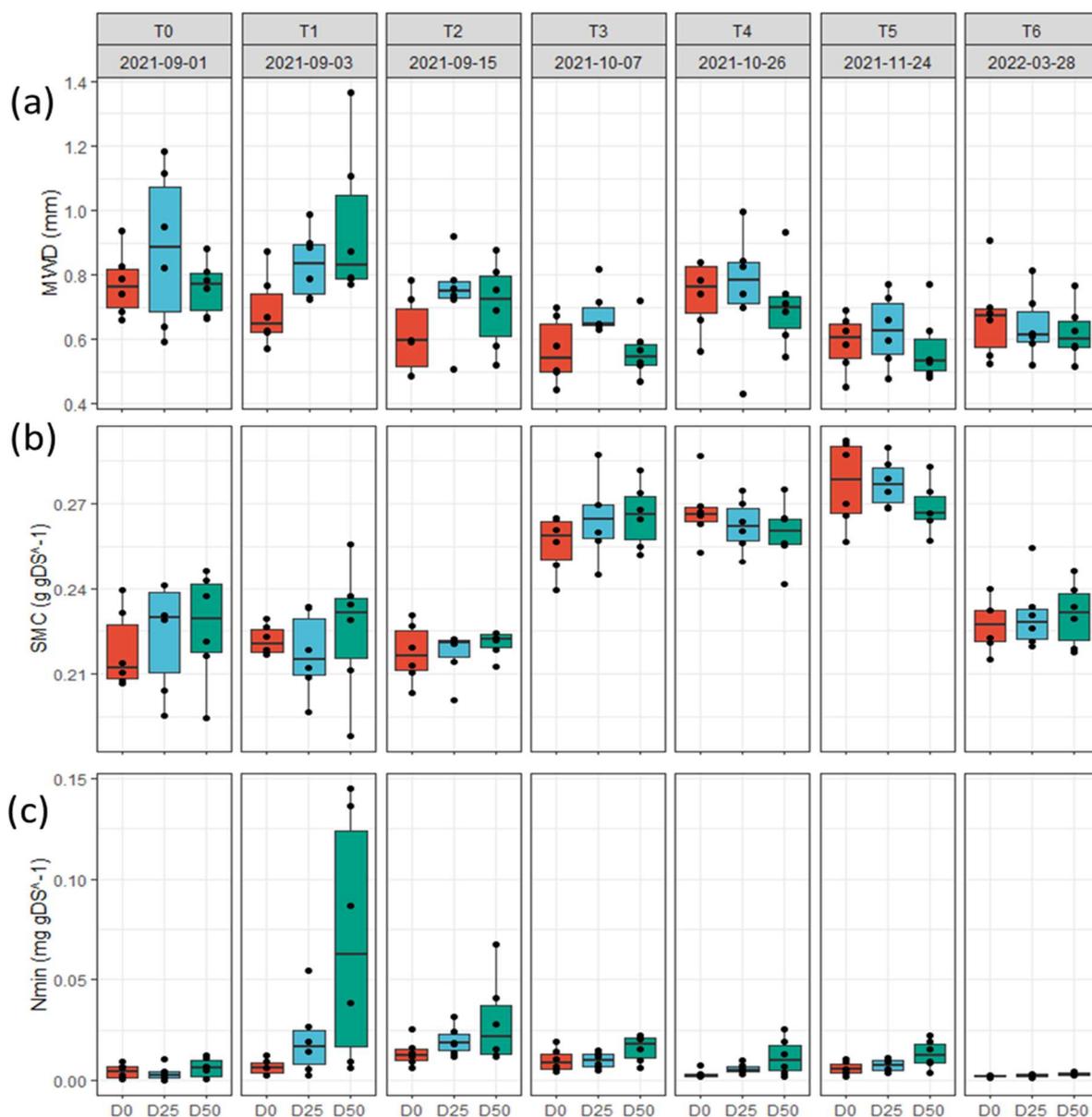


Fig. 5.1 Boxplots of the measured dynamics in a) mean weight diameter (MWD), b) moisture content (SMC), and c) mineral nitrogen (Nmin) of field soil receiving three different digestate treatments (D0, D25, D50) taken across seven sample dates from September 2020 to March 2021

### 5.3.3 Comparison of spatial variation in soil characteristics

The significant ( $p < 0.05$ ) correlations between different soil properties are represented by the correlation matrices in Fig. 5.2, for samples from all samples dates (Fig. 5.2(a)) and from the only date where statistically significant differences in MWD were observed between treatment plots at T1 (Fig. 5.2(b)). Significant correlations were observed between the soil MWD and soil total carbon (TC),

dissolved organic carbon (DOC), ammonium nitrogen ( $\text{N-NH}_4^+$ ), conductivity (Cond) and pH for data from all sample dates, though all correlations were stronger when only taking into account the samples from T1. Representation of the correlated data by the scatter plots shown in Fig. 5.3 revealed that the significant correlation between MWD and these soil properties was due to those samples containing the highest amount of digestate under treatment D50. Several samples taken from D25 and D50 did not contain high concentrations of DOC or  $\text{N-NH}_4^+$ , which would suggest that the soil sampled, due to the highly heterogeneous real-world conditions, had not received digestate. A lack of digestate present in these samples would explain why the MWD was also low, and at a level comparable to the control soil in D0 plots. Across the whole data set for all sample dates, there was also a small but significant negative correlation found between the MWD and the soil moisture content (SMC) at sampling ( $r = -0.22$ ), as well as the concentration in total oxidised nitrogen  $\text{N-NO}_x (= \text{N-NO}_2^- + \text{N-NO}_3^-)$ .

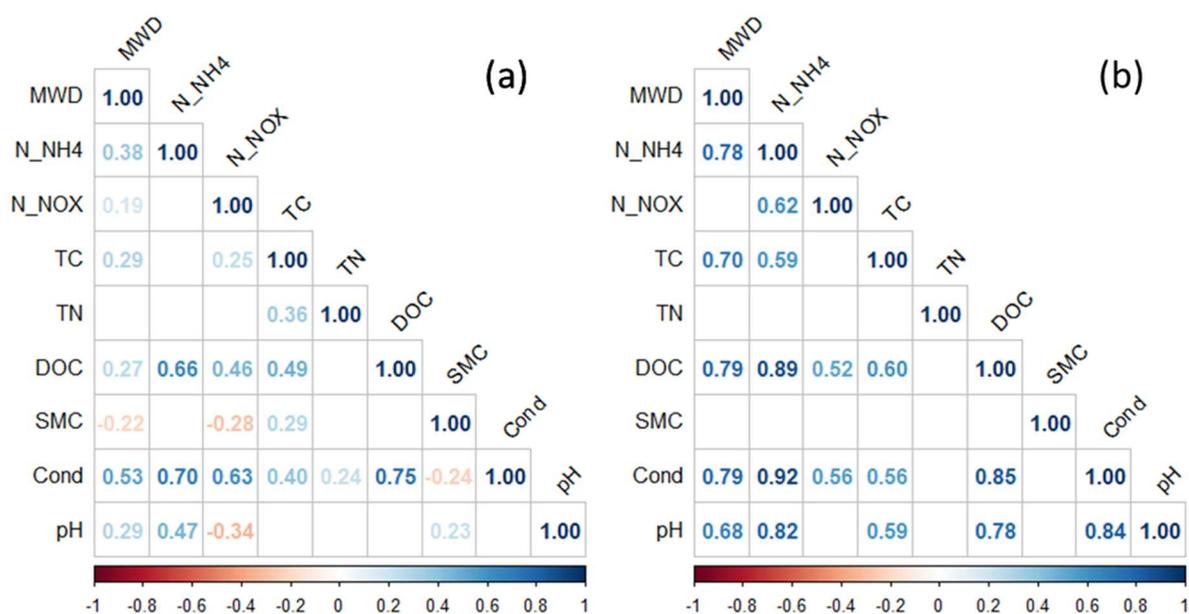


Fig. 5.2 Correlation matrix of soil characteristics measured a) for all sample dates (1, 22, 35, 54, 83, and 207 days after digestate injection) and b) for samples taken at T1 (1 day after injection). MWD = Mean weight diameter,  $\text{N\_NH}_4$  = Ammonium nitrogen,  $\text{N\_NO}_x$  = Total oxidised nitrogen ( $\text{NO}_2^- + \text{NO}_3^-$ ), TC = Total carbon, TN = Total nitrogen, DOC = Dissolved organic carbon, SMC = Soil moisture content, Cond = Conductivity. Only significant ( $p < 0.05$ ) corrected Pearson's correlation coefficients are shown.

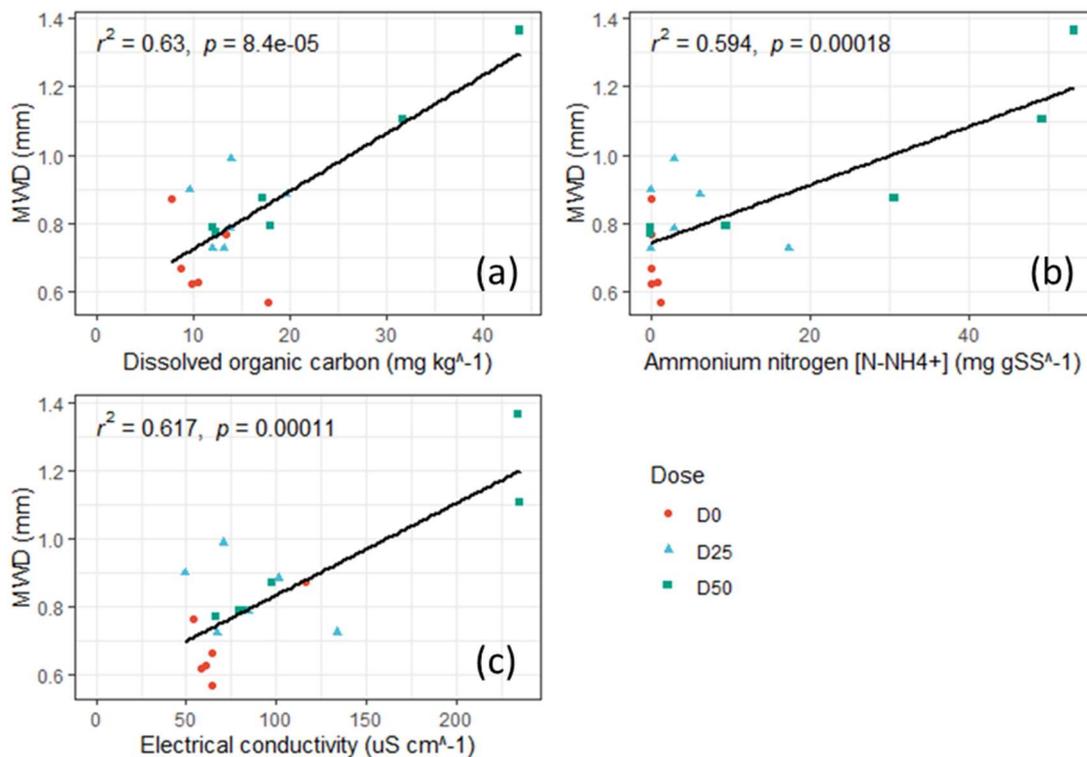


Fig. 5.3 Scatter plots of soil MWD vs. (a) Dissolved organic carbon (DOC), (b) Ammonium nitrogen ( $N-NH_4^+$ ), and (c) Electrical conductivity, sampled just after digestate injection (1 day). In the top left of each plot are given the  $r^2$  and  $p$ -value for the linear regression traced by the black line.

## 5.4 Discussion

### 5.4.1 Effect of digestate spreading on soil aggregate stability

According to the soil stability and crustability classification proposed by Le Bissonais (1996), the majority of sampled field soil could be classified as “unstable” ( $0.4 < MWD < 0.8$ ), meaning this soil is at a high risk of runoff and erosion. For the soil at T1 having received digestate (D25 and D50) where the average MWD was slightly higher, the soil was elevated to a “medium” stability classification ( $0.8 < MWD < 1.3$ ), indicating only a moderate risk of runoff and erosion dependent on climate and topography. Analysis using non-parametric statistical tests revealed only slight statistical ( $p=0.088$ ) evidence for an increase in soil MWD at T1 on the D50 plots receiving the highest dose of digestate relative to the control soil D0. However, the MWD of soil in the digestate treated plots was highly heterogeneous, and it was uncertain if this result was purely a result of uncontrolled factors (e.g.

historical differences in soil management, or inherent heterogeneities in soil properties). Further analysis showed significant positive correlations ( $p < 0.05$ ) between MWD and soil mineral N, DOC and EC (see Fig. 5.3). It was most likely that an increase in these soil properties was due to the presence of digestate in the sample. Thus, where soil fell in contact with digestate following application, a stabilisation of soil aggregates occurred shortly afterward (1 day).

Given the short time interval between digestate application and the observed increase in MWD at T1 (1 day), evidence would point to a direct, abiotic stabilisation of aggregates. Raw and liquid digestates have been shown to have an immediate and direct impact on soil aggregates (Cooke et al., 2023) under controlled conditions. Direct coating of soil aggregates by digestate leads to increased resistance to water penetration (Voelkner et al., 2019). Organic fertilisers such as compost and digestate are also believed to increase soil humic substances that directly improve aggregate cohesion (Rezacova et al., 2021). However, the indirect influence of microbial activity on aggregate stabilisation at this time interval may not entirely be ruled out. A rapid but transient aggregate stabilising effect has been observed in other field trials with application of low C-N ratio raw digestates (Alburquerque et al., 2012b; Frøseth et al., 2014), where this effect was attributed to stimulation of soil biological activity and subsequent deposition of microbial exudates. Regardless of the exact mechanisms of aggregate stabilisation involved, the transient nature of the increase in MWD at T1 is most likely due to microbial activity rapidly consuming organic binding agents (Abiven et al., 2007; Tisdall and Oades, 1982). The DOC was at its highest in D50 soil at T1, but by T2 the soil DOC was found to have dropped to levels similar to those prior to digestate injection (data not shown), in line with the changes in soil AS. The DOC in the soil is considered a labile OC pool, and has previously been correlated with increased MWD following organic amendment with plant residues (Zhang et al., 2021).

Despite the difference between treatments at T1, there was no observable difference in the average MWD from T0 to T1. Disruption of soil aggregates from ploughing reduces the structural stability of

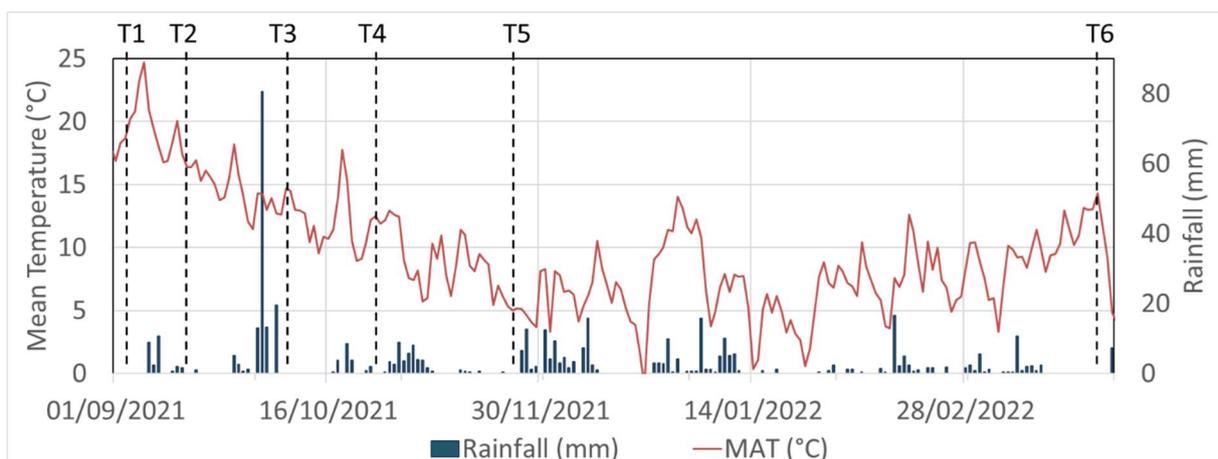
the soil (Plante and McGill, 2002), which would explain the marginal reduction in MWD for the D0 plots from T0 to T1. Application of digestate OM in sufficient quantity may have counteracted this effect for D25 and D50 treatments through stabilisation and protection of soil aggregates. For the D50 treatment at this date, several samples display very high MWD, while for several others the MWD remains comparable to samples from the same plots at T0. It is clear from the spread of results from different analyses that, under field conditions and using full-size agricultural equipment, digestate is not homogeneously mixed into the soil and so regions develop where impacts on soil physical properties may be elevated compared to the surrounding soil.

The depth of ploughing (20-25 cm) used during this trial also increased the distribution of digestate in the soil profile. Indeed, in a no-till system, the digestate would have been only injected in the first 5cm of soil, and the digestate concentration in this profile would have been five times higher. The highest digestate application rate (D50) would not normally be used in agriculture due to the excessive rate of N added ( $330 \text{ kg}_N \text{ Ha}^{-1}$ ). However, this treatment provides some insight into the potential effect of reduced tillage where more digestate would be concentrated into a smaller soil profile. Furthermore, it may be assumed that the proven positive impacts of reduced tillage on soil AS (Abid and Lal, 2008; Bottinelli et al., 2017) coupled with digestate application would lead to even higher observed MWD.

Comparison of MWD before and after digestate treatment was complicated by the high variation in values across the trial plot at T0 (0.57 to 1.18 mm), which meant that statistical analysis found no significant difference between MWD for soil in the various treatment plots between T0 and T1. The wide range of MWD was assumed to be due to differences in fertilisation and crop development from the year before, and significant correlations were found between MWD, soil TC and conductivity at this date (data not shown). An alternative hypothesis is that some areas where soil had been sampled might have been subjected to repeated vehicle passage during the previous harvest that weakened the soil structure (Barik et al., 2014).

## 5.4.2 Impact of climatic conditions on soil MWD

The differences in the MWD of the control soil from its highest in September (= 0.77 mm at T0) to its lowest in October (= 0.57 mm at T3) was much greater than the largest difference in mean MWD between treatment plots at any given date. The small but significant correlation between soil moisture content (SMC) and the MWD shown in fig. 4 would confirm that the saturation of the soil from T3 onwards had a negative impact on soil AS. This is in agreement with the findings of Perfect et al. (1990), who were able to explain a considerable amount of the seasonal variations in soil AS in terms of the soil moisture content. However, in the current study, the MWD varied between T3 and T5 varied seemingly independent of the soil moisture content, which remained constant. It was decided to draw on external weather data from a nearby weather station (located 4 km from the site) in order to attempt to account for some of the variation caused by seasonal changes and occasional weather events. The daily mean air temperatures (MAT, °C) and rainfall (mm), from the start to the end of the trial are shown in Fig. 5.4.



*Fig. 5.4 Daily rainfall and mean air temperature data from a local weather station (Source: Coopérative Agricole Le Guessant, 2023) covering the field trial period. Vertical dashed lines indicate the soil sampling dates.*

Comparison of the weather data at the sample dates with data from soil analysis revealed several correspondences between the trend of climatic data and the changes in the soil MWD. The *MAT* followed a gradual decline that matches the decline in MWD up to the last sample date of 2022 at the end of November. Reduced soil temperatures lead to reduced soil microbial activity (Rodrigo et al., 1997), and low temperatures coupled with rainfall have been directly correlated with measures of soil AS (Dimoyiannis, 2009).

Episodes of rainfall at the beginning and end of September prior to sampling may explain the decline in average MWD across all treatments between T1 and T2, and T2 and T3. Despite the trend of higher rainfall and lower temperatures along with declining soil AS towards the end of 2022, there was a relatively warm and wet period followed by a short cold and dry period just before T4 at the end of October. This may explain why the average soil MWD of samples was slightly higher at T4 than at T3. Despite the rising temperature from February to the end of March, the MWD was not greatly increased at T6, possibly due to a lag between soil biological activity and improving climatic conditions. Algayer et al. (2014) experienced similar difficulty in explaining all variations in short-term soil AS dynamics, despite finding a strong correlation with soil moisture content and the hydric history of the test site.

#### 5.4.3 The interaction between digestate application, climatic conditions and soil conditions on aggregate stability

As already discussed, the lack of differences in MWD between treatments from sample date T2 onwards was possibly due to rapid mineralisation of the added OM and a return of all treatments to an approximately similar state. Albuquerque et al. (2012a) noted that certain digestates may still contain high concentrations of labile C, which leads to rapid C mineralisation in incubation with soil under controlled conditions. High emissions of CO<sub>2</sub> in the days following digestate spreading has also been noticed in field conditions on sandy soil in late summer (Czubaszek and Wysocka-Czubaszek, 2018). Abiven et al. (2008) proposed that, due to the more favourable soil conditions for rapid

decomposition of the additional organic material (high soil moisture content and soil biological activity), organic amendments applied later in the year would generally lead to a more rapid but brief increase in soil AS than application in the spring. For the present field trial, as the average soil moisture content remained close to or at the estimated soil water holding capacity for the whole trial period, conditions would have been optimal for C and N mineralisation (Rodrigo et al., 1997). The contribution of digestate to stable SOC stocks should not be overestimated, particularly in the case of different soil conditions (Badagliacca et al., 2020) and digestate types (Cattin et al., 2021). However, increasing stable SOC should not be the only objective in organic amendment, and labile C is useful in maintaining healthy soil physical functions such as AS (Chaplot and Cooper, 2015).

## 5.5 Conclusion

Heterogeneity of the soil at a field scale, coupled with shifting climatic conditions makes observation of subtle changes in soil aggregate stability under field conditions difficult. The application of digestate at two rates, equivalent to  $165 \text{ kg}_N \text{ Ha}^{-1}$  and  $330 \text{ kg}_N \text{ Ha}^{-1}$ , did not have significant negative impacts on soil aggregate stability over the course of 8 months, but appear to have improved the soil aggregate stability for a very brief period following amendment. It is unlikely that the positive impact was due to stimulation of biological activity given the very short time from spreading, but rather appears to be linked to a direct coating and stabilisation of soil aggregates in contact with digestate. This effect was also highly dependent on the quantity of digestate in contact with the soil, and the heterogeneous application and mixing of digestate by conventional farming equipment led to concentration of digestate in specific spatial regions where impacts on soil structure were consequently elevated. Seasonal variations in MWD, linked mainly to rainfall and soil moisture content, were greater than the variation that could be attributed to the effect of digestate application. Timely soil amendment with a similar raw digestate could be used to counteract negative effects on the soil structure from ploughing and exposure of bare soil.

## 6 Interaction between digestate and soil types on soil aggregate stability dynamics

Up to this point of the manuscript and the research reported therein, study had been focussed on the importance of the origin of the digestate and its characteristics, and their impact upon application to silt loam soils. However, from our bibliographic research, summarised in Table. 1.1, it was apparent that the soil properties could be of equal or greater importance in determining the response in AS as the digestate properties. For the final phase of experimentation conducted during this PhD project, it was decided to incubated digestates with other types of soil sampled from experimental sites in Brittany. Several of the digestates sampled and tested (namely CS7, RD8 and RD9) and two of the soils tested (EFELE, and KERGUE), form part of longer-term field trials investigating the impact of various organic amendments on soil properties including AS. Though not possible to discuss this in the current document, it is intended that the data gathered during our experiments might one day provide useful information for interpreting the findings of these long-term trials.

## 6.1 Introduction

Soil organic amendment with digestate has been shown to have varying effects on the AS of different soils types (Badagliacca et al., 2020; Rivier et al., 2022; Sarker et al., 2018). Recent research has shown that digestates with differing characteristics can even have contrasting effects on AS of the same soil, and this has led to the establishment of a first typology of impact of digestate on soil AS (Cooke et al., 2023). However, to our knowledge, no studies to date involve testing of multiple different digestates incorporated into different soils, and so it remains to be seen if the typology holds true for soil types other than the reference soil used (a silt loam).

It is believed that soils of different texture and mineralogy will react differently to organic amendment due to the variability of interaction between soils clays, dissolved ions and organic matter (Amézqueta, 1999; Emerson, 1967; Six et al., 2000a). Soil texture and quality appears to influence the soil biological response to digestate amendment in terms of the microbial community dynamics (Panuccio et al., 2021), as well as the relative effectiveness of the biotic and abiotic aggregate binding agents responsible for soil aggregate stabilisation (Badagliacca et al., 2020; Zhang et al., 2021). In some cases, the incorporation of mineral rich organic wastes such as sludges and digestates may lead to dispersion of soil clays and disruption of soil aggregates (Mbagwu et al., 1991; Voelkner et al., 2015b).

Incubation trials were conducted, composing two other soils of differing origin (a loam and clay loam), and four digestates (representative of the four groups in the typology of impact, in order to:

- Investigate the interaction between the soil texture and the type of digestate amendment, and their combined effect on short-term soil AS dynamics,
- Determine whether the typology of impact established in our previous publication (Cooke et al., 2023) may be applicable to other soil textures.

## 6.2 Materials and method

### 6.2.1 Incubation protocol

In spring 2022, soils were sampled by spade to a depth of 30 cm, from agricultural fields located in Brittany, North-west France. Whole soil was stored at 5°C before preparation of aggregates. The aggregates, 3.15-5 mm in size, were obtained by dividing clumps of soil along lines of weakness followed by gentle sieving, and then stored at cool ambient temperatures before incubation. The characteristics of the soils are given in Table. 6.1. The soil KERGUE, a loam soil classified as a brunisol, was sampled from the Kerguéhennec Experimental Station of the “Chambre Régionale d’Agriculture de Bretagne” (47 °53’ N, 02°44’ W, Bignan, France). The soil NAIZIN, a clay loam classified as a humic cambisol, was sampled from a field located in the 12 km<sup>2</sup> watershed of a French Ministry for Research’s Observatory for Research on the Environment (48°00’ N, 02°49’ W, Kervidy-Naizin, France), part of the French network of agricultural watershed research (SOERE RBV - [http://www6.inra.fr/ore\\_agrhys](http://www6.inra.fr/ore_agrhys)). The soil EFELE, a silt loam soil classified as a luvisol-redoxisol, was the soil previously used in the incubation trials reported in chapters 3 and 4, sampled from the EFELE SOERE PRO field trial site (48°06’ N, 02°47’ W, Le Rheu, France). The results from previous incubation trials were combined with the results of the present trial to allow comparison between the three soil types.

Four agricultural digestates were selected, one from each of the four groups of the typology of impact (Cooke et al., 2023). These were:

- RD3 (Group 1) - a raw digestate of primarily cow slurry and dairy wastes,
- RD1b (Group 2) - a raw digestate of primarily cow/pig manure and crop residues,
- manure LP4 (Group 3) - a liquid phase from phase separation of a digestate of primarily maize cane and cow manure,
- CS7 (Group 4) - a composted solid phase of a digestate of sewage sludge and abattoir wastes.

Furthermore, two other raw digestates RD8, from AD of pig slurry, and RD9, from AD of a cover crop, were also incubated. However, in order to minimise the size of the experiment, these digestate were only incubated with EFELE or KERGUE soil, and samples only taken at three of the sample dates. The properties of all digestates are given in Table. 6.2.

*Table. 6.1 Properties of the soils tested in incubation trials*

<b>Nom</b>		<b>EFELE</b>	<b>KERGUE</b>	<b>NAIZIN</b>
Taxonomy		Luvisol-redoxisol	Brunisol	Humic cambisol
Clay (0 – 0.002 mm)	%	15	17	27
Silt (0.002 – 0.05 mm)	%	69	42	51
Sand (0.05 - 2 mm)	%	16	41	22
Organic matter	g/kg	15	32	48
N total	g/kg	1.0	1.7	2.7
CaCO <sub>3</sub>	g/kg	<1	<1	<1
COT	g/kg	8.7	18.7	28.0
C/N	-	8.4	10.9	10.5
pH (water)	-	6.9	5.6	6.3
Phosphorus (P) Olsen	g/kg	0.1	0.1	0.0
CEC Metson	cmol+/kg	6.4	9.3	10.9
Copper (Cu)	mg/kg	6.2	4.0	3.8
Iron (Fe)	mg/kg	284	158	105
Manganese (Mn)	mg/kg	50	31	16
Zinc (Zn)	mg/kg	6.3	2.5	2.3
Silicon (Si)	g/100g	0.0	0.0	0.0
Aluminium (Al)	g/100g	9.3	8.2	9.2
Calcium (Ca)	g/100g	1.4	1.9	1.5
Magnesium (Mg)	g/100g	0.7	1.1	0.8
Potassium (K)	g/100g	2.3	2.4	2.2
Sodium (Na)	g/100g	1.7	1.8	1.8

The same incubation protocol as had been used during the previous chapters was adapted to allow incubation of two other differently textured soils with four digestates. Briefly, digestates were mixed with prepared soil aggregates at a rate of 6 g<sub>C</sub> kg<sub>dry soil</sub><sup>-1</sup> and incubated in sealed containers in the dark at 20°C (and approximately 0.18 – 0.3 g g<sup>-1</sup> soil moisture content, depending on the soil) for 174 days.

Incubation containers were destructively sampled at days 0, 7, 42, 98, and 174, and soil either dried at 40°C for 24h then stored at 4°C, or stored humid at -20°C, ready for analysis.

*Table. 6.2 Physico-chemical characteristics of the incubated digestates*

<b>Characteristic</b>	<b>Units</b>	<b>RD1b</b>	<b>RD3</b>	<b>LP4</b>	<b>CS7</b>	<b>RD8</b>	<b>RD9</b>
TS	g kg <sub>FM</sub> <sup>-1</sup>	97	78	95	429	84	60
VS (OM)		584	600	519	518	631	695
TC		339	314	287	282	338	393
TKN	g kg <sub>TS</sub> <sup>-1</sup>	57.4	74.4	84.5	27.0	56.9	73.8
N-NH4		32.4	49.2	59.3	2.1	26.8	41.3
TC/TKN		5.9	4.2	3.4	10.4	5.9	5.3
pH		8.5	8.1	8.1	9.0	-	7.9
TP		19.4	30.1	16.2	12.0	18.2	7.2
TK		7.9	8.0	8.6	3.9	11.6	4.2
Ca		22.5	32.8	24.1	20.0	33.4	33.7
Mg		7.1	1.9	7.6	14.4	3.0	2.5
Na	g kg <sub>TS</sub> <sup>-1</sup>	29.3	37.7	18.6	2.1	21.5	3.2
Fe		6.5	19.7	7.0	18.1	5.5	3.3
Al		58.9	66.3	51.2	9.6	71.1	54.9
Zn		0.2	0.6	0.2	0.3	0.9	0.1
Cu		0.04	0.10	0.03	0.06	0.21	0.03

## 6.2.2 Analysis of incubated soil

Carbon mineralisation from the soil was monitored as per Cooke et al. (2023). Soil aggregate stability was analysed as per Le Bissonnais (1996) using the “slow wetting” and the “fast wetting” tests. The slow wetting test is well adapted to detecting changes in AS due to agricultural practices on fragile silt soils (Le Bissonnais and Arrouays, 1997), focussing on aggregate stability toward differential swelling free of slaking. For more clay rich soils that have a naturally higher AS and greater resistance to slaking, rapid wetting of aggregates as is performed during the fast-wetting test may provide insight into the mechanisms of aggregate stabilisation (Pituello et al., 2018).

Soil clay mineralogy was determined on clays samples placed on orientated plates by X-ray diffraction using a Bruker D8 Advance diffractometer. Clay samples were prepared by destruction of OM (through

immersion in 5 % hydrogen peroxide ( $H_2O_2$ ) at 40°C for one week), chemical dispersion in strong NaCl solution (4 hours), followed by sieving at 50  $\mu m$  and flocculation of clays in 0.5M  $CaCl_2$  solution.

## 6.3 Results and discussion

### 6.3.1 Soil aggregate stability dynamics following digestate incorporation to different soils types

#### 6.3.1.1 Comparison of aggregate stability dynamics of control soils

The results of analysis of aggregate stability dynamics for soils using the slow wetting test ( $MWD_{sw}$ ) are shown in Fig. 6.1. The average  $MWD_{sw}$  for the three soils was fairly different. The EFELE soil for this incubation trial (Incub4) was slightly more stable ( $MWD_{sw} > 0.5$  mm) than the same soil sampled for the previous incubation trials (Incub2, Incub3), while KERGUE and NAIZIN were both much more stable soils overall ( $MWD_{sw} > 2$  mm at most dates). Though only significant for KERGUE and NAIZIN soils, a slight increase in MWD was observed for all soils over the course of incubation. This was attributed to a slight loss of soil moisture over time due to evaporation.

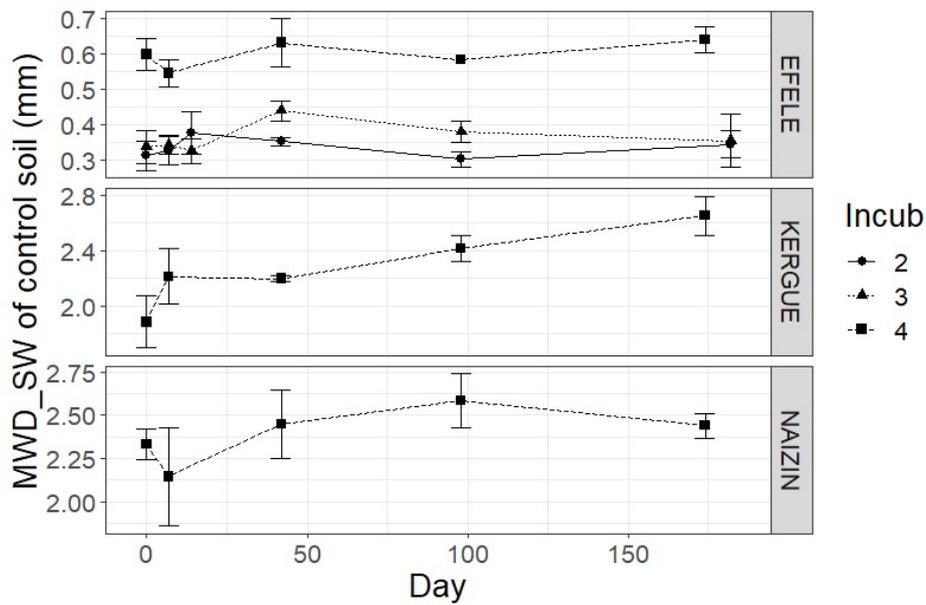


Fig. 6.1 Aggregate stability dynamics of control soil as per the slow wetting test (Le Bissonnais, 1996), monitored over the course of three incubation trials (Incub2, Incub3 = incubation trial reported in (Cooke et al., 2023), Incub 4 = current incubation trial). Error bars denote standard deviation ( $n=4$ ).

The dynamics in soil moisture content (SMC) over the course of the incubations are given in Fig. 6.2.

There was a significant ( $p>0.05$ ) correlation between the average SMC and the average  $MWD_{SW}$  of the EFELE ( $R^2= 0.31$ ) and KERGUE ( $R^2= 0.21$ ) soils but not for NAIZIN. Drying of soil may precipitate hydrophobic organic matter on the surface of aggregates, thus decreasing surface wettability and increasing AS (Bottinelli et al., 2017; Cosentino et al., 2006). Since the effects of aggregate drying on SMC were mainly significant after day 174, we decided not to take into account the results obtained at this date in the discussion.

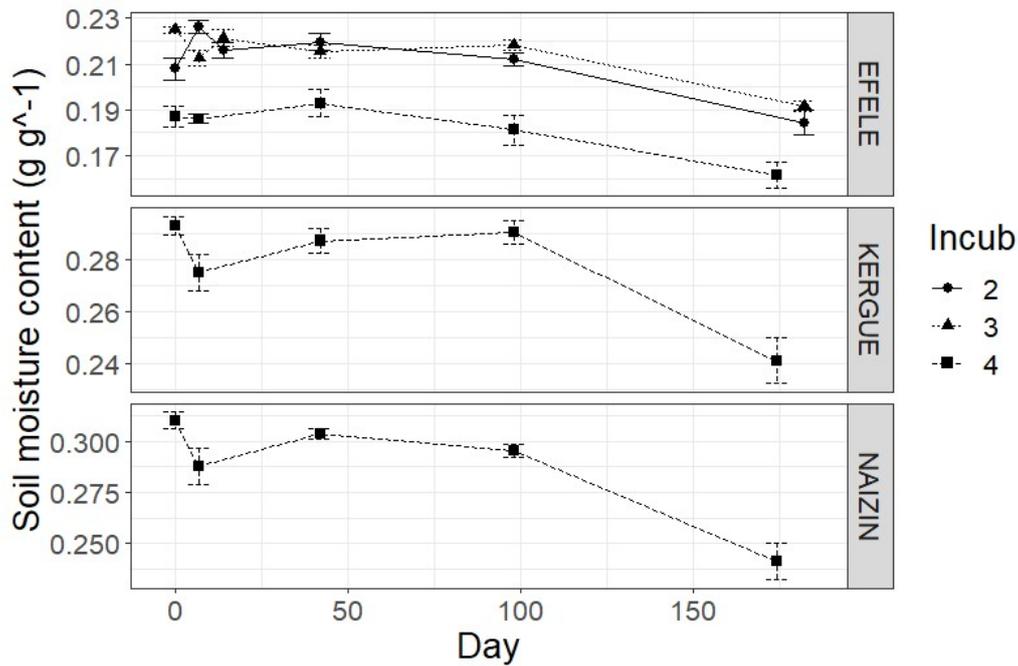


Fig. 6.2 Dynamics in soil moisture content (SMC) for the three incubated soils (all modalities) monitored over the course of three incubation trials (Incub2, Incub3 = incubation trial reported in (Cooke et al., 2023), Incub 4 = current incubation trial). Error bars denote standard deviation ( $32 \leq n \leq 48$ ).

### 6.3.1.2 Comparison of aggregate stability dynamics of soils amended with digestates

The results of analysis of aggregate stability dynamics for soils following digestate incorporation, using the fast wetting (FW) and slow wetting (SW) tests, are shown in Fig. 6.3 and Fig. 6.4, respectively. The mean MWD of the control soil at each date was subtracted from the MWD of the soil-digestate mixtures in both cases, in order to facilitate interpretation of the figures. The incorporation of digestate appears not to have had a significant impact on  $MWD_{FW}$  at most sample dates, and so this is not discussed here in much detail. Only digestate LP4 had a statistically significant positive effect on  $MWD_{FW}$  at most other sample dates when incorporated to NAIZIN soil.

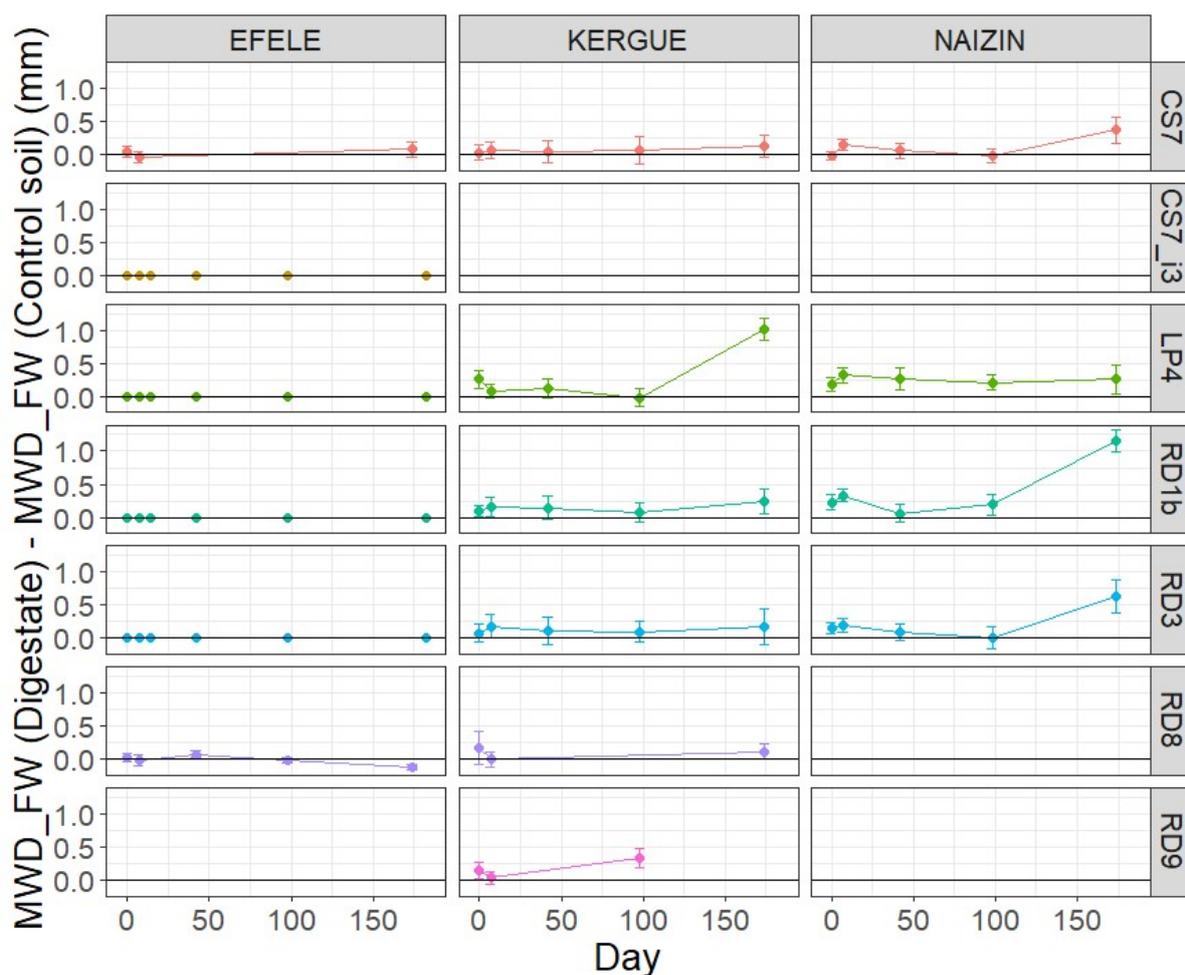


Fig. 6.3 Aggregate stability dynamics for digestate soil mixtures as determined using the fast wetting test of (Le Bissonnais, 1996), plotted as the difference between the MWDs of the soil-digestate mixture and the control soil. Error bars denote standard deviation ( $n=4$ ).

The increase in  $MWD_{FW}$  and  $MWD_{SW}$  for digestate amended soil at the final sample date, in particular for the KERGUE and NAIZIN soils, was very likely due to the drying of soil aggregates over the course of incubation as already mentioned. Drying of aggregates coupled with precipitation of OM deposited on the aggregate surface may lead to even greater increases in AS of a soil with organic amendment relative to the unamended control. This effect has already been noted in incubation of soil with maize straw, where the effects were found to be from the deposition and precipitation upon drying of microbial polysaccharides and hydrophobic molecules (Cosentino et al., 2006).

In contrast to the  $MWD_{FW}$ , significant changes in the  $MWD_{SW}$  were observed for all digestates in all soils at one or more sample dates. The most significant positive improvements to  $MWD_{SW}$  were observed for soils amended with digestates LP4 and RD1b, including at day 0. Incorporation of digestate RD3 resulted in a reduction in  $MWD_{SW}$  relative to the control soil for KERGUE and NAIZIN at day 98. These results confirm that the slow wetting (SW) test of (Le Bissonnais, 1996) appears to be a more sensitive indicator of the effects on soil structural stability brought about by organic amendments (Abiven et al., 2008; Cosentino et al., 2006).

Using the  $MWD_{SW}$  data for soil amended with the digestate CS7, which was incubated twice, in the prior incubation (CS7\_i3) trial and the subsequent incubation trial (CS7), the comparability of the results could be tested. Overall, the digestate CS7\_i3 did not significantly change soil AS at nearly all dates apart from a minor improvement slightly after incorporation (7 days) and a gradual improvement towards 182 days. In a similar way CS7 did not significantly improve AS at the measured dates (0, 7 and 174 days after incorporation), though a slight rise in AS was observed at day 174. In fact, CS7 did not have any positive effect on soil AS for the three different texture soils until the final date of incubation. These dynamics are typical of amendment with a mature compost (Abiven et al., 2009), where improvements in the soil AS are expected to be slow but prolonged.

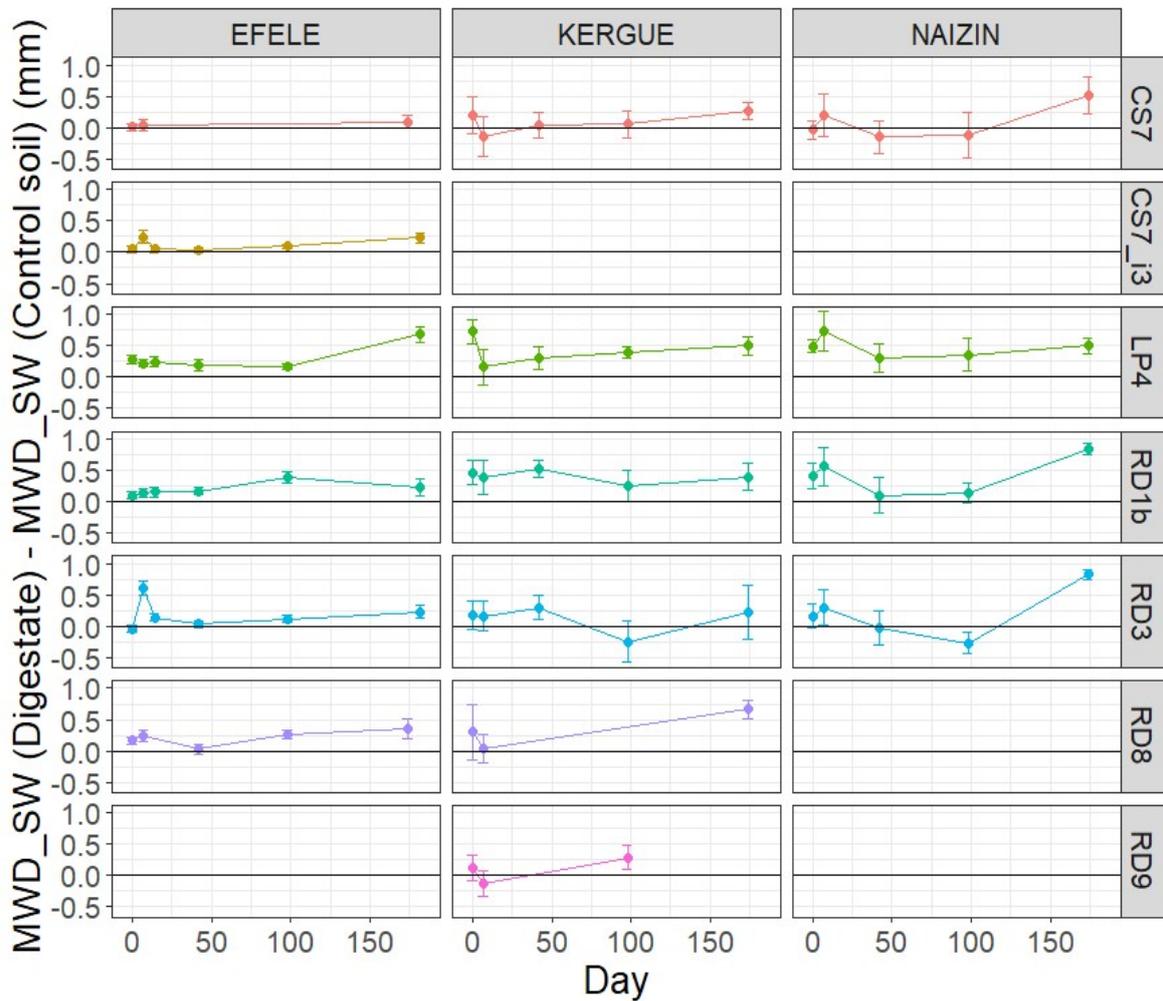


Fig. 6.4 Aggregate stability dynamics for digestate soil mixtures as determined using the slow wetting test of (Le Bissonais, 1996), plotted as the difference between the MWDs of the soil-digestate mixture and the control soil. Error bars denote standard deviation (n=4).

### 6.3.1.3 Relevance of the digestate typology of impact to different soil textures

As previously discussed, all soils amended with digestate CS7 showed little improvement in MWD at most dates, as typical of the digestates in group 4 of the typology. A direct abiotic stabilisation of soil aggregates at day 0 was observed for all soils amended with digestate LP4, as expected of a group 3 digestate. For the KERGUE and NAIZIN soils, this initial stabilisation was even stronger than for the EFELE soil. However, after this sample date the subsequent AS dynamics following amendment with LP4 were fairly different between the three soils. The digestates RD1b and RD3 also did not result in

the same dynamic in soil AS when added to the other two soils as they did for EFELE soil, and in some cases even led to a slightly reduced  $MWD_{SW}$  after around 98 days of incubation. Indeed, for NAIZIN soils, it would appear that the AS dynamics (both for  $MWD_{SW}$  and  $MWD_{FW}$ ) were similar for all digestates (though differing in the size of impact). This would suggest that, in the case of this clay and OM rich soil, the soil type had a predominant influence on soil AS dynamics over the type of digestate. For the KERGUE soil, the digestate RD1b had a strong direct effect on  $MWD_{SW}$  similar to digestates in group 3 of the typology, though overall it had a constant positive impact at all dates (over this short-term of several months) typical of a digestate in group 2.

Despite the limited data for the two previously untested raw digestates RD8 – a digestate of primarily pig slurry, and RD9 – a digestate of primarily cover crops, both appeared to induce similar dynamics in AS when added to KERGUE soil. In the case of RD8, the AS dynamics in EFELE and KERGUE soils were similar to that of digestate LP4. It is likely that RD8 and RD9 would fit into group 4 of the typology of impact, though this would require sampling and analysis of MWD at more dates in order to have a more complete image of the AS dynamics. The group 3 appears to compose of raw liquid and liquid phase of digestates with low starting TS (<9%) and low C-N ratios (<7), properties that these two digestates appear also to have (see Table. 6.2).

### 6.3.2 Carbon mineralisation dynamics following digestate incorporation to different soils types

#### 6.3.2.1 Comparison of carbon mineralisation from control soils

Plotting the cumulative C mineralised from the control soils over the course of incubations (see Fig. 6.5), it is possible to see that the overall rate of C mineralisation was relatively low and steady for each soil. For the control soils incubated in the current trial (Incub 4), in terms of the total C mineralised, they followed the descending order KERGUE>NAIZIN>EFELE. The C mineralisation from the EFELE control soil in the previous incubations (Incub2, Incub 3) was higher than for the current incubation

(Incub4). This may possibly be explained by a difference in soil moisture content (SMC) between the EFELE soil used in the separate incubations. The water content of soil is an important factor in regulating soil microbial activity and therefore in determining rates of C mineralisation, though taking this into account in experimentation can be complicated (Rodrigo et al., 1997). In our case, maintaining high SMC to obtain ideal conditions for C mineralisation needed to be weighed against the potentially detrimental effect of rewetting aggregates on the AS, and so some drying of the incubated soil had to be accepted. Differences in the C mineralisation between control soils were minimal when compared to the C mineralisation from the digestate soils, where total cumulative mineralised C ranged from approximately 100 to 180 mg depending on the soil and digestate.

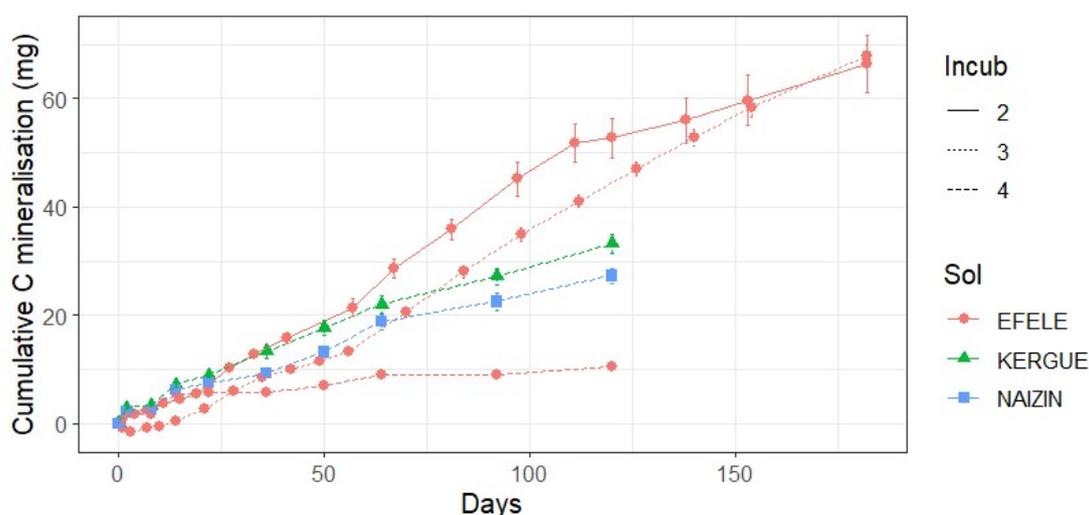


Fig. 6.5 Cumulative C mineralisation from control soils over the course of three separate incubation trials (Incub2, Incub3 = incubation trial reported in (Cooke et al., 2023), Incub 4 = current incubation trial).

### 6.3.2.2 Comparison of carbon mineralisation from soils amended with digestates

The C mineralisation from the soils was monitored for all modalities in the current incubation trial up to day 121, and the results, calculated as the cumulative percentage of the total added C mineralised, for each digestate-soil mixture are presented in Fig. 6.6. There were issues with the analyses at day

182 and so this data is not included. For CS7 and LP4, C mineralisation was similar regardless of the soil, and likewise for the raw digestate (RD1b, RD3 and RD8) in the first week of incubation. However, after this, some differences emerged between the rates of C mineralisation depending on the soil, with the total C mineralisation after 130 days for each soil following the order EFELE > NAIZIN > KERGUE. On average, digestates CS7 and RD9 showed lower C mineralisation (<10% and <15% of added C, respectively), than the raw digestates RD1b, RD3 and RD8 (15 – 30 % of added C depending on the soil type).

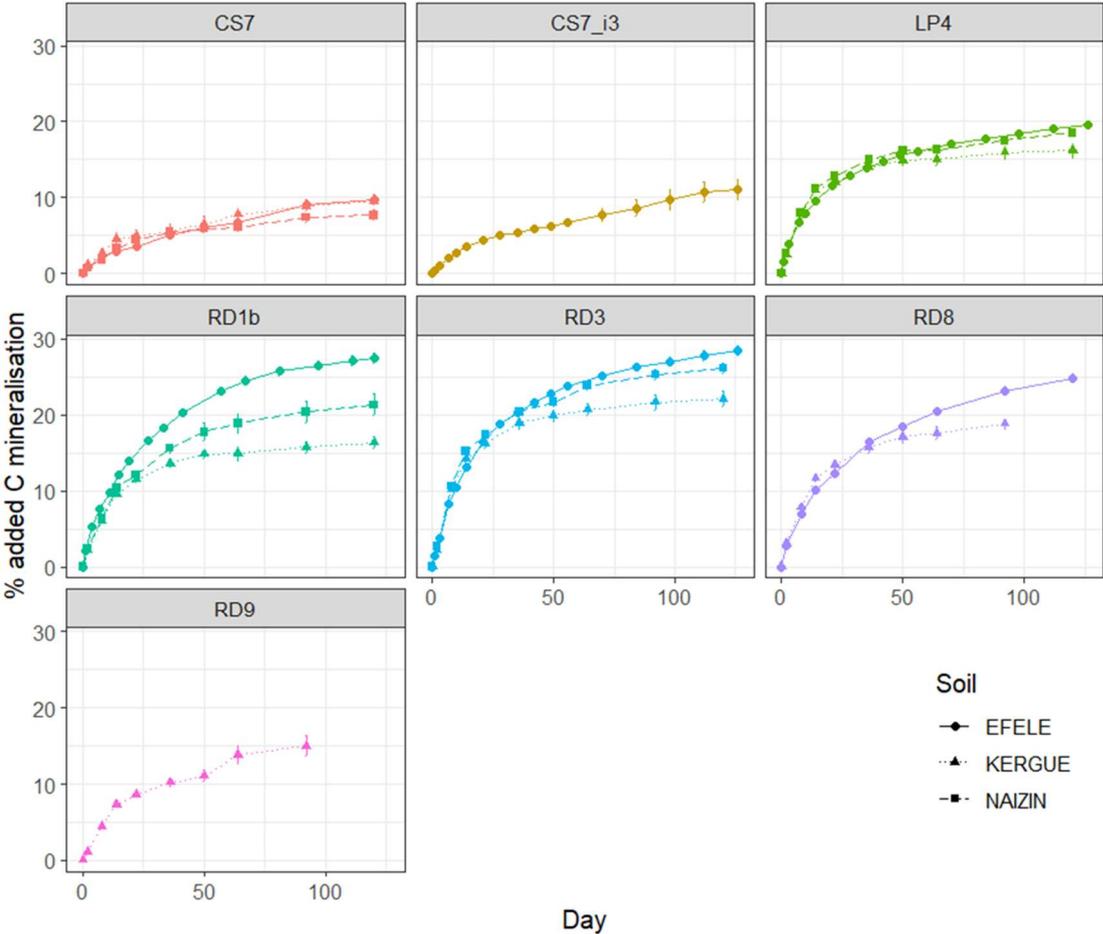


Fig. 6.6 Cumulative C mineralisation (as a percentage of added C in the form of digestate) for the incubated soil-digestate mixtures, grouped by the digestate. Error bars represent standard deviation (n=4).

Utilising a first order + zero order two compartment model fitted to the C mineralisation data, the constants  $C_0$  (labile C in digestate, %TC),  $b$  (labile C decomposition rate constant,  $\text{day}^{-1}$ ), and  $A$  (recalcitrant C decomposition rate constant %TC  $\text{day}^{-1}$ ), were calculated and are given in Table. 6.3, along with the RMSE as an indicator of the performance of the model in each case. Overall, model predictions were good and RMSE values fairly similar across all digestates, though for low C mineralisation digestates such as CS7 and RD9, this level of accuracy was fairly low. The estimated values from the model correspond with those obtained by other studies on the C mineralisation from soil amended with digestates (Askri et al., 2016; Häfner et al., 2022).

The estimated constants for the digestate CS7 in the EFELE soil for the two incubations (CS7 and CS7\_i3) were approximately the same, as was the cumulative C mineralisation at each date, as represented in Fig. 6.6. It should be noted that the calculated constants from the model are subtly different from those estimated in (Cooke et al., 2023) for the same digestate and soil, as the time interval used here is shorter, only 121-128 days (depending on the digestate). For all digestates except RD1b, the labile C component ( $C_0$ ) and the labile C mineralisation rate constant ( $b$ ) were estimated to be similar for all soil types, which confirms the robustness of the chosen model. For RD1b, there were significant differences between the estimated  $C_0$  depending on the soil type, with a much higher estimate for EFELE soil than for KERGUE and NAIZIN, which may be linked to different levels of microbial activity in these soils, or different microbial communities able to better access the OM in the digestate. However, microbial markers were not tested in this experiment and so this hypothesis could not be confirmed or disproved. The differences in the recalcitrant C rate constant ( $A$ ), while not greatly significant, may again be due to differences in the soil microbial dynamics in each soil, or due to better protection of the recalcitrant C in the case of the KERGUE and NAIZIN soils with their higher clay content (though this cannot be proven).

Table. 6.3 Estimated parameters of a first order + zero order model fitted to cumulative carbon mineralisation data for digestates added to three different soils

Model equation : $\%C_{min} = C_0 \cdot (1 - e^{-bt}) + A \cdot t$					
Digestate	Soil	C <sub>0</sub> (%)	b (day <sup>-1</sup> )	A (% day <sup>-1</sup> )	RMSE (%)
CS7 (i4)	EFELE	2.86	0.10	0.06	0.85
CS7	KERGUE	4.38	0.11	0.05	1.38
CS7	NAIZIN	5.02	0.06	0.02	0.83
CS7 (i3)	EFELE	3.22	0.10	0.06	1.22
LP4	EFELE	12.74	0.09	0.06	0.43
LP4	KERGUE	13.47	0.10	0.02	1.06
LP4	NAIZIN	14.01	0.10	0.04	0.71
RD1b	EFELE	20.29	0.05	0.06	0.93
RD1b	KERGUE	13.87	0.07	0.02	0.98
RD1b	NAIZIN	16.14	0.06	0.04	1.66
RD3	EFELE	20.59	0.06	0.06	0.71
RD3	KERGUE	18.64	0.09	0.03	1.28
RD3	NAIZIN	19.26	0.09	0.06	0.95
RD8	EFELE	15.01	0.06	0.09	0.83
RD8	KERGUE	14.61	0.09	0.05	1.14
RD9	KERGUE	7.79	0.09	0.08	1.34

### 6.3.3 The factors affecting the impact of different digestates on different soils

#### 6.3.3.1 Soil texture and OM content

The soil texture, and in particular the clay content, is a governing factor in soil aggregate formation and stabilisation (Emerson and Greenland, 1990). For silt rich soils, aggregate stability is often low and factors such as the soil organic matter content become highly important in the formation and stabilisation of aggregates (Le Bissonnais and Arrouays, 1997). Conversely, for soils with high clay contents (>30%), aggregate stability is generally very high (Kemper and Koch, 1966), and the arrangement of clay particles and the electrostatic forces between them are the dominant factor in the formation of stable aggregates (Six et al., 2000a). The soil texture would partly explain the differences in AS of unamended soils, with both soil clay content and average MWD<sub>sw</sub> of the control soils following the order NAIZIN>KERGUE>EFELE. However, the KERGUE and EFELE soils had very close

clay contents (see Table. 6.1), but very contrasting soil AS, which is almost certainly due to the higher OM content of the former. The difference in OM content between these two soils may be due to the differences in the history of management practices used at the two sites from which the soil was sampled.

Soil texture may affect the microbial communities responsible for soil aggregate stabilisation following organic amendment. Fungi appear to be much more important in aggregate stabilisation following organic amendment of coarser textured soils (De Gryze et al., 2006; Metzger and Yaron, 1987). Zhang et al. (2021) also remarked that fungal populations appeared to dominate aggregate stabilisation following digestate incorporation to sandy soil, but that bacterial populations were dominant in digestate amended clay soil. The transient increase in  $MWD_{SW}$  (at day 7) for the clay rich NAIZIN soil may well have been due to the deposition of microbial exudates that were rapidly broken down. Conversely, for the KERGUE soil lower in clay, the increases in  $MWD_{SW}$  were longer lasting, and therefore consistent with fungal stabilisation of aggregates. Analyses of the fungal and bacterial population dynamics in the different soils would be needed to assess the relative contribution of different taxonomic groups to soil AS dynamics.

#### 6.3.3.2 Clay mineralogy

The four soils were found to have very similar mineralogy, dominated by chlorite, illite-mica (muscovite), and kaolinite (see Table. 6.4). They were not found to contain expansible clays such as smectites. The KERGUE and NAIZIN soil are distinguished from the EFELE soil by the presence of a non-expansible interstratified regular illite vermiculite. This mineral originates from the first stages of alteration of mica (muscovite).

Very low reactivity clays such as kaolinite show a very high stability and resistance to potential dispersive effects of high levels of dissolved sodium (McNeal and Coleman, 1966), although this may also be due to association with high levels of iron and aluminium sesquioxides within this clay. Illite

clays, however, display high sensitivity to dispersion (Shainberg and Letey 1984). Though the overall importance of clays in soil aggregation may be reasonably well established, the effect of the mixture of different clay minerals generally present in a soil is much less easy to predict (Amézketa, 1999).

*Table. 6.4 Composition of clay fraction (<50um) in incubated soils following analysis by x-ray diffraction on orientated plates*

<i>EFELE</i>	<i>KERGUE</i>	<i>NAIZIN</i>
Chlorite	Chlorite	Chlorite
Mica illite	Mica illite	Mica illite
Kaolinite	Kaolinite	Kaolinite
Interstratified illite smectite	Interstratified illite vermiculite	Interstratified illite vermiculite
Quartz	Quartz	Quartz

Given that the clay mineralogy of the three soils was found to be largely similar, it is expected that the differences in the total proportion of clay in the soil may play a more important role in determining the differences in AS between the soils. Nonetheless, it is possible that the NAIZIN and KERGUE soils, with their content in illite vermiculite clays, suffered greater negative effects through dispersion from the incorporation of digestates RD3 and RD1b with their high content in minerals forming monovalent cations (K and Na). When present in excess, Na<sup>+</sup> cations can replace K<sup>+</sup> between the lamellae of illite clays, generating a double-layer around the clay particles and facilitating their dispersion (Rengasamy, 2018; Tessier, 1990). The interference of monovalent cations in clay bonding may subsequently disrupt the stability of macro-aggregates (Emerson, 1967; Levy and Torrento, 1995). Whether Na<sup>+</sup> ions did interact with soil clays during incubation and at what level these interactions would have been disruptive to the soil AS would further detailed testing.

## 6.4 Conclusions

The digestate typology of impact on soil AS developed by Cooke et al. (2023), while still relevant in indicating a certain outcome of digestate incorporation in silt loam soil, is not completely applicable to other soil types, and may require some improvement to account for different effects of digestate in clay and OM rich soils. Further development of the typology would require an in-depth characterisation of the soil biological, chemical and physical processes involved in aggregate stabilisation in each case in order to provide better explanation of the complex outcomes.

## 7 General discussion and conclusion

In this chapter, we summarise the experimental results of this thesis research project, and discuss how they have responded to the scientific objectives that were laid out at the conclusion of chapter 1. Furthermore, we consider what potential implications our findings have for future research in the field as well as what guidance may be provided based on these results to the agricultural AD sector in general.

## 7.1 Summary and discussion of the principal results

### 7.1.1 What are the impacts of digestate application on short-term dynamics in soil aggregate stability, and how are these influenced by the various anaerobic digestion process parameters?

Combining a laboratory incubation protocol tested and refined in preliminary experiments with a precise method for monitoring of the soil aggregate stability of a silt loam soil (chapter 2), it was possible to explore and compare the impact on soil AS of 15 digestates sampled from seven AD plants and produced under a wide range of process conditions (chapter 3). To our knowledge, ours is the most complete investigation to date of the short-term effects on soil AS following application of multiple digestates. With this in mind, perhaps the most significant finding from our research was that we observed a range of different dynamics in AS specific to the type of digestate incorporated into the soil. Using hierarchical clustering analysis, we were able to establish an initial typology of impact, classifying digestates into four groups corresponding to four different observed “dynamics” (see Fig. 3.5), that were defined as:

- Those digestates inducing a rapid but transient improvement of AS (Group 1),
- those inducing a slower, temporary improvement of AS (Group 2),
- those inducing an immediate improvement followed by a longer-lasting increase in AS (Group 3), and,
- those inducing a minor or non-significant improvement in AS (Group 4).

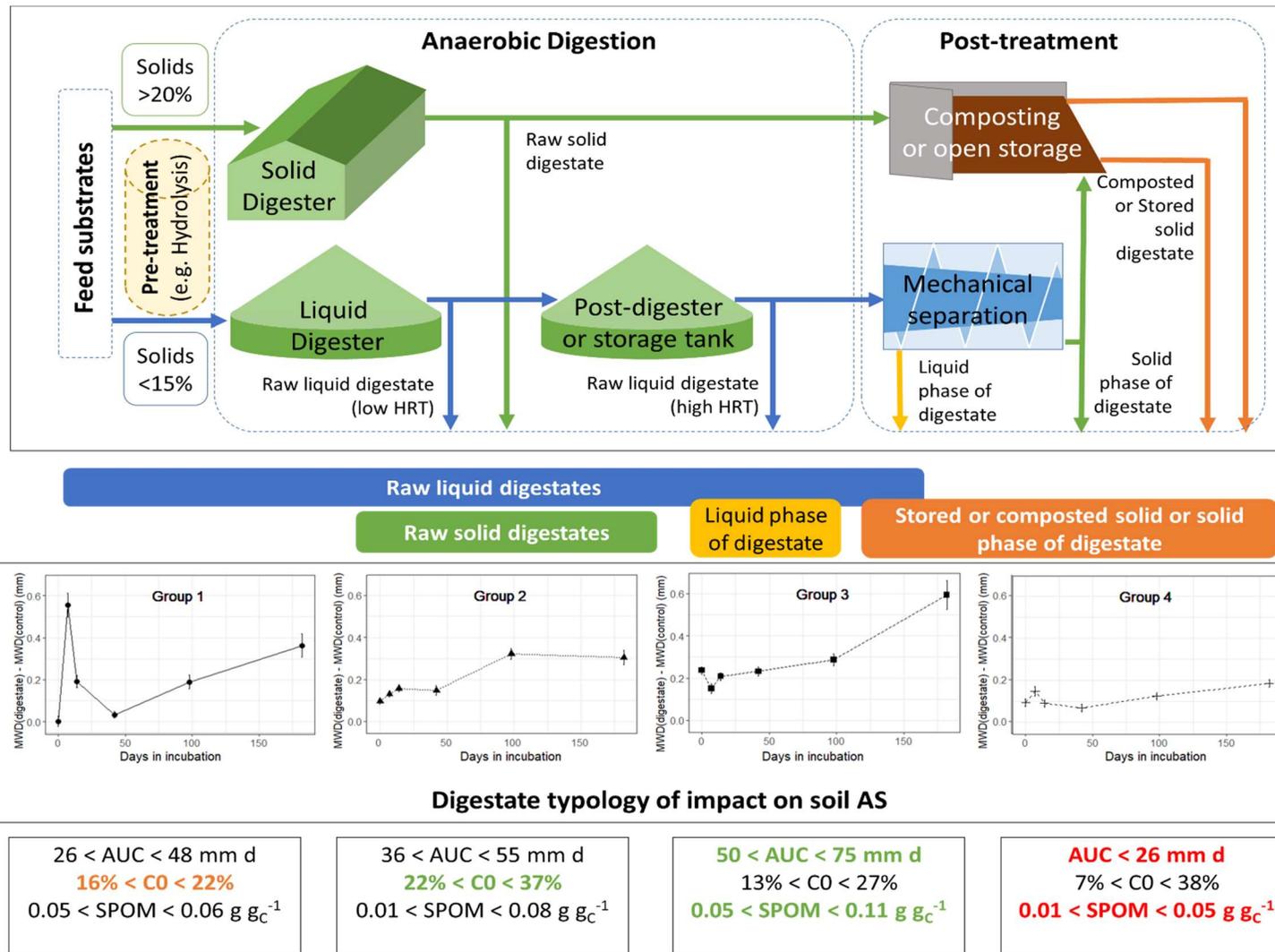


Fig. 7.1 Schematic of the AD process parameters leading to the production of different digestates, and their subsequent impact on silt loam soil AS dynamics as defined by the developed typology of impact. The calculated AUC (or overall impact on soil AS) as well as the range of characteristics (CO = % labile carbon content, SPOM = soluble and particulate OM extracted by CaCl solution) of the digestates in the different groups are given in the boxes at the bottom.

It is proposed that a digestate typology of impact on soil AS, such as the one proposed here, might be used as a guidance tool in decision making for digestate application in agriculture. This may include the selection of digestates for soil incorporation with the intent of:

- maximising short-term benefits to soil AS to improve soil physical fertility (for example, the use of digestates in typology 2 or 3 may be recommended for marginal or degraded soils to provide significant temporary, and perhaps long-term, improvements in soil AS),
- providing transient improvements to soil AS timed to protect soil at times when the risk of crusting or erosion may be at its highest (for example, applications of a digestate in group 2 at planting in early spring may protect the soil from heavy rainfall events in subsequent months while plant cover develops).

It was possible to describe the developed digestate typology of impact based on key AD operating parameters (see *Fig. 7.1*), notably in terms of the post-treatment applied. Liquid phases of digestate generated from mechanical separation (n=3), all fell into group 3 of the typology, while solid digestates having undergone composting or open-air storage (n=4) fell primarily into group 4. Raw digestates (n=6) fell into three of the four groups of the typology, and it was suspected that the differences were due to the varying feed substrates employed in the production of the digestates. However, the limited size of the dataset, the greater effect of the other factors on the typology, and the difficulty in defining complex feed mixtures in this way, meant it was not possible to determine a clear connection.

In complement to the typology, and in order to facilitate comparison between digestates, we also adopted a useful measure for comparison of digestate impact originally proposed by Annabi et al., (2007): the Reimann's sum or area under the curve (AUC) described by the soil AS dynamic (see *Fig. 7.1*). The advantage of using the AUC over other measures such as the mean, minimum or maximum MWD was that it took into account the duration of the aggregate stabilisation as well as the amplitude. As such, digestates with only a transient impact on soil AS would have only a low AUC, while those with lower but more constant impact on soil AS would have a higher AUC.

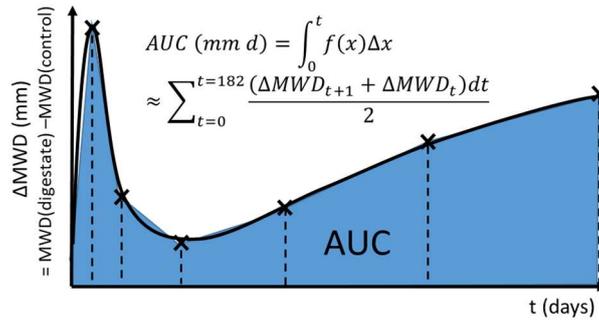


Fig. 7.2 Diagram explaining the method for calculating the AUC or area under the curve of aggregate stability for an incubated digestate

The AUC proved useful in defining the connection between AD operating parameters and post-treatments applied to digestates and their classification in the typology of impact. Again, phase separation and/or composting of digestate had a significant effect in this regard, with the impact of different post-treated digestates on soil AS (as defined by the AUC) following the order: Liquid phase  $\geq$  Raw  $\geq$  Solid phase = Composted/Stored. The level of stabilisation of the digestate, in terms of the length of time spent in anaerobic digestion (the hydraulic residence time - HRT) plus the presence of feed substrate pre-treatments (see chapter 3), also provided some explanation of the variation in values of AUC for the panel of digestates tested. The effect of these process parameters appears to be preponderant over the effect of the mixture of feed substrates used to produce the digestates, and so a clear connection between the principal feed materials and the AUC could not be established. It was concluded that detailed physico-chemical characterisation would provide a greater degree of precision in predicting digestate impact on soil AS, and some insight into the effect of the feed materials used (in terms of their contribution to the characteristics of the digestates). The greater part of this analysis formed the basis of a second article, presented in chapter 4.

7.1.2 What are the connections between digestate characteristics and the observed impacts on soil aggregate stability dynamics?

7.1.2.1 What indicators can be determined from digestate characteristics that may be used to predict their impact on soil aggregate stability?

We adopted two principal approaches in our analysis and interpretation of the link between digestate characteristics and soil aggregate stability, namely:

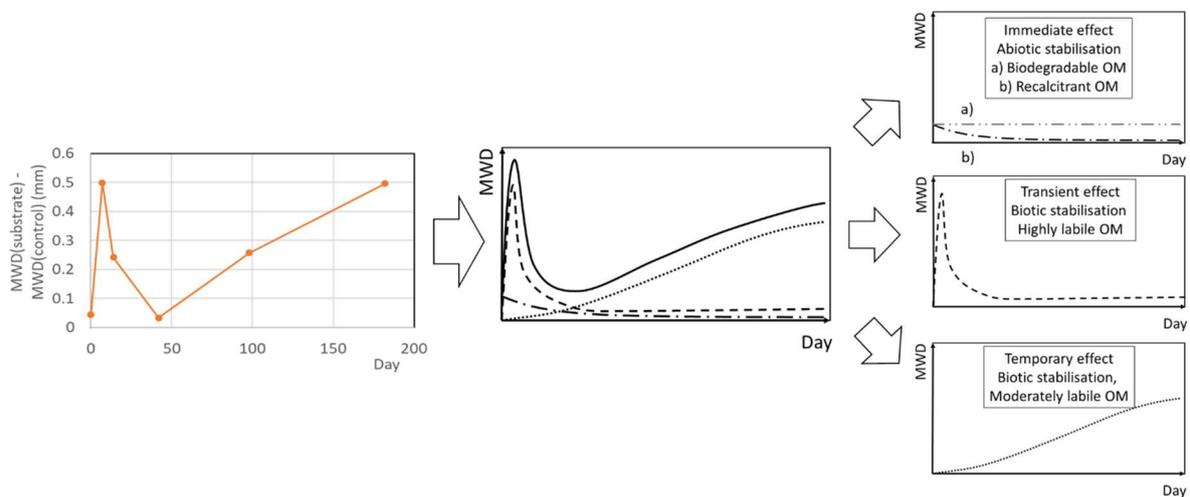
- Modelling of soil C mineralisation following digestate amendment (using a dual first-order/zero-order equation) in order to describe the dynamics observed, followed by correlation of the model parameters (estimated labile C content and mineralisation rate constants) with the AUC (chapter 3),
- Sequential extraction of OM fractions using the method developed by Jimenez et al. (2015) followed by PLS-R modelling to predict AUC (chapter 4),

Using the results of these analyses, we were able to provide evidence of a connection between the biodegradability and bioaccessibility of the OM contained within digestates and their subsequent impact on soil AS. We determined that much of the variation between digestates in terms of their impact on soil AS could be explained by their content in organic matter forms of varying accessibility to soil microorganisms. PLS-R modelling using the sequential extraction model was able to explain up to 70% of the variation in the AUC for the digestates tested. Readily extracted carbon and nitrogen (SPOM and REOM) are believed to be important in initiating and maintaining microbial activity leading to production of aggregate binding agents acting over the short-term. In particular, the “bioaccessibility” of organic nitrogen forms in the digestate appeared to determine the level of impact of digestate, possibly by providing important N supply for microbial development – though it should be noted that we did not supply mineral N to incubation jars and so were in a context of limited free N availability.

The results from monitoring and modelling of carbon mineralisation presented in chapter 3, confirmed that biodegradability of digestate OM was key to their impact on soil AS, with rapid mineralisation indicative of higher concentrations of labile C observed for the digestates also initiating stronger increases in MWD. This supports the theory that modelling of soil AS dynamics coupled with existing models for C mineralisation may be possible (Chenu et al., 2011; Dénès et al., 2014). Studying more closely the typology of impact, and the “typical” soil AS dynamics that are described by each of the groups, gives a possible insight into the nature of the effects of digestate quality on the soil structural stability.

Digestates in two of the groups of the typology resembled closely those dynamics in AS typically observed for manures (group 2), having a temporary effect, and for mature composts (group 4), having little or no effect over the short-term. However, many of the AS dynamics observed (particularly those in group 1 and group 3) did not appear to follow a typical “log-normal” curve as has proven to be the case for many other organic amendments (Abiven et al., 2008). We hypothesised that the OM contained within the digestate had varying rates of biodegradability/bioaccessibility, which sometimes resulted in multiple peaks in aggregate stabilisation (see *Fig. 7.2*) - a phenomenon previously observed by Metzger and Yaron (1987) for sewage sludge. This was particularly evident for the two digestates in Group 1 where, in incubation, a sharp peak in soil MWD after 7 days was rapidly lost, but then was followed by a gradual increase in MWD towards 182 days. Furthermore, the digestates in Group 3 of the typology initiated a significant increase in soil AS directly after incorporation, which suggests the presence of components contributing to direct, abiotic stabilisation of aggregates. In many cases, this initial stabilisation was lost after 7 days, as if the binding agents involved were rapidly broken down or otherwise removed, while in other cases the effects may well have persisted. In either case, this initial aggregate stabilisation would also overlap with the AS dynamics due to microbial consumption of various OM fractions and subsequent generation of binding agents, to generate a composite curve, as described conceptually in *Fig. 7.3*. It is probable that testing of other digestates through similar incubation trials would reveal other variations of the dynamics in soil AS observed during our

experiments. Indeed, the dynamics observed for several of the digestates tested (RD5, CS5 and SP2), did not fit perfectly the “ideal” curves described by the groups of the typology, falling somewhere in between.



*Fig. 7.3 Conceptual breakdown of the complex dynamic in soil AS observed for a raw liquid digestate (RD4) in terms of soil AS effects resulting from microbiological degradation of different OM fractions*

7.1.2.2 What are the mechanisms by which digestate characteristics affect soil aggregate stability?

The sequential extraction technique and characterisation methods applied in chapter 4 did not allow precise identification of specific OM forms or compounds that may have been connected to soil aggregate stabilisation. Furthermore, we did not perform soil biological or chemical analyses that may have allowed identification the precise biotic mechanisms involved in soil aggregate stabilisation, i.e. the microbial communities responsible (fungi or bacteria) or the binding agents involved (hydrophobic OM, microbial exudates, fungal hyphae). Nonetheless, the results did prove helpful in the advancement of our understanding of the connections between digestate characteristics and changes in the soil leading to aggregate stabilisation, and this is summarised in Table 7.1.

Table. 7.1 Digestate characteristics and the mechanisms by which they may alter aggregate stability of a silt loam soil– what conclusions can be drawn from the results of the present research?

Possible digestate characteristics	Advancement in understanding of the impact of the digestate characteristic
<b>Characteristics linked to biotic (microbial) stabilisation mechanisms</b>	
1. Remaining undigested labile OM (simple carbohydrates, amino acids, soluble proteins) + N <sub>min</sub>	<ul style="list-style-type: none"> <li>● A rapid transient response in soil AS was observed for two digestates – possibly due to microbial deposition of exudates (bacterial?) that were then broken down</li> <li>● Slower responses were also observed, possibly due to evolution toward fungal communities (and a growth of mycelial networks binding soil aggregates), or else the gradual accumulation of more resistant microbial residues (either modifying aggregate pore structure or increasing particle cohesion). However, no proof of shifts in microbial communities was found from analysis of soil after 182 days of incubation (see appendix).</li> <li>● Highly bioaccessible OM fractions from sequential extraction (SPOM and REOM), plus rate of mineralisation of labile C, linked to increased aggregate stabilisation over short-term.</li> </ul>
2. Slowly biodegradable OM (ligno-cellulose, complex proteins)	
3. All biodegradable OM	
4. High labile C + N content	<ul style="list-style-type: none"> <li>● As above, the quantity of N in bioaccessible OM fractions appears to be important in driving soil aggregate stabilisation over the short-term. High levels of N<sub>org</sub> may well facilitate microbiological activity and lead to greater deposition of binding agents.</li> <li>● Negative effects of excessive mineral N were not observed, though all digestates were dried for incubation, and so N availability was less than for fresh digestates. In all cases, digestates increased soil OC after 6 months of incubation</li> <li>● The analytical techniques used could not determine if priming effects occurred upon incorporation of digestate to the soil, although in all cases SOC was increased by digestate addition over the time-scale of the trial</li> </ul>
5. Toxins or heavy metals	<ul style="list-style-type: none"> <li>● Though not specifically targeted by analysis, no observable evidence of toxicity leading to reduced aggregate stability was observed, despite the high doses of digestates used</li> </ul>
<b>Characteristics linked to abiotic stabilisation mechanisms</b>	
6. Large stable OM (Microbial residues, High M <sub>R</sub> aromatic compounds, “Humic” substances)	<ul style="list-style-type: none"> <li>● Non-extracted (Humin?) and strong alkali extracted (Humic acids?) OM was found to be either negatively or not significantly correlated with AUC. Therefore, there was no evidence of humified OM from digestate directly contributing to AS.</li> <li>● Evidence points towards improved aggregate cohesion in some cases, and possibly hydrophobicity in others (however, no correlation was found between changes in initial soil AS and initial soil wettability).</li> <li>● Immediate stabilisation of aggregates was reduced by day 7 of incubation for certain digestates, therefore some of the OM responsible is likely highly biodegradable.</li> </ul>
7. Hydrophobic OM (Lipids, amphiphilic molecules, microbial residues, extracellular polysaccharides)	
8. Polyvalent cations (Ca <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>3+</sup> , Al <sup>3+</sup> )	<ul style="list-style-type: none"> <li>● Some negative effects were observed upon incorporation of digestate with a high Na and K content to soils containing dispersible clays. However, this was temporary and may have been compensated for by positive effects from added labile OC.</li> <li>● No evidence of positive effects on soil AS from polycations were observed. In many cases, digestates contained an abundance of both minerals forming monocation and those forming polycations. More detailed analysis of exchangeable minerals released from the digestate when added to the soil may be required.</li> </ul>
9. Monovalent cations (Na <sup>+</sup> , K <sup>+</sup> , NH <sub>4</sub> <sup>+</sup> )	
10. Digestate pH (> or < than soil pH)	<ul style="list-style-type: none"> <li>● Shifts in soil pH from mildly acid (6.5) to basic (9) were observed after digestate addition during incubation trials though there was no obvious correlation with changes in soil AS.</li> </ul>
<b>Characteristics linked to biotic (plant) stabilisation mechanisms</b>	
11. Minerals / Plant nutrients (N, P, K, etc.)	<ul style="list-style-type: none"> <li>● No short-term effect of digestate application or plant development on soil AS (after initial sample date) was observed during a small field trial over an oilseed rape crop growing season, despite the apparent beneficial effect of digestate fertilisation on the crop development.</li> <li>● However, more field trials involving a greater diversity of crop types, along with observation of soil stabilisation in the rhizosphere compared to the rest of the soil, are needed.</li> </ul>

Our results from incubation trials and the in-field trial reported in chapter 5 indicate that abiotic mechanisms likely contributed to initial aggregate stabilisation following the incorporation of raw liquid, liquid phase, and composted digestates to the soil. However, the characterisation carried out did not permit identification of any specific molecules involved in either case. It is possible that between composted/stored digestates and liquid digestates, the abiotic binding agents responsible for initial stabilisation of soil aggregates are not the same. As the solid phases of digestate tested did not show much effect on initial soil AS, it is probable that those binding agents that are present in the liquid phase are less present (in terms of the proportion of OC) in the solid phase. Composting of the solid phase may then have led to renewed production of binding agents (Annabi et al., 2007), hence their observed impact on initial soil AS. The initial effects did not last for many of the digestates, with MWD decreasing to the same levels as the control soil, it could be presumed that the organic binding agents involved were rapidly altered or broken down by soil microorganisms. Aggregate wettability, analysed by the water droplet penetration time (WDPT), did not show correlation with the initial increases in soil AS, suggesting that components of the digestate may have increased aggregate cohesion rather than increasing hydrophobicity.

Understanding how specific digestate characteristics may affect soil AS, or at least which characteristics may be useful as indicators to predict the effect of digestate incorporation on soil AS, may be of practical interest in the design and conception of agricultural AD plants. It could be possible in future to modify AD plant process conditions to obtain digestates with optimal impact on soil AS. For example, production of digestate with higher levels of soluble, labile OM would increase the stimulation of soil microorganisms and the production of biotic aggregate binding agents.

However, there are numerous priorities to be taken into consideration with the installation and operation of an AD plant, such as optimising biogas production, managing incoming substrates to maintain plant operation, storing and processing digestate while avoiding excessive accumulation, and meeting the necessary directives around sanitisation and environmental emissions to name a few.

Producing digestate adapted for improvement of soil AS may come at a compromise to biogas yields, for example. Changes to digestate quality should also be balanced with the risks of soil application of unstabilised digestates in terms of nitrogen immobilisation (Alburquerque et al., 2012a) and phytotoxicity (Drennan and DiStefano, 2010). Going further, an opportunity exists for optimisation of digestate agronomic properties using combinations of existing post-treatment processes. For example, ammonia stripping of the liquid phase of digestate may leave behind a digestate with a high proportion of soluble OM that may be applied in greater quantities for improved effects on soil AS.

### 7.1.3 How can these findings be applied to digestate application in a wider range of agricultural contexts?

#### 7.1.3.1 What comparability do laboratory results have to the effects of digestate application in field conditions on the soil aggregate stability?

We conducted a field trial, as reported in chapter 5, intended to test whether short-term dynamics in soil AS associated with digestate application could be observed under field conditions, for a similarly textured silt loam soil as that used in laboratory incubation trials. While there may well be an upper limit to the quantity of digestate incorporated to the soil after which there is no further improvement to soil AS, as was apparent from the initial experiment reported in chapter 2, these doses are unlikely ever to be reached in an agricultural context. At agronomic application rates, the dose of digestate is likely an important factor in determining the scale of their effect on soil AS, as has been observed for other organic amendments (Cosentino, 2006; Metzger and Yaron, 1987; Özdemir et al., 2007). As we hypothesised that the application rate would be important in the comparative amplitude, and therefore the observability, of any effect from digestate on soil AS in relation to the effects of other factors (climatic conditions, soil heterogeneity), the study involved testing with both a simple ( $165 \text{ kg}_N \text{ Ha}^{-1}$ ) and double ( $330 \text{ kg}_N \text{ Ha}^{-1}$ ) agronomic dose of applied digestate.

Despite the highly heterogeneous soil conditions, and the fact that seasonal changes to soil AS did indeed outweigh the effects from the application of digestate for the most part, it was still possible to

draw a number of conclusions that supported the findings of the laboratory incubations. Firstly, no short-term negative effects that could be linked to the application of digestate were observed over the course of 8 months, even at a dose double ( $330 \text{ kg}_N \text{ Ha}^{-1}$ ) of that typically applied. This supported the conclusion from the laboratory trials that potential negative effects from high mineral content, and particularly high N content appeared not to outweigh the benefits from the additional OM incorporated. Secondly, correlation analysis of the data revealed a slightly higher MWD just after injection might have been associated with concentrated pockets of digestate in the soil profile. The restriction of application rates of manure-based digestates (not qualifying as an organic amendment) by the Nitrates directive n° 91/676/CEE, means that the quantities of raw or liquid phase of digestate applied in agriculture may rarely provide large benefits to soil AS over the short-term.

Much of our findings, obtained under controlled laboratory conditions, are related to the “internal potential” of the digestate in stabilising aggregate. This potential can be compared and contrasted against the wider potential of digestates, such as their ability to contribute to SOC and/or to their ability to supply nutrients for crop growth. How these various aspects of digestate potential, characterised under controlled laboratory conditions, might be extrapolated to application in the field is still under question. The modification of soil AS by digestate over the short-term as assessed through laboratory trials could ultimately provide an indication of the tendency in soil AS that multiple amendments of digestate are likely to produce over the medium- to long-term.

It might be concluded that, for the digestates studied in our incubation trials, the positive (or non-negative) short-term effects on soil AS observed should not logically lead to negative effects in the long-term. At the same time, the improvements to soil AS will be limited at agronomic application rates, will not be easily observed over the effects of climatic factors (soil humidity), and may well be outweighed by detrimental impacts from excessive tillage or over-fertilisation.

7.1.3.2 What are the interactions between soil conditions and digestate characteristics that may alter the observed impacts on soil aggregate stability?

Further to the question of whether the results obtained from initial experimentation were applicable in field trials, was whether the same impacts on soil AS would be observed in the case of different soil types (other than the silt loam soils tested throughout most of the project). In a final phase of laboratory experimentation, reported in chapter 6, we tested digestates representative of each group of the typology through incubation with two other soils (an OM rich loam KERGUE, and a clay loam (NAIZIN) in a similar manner to the incubations conducted previously. The observed changes in AS over time for the other soils following digestate incorporation were often very different for these other soils, in terms of the amplitude of effect and the form of the dynamic. Increases in  $MWD_{SW}$  relative to the control soil were around 20-30% for the naturally more stable NAIZIN and KERGUE soils, while for the fragile EFELE soil the increases were temporarily over 100% of the control soil  $MWD_{SW}$  in certain cases. Some transient negative effects on soil AS were observed for a digestate of dairy wastes (including Na and K rich milk permeate) in the other two soils. This stresses the need to look beyond the positive impacts of digestate OM (as appears generally to be the case) and consider the potential risks in the use of very mineral-rich digestates on dispersible clay soils.

Our results demonstrated that the interaction between digestate and soil is potentially quite complex and requires testing of a greater number of digestates incorporated into a wider range of soil types. They highlight a need to understand the precise direct interactions of digestate with soil clays and minerals, and indirect effects on the microbiological communities in the soil to build a better image of the resulting dynamic effects on soil AS.

## 7.2 Perspectives

Over the course of the project, there were many discussions around the possibilities for improvement of the experimental methods used, and the potential wider implications of the findings made. Several of what we consider to be some of the more pertinent points are considered in this section, with a

view to providing perspectives other than those already covered elsewhere in the manuscript, as well as (we hope) ideas and inspiration for future research efforts.

## 7.2.1 Potential for advancement of analytical techniques

### 7.2.1.1 The future relevance of studying soil AS

A central premise of this thesis project was the importance of the study of soil aggregates and their stability against the action of water as an indicator of soil physical health. Nonetheless, some consideration was given as to whether other measurements could have been selected other than the soil AS. Indeed, following on from our research, the question remains as to which methods of assessment of soil physical health are likely to be useful to future research conducted into the agronomic impact of digestates and other organic amendments. While still a rich ongoing area of research, the study of aggregate stability has been criticised over recent years as being more of an artefact of soil treatment than representing the real state of the soil (Rabot et al., 2018; Young et al., 2001). Proponents are pushing for a new soil structure model that considers, rather than being composed of an assembly of aggregates, the soil is more of a long, twisted pore network (Young et al., 2001). Nevertheless, in these critiques little thought is given to the question of how to study the resistance of the soil to various stresses (the “structural stability”) other than by way of the aggregate stability, and it remains a practical and simple quantitative measure even if the limitations of this measurement should be recognised (Young et al., 2001). A more progressive approach may well be to integrate the measurement of aggregate stability with measurement of soil porosity (the “structural form”) in future studies, therefore taking into account both the solid and void spaces of soil structure and providing complementary information (Schlüter et al., 2020). Indeed pore modelling of macroaggregates has shown that aggregate stability can be linked to the distribution of pore sizes and forms (Menon et al., 2020). In order to anticipate and adapt to a changing climate, integrated models are desperately needed, coupling the science of soil structural formation, stabilisation, and resilience, soil C and N dynamics, soil biology (microbial abundance and diversity, effect of macro-flora), and

hydrology. This would allow us to check, for example, if there is synergy or antagonism between a higher aggregate stability and related processes of soil crusting, soil erosion, water storage and water infiltration that would assist in managing both soil degradation and drought risk.

#### 7.2.1.2 Improvement of digestate characterisation and modelling of their impact on short-term soil AS

During the research reported in chapter 4 of this manuscript, it was possible to identify several characteristics of digestate that may be used as indicators of their potential to improve soil AS, in particular the organic matter fractions “SPOM” and “REOM” that may be obtained by simple sequential extraction methods. However, chemical fractionation methods are known to have certain limitations, as they can lack of selectivity in extraction of the targeted organic compounds or groups of compounds, extracting both “complex” recalcitrant OM at the same time as labile OM from organic materials such as digestate (Jimenez et al., 2015; Laera et al., 2019).

It may be possible to use alternative (spectroscopic) methods, such as NMR (Sarker et al., 2018) or NIR spectroscopy (Peltre et al., 2011) to characterise digestate OM, coupled with statistical techniques to identify relations between spectral peaks and temporal dynamics in soil AS. These methods may even permit identification of the hydrophobic or complex OM in digestate that may coat and protect soil aggregates (Voelkner et al., 2015a). However, spectral methods are also limited in that they provide only qualitative data, and therefore would not be useful in predictive models taking into account organic amendment at varying doses. Concerning the improvement of our understanding of soil AS dynamics linked to biotic factors, innovative methods such as Rock-Eval® might be employed to better characterise the active and recalcitrant organic carbon fractions in a digestate and predict their potential to stimulate soil microbial activity. Indeed, Rock-Eval® has already been successfully used in predicting digestate OM biodegradability in the soil (Béghin-Tanneau, 2020; Ducasse et al., 2023). Perhaps these innovative techniques could be used in future to increase the accuracy of prediction of statistical models of soil AS dynamics induced by digestate, through more precise identification of

organic matter biodegradability, and may even provide the data to adequately parametrise a new mechanistic model.

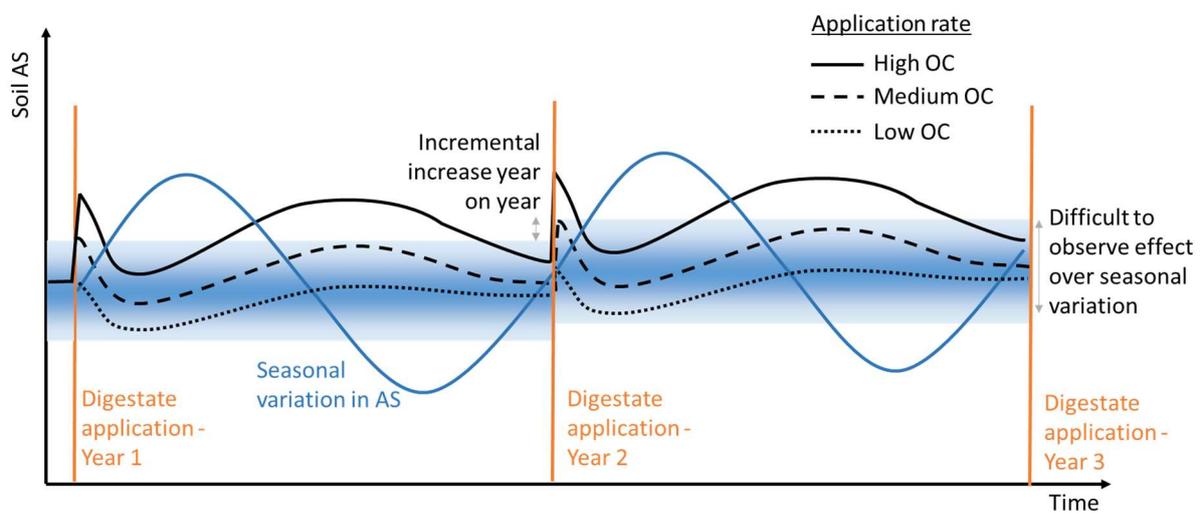
Furthermore, as was already suggested in chapter 4 and chapter 6, it may be equally necessary to account for the abiotic impact of digestate on soil exchangeable cations as a potential factor in aggregate cohesion/dispersion, when considering application to dispersible (clay) soils. Rengasamy (2018) propose using the “cation ratio for soil structural stability” (CROSS) for assessment of the balance of K, Na, Mg, and Ca in solution for irrigation water. This method might equally be applied to assessment of liquid digestates high in minerals. To summarise, future efforts to model short-term AS dynamics following organic amendment could be adapted to account for three aspects in particular:

- To factor in an initial, abiotic stabilisation of soil aggregates and the fact that this may also lead to a certain dynamic in AS, being either transient (degradable lipids) or recalcitrant (high molecular weight aromatic molecules, polycations),
- To account for multiple peaks in aggregate stability over time, linked to degradation of different OM fractions contained within complex organic residues (see Fig. 6.1),
- To be adjustable in terms of the link between aggregation, aggregate stabilisation, and the quantity of C and N pools available/accessible to soil microbial communities.

#### 7.2.1.3 Observing and predicting long-term impacts of digestate on soil AS

While this thesis project has focussed on understanding the short-term dynamics in soil AS following digestate amendment, this could also help in developing an understanding of cumulative long-term dynamics in soil AS from repeated digestate applications. For example, it may prove possible to generate a model based on the conceptual model proposed by Annabi et al. (2007) (and described in the state-of-the-art at the beginning of this manuscript), so that various AS dynamics, different application rates and seasonal variations may be accounted for. For many of the digestates studied (in groups 1 and 3 of the typology of impact) the MWD of soil had increased up to the end of experimentation after 6 months, and it is possible that this effect may well have persisted further,

though we cannot verify this from our data. Other studies do indeed show that certain digestates may induce increases in soil AS well beyond 6 months (Badagliacca et al., 2020; Sarker et al., 2018). However, it is not always easy to provide evidence of medium- term effects (2+ years) of digestate (and other organic amendments) application which may be due to their sometimes limited impact after this time (refer back to the summary in Table. 1.2). Conversely, long-term positive effects may be observed after >5 years of repeated applications (Eich-Greatorex et al., 2020; Rezacova et al., 2021), suggesting that a gradual increase in soil AS is occurring over time.



*Fig. 7.4 Conceptual diagram of the minor incremental improvement to soil AS from consecutive applications of a raw digestate (type 3) observable depending on the application rate and the seasonal variation in soil AS.*

Much of the differences in impact of digestate on soil AS observed in long-term trials, as is the case for the short-term impacts, likely depend the agricultural context and the soil type (texture, mineralogy, biology). The silt loam soil, used in the majority of incubation experiments of our research, was sampled from a long-term research site (EFELE SOERE PRO) investigating the long-term effects of organic and synthetic fertilisation on soil properties. Reports from this trial have revealed only a very small evolution in soil AS, even after almost 10 years of repeated applications of various organic amendments (ADEME et al., 2016; Morvan et al., 2022), linked to a similarly limited increase in SOC over this time. Nonetheless, there is some anecdotal evidence that, over the very long-term, increases in soil AS from digestate incorporation may be achieved under these conditions. The soil used in the

field trial in chapter 5 (referred to hereby as GEVEZE, in reference to the location of the trial site), was a silt loam of very similar properties (clay mineralogy, exchangeable minerals - see data in Appendix 1), to the EFELE soil. The two have been subjected to relatively similar agricultural practices over the recent past (wheat-maize crop rotation, conventional tillage, etc.) and are subject to the same climatic conditions. Nonetheless, having received regular applications of digestate for more than 15 years, the GEVEZE soil has both higher OC content and higher AS (in spring 2022: SOC = 1.26%,  $MWD_{SW} = 0.68$  mm) than the EFELE soil (in spring 2022: SOC = 0.87% and  $MWD_{SW} = 0.4$  mm). With a better understanding of short- to medium- term effects of digestate on soil AS, undoubtedly a model could be constructed to allow prediction of effects up to the longer term (see Fig. 7.4).

#### 7.2.1.4 The impact of digestates on microbial communities and the connection with soil structure

The study of this thesis has been focussed upon assessment of the link between biochemical characteristics of digestate and their impact on soil aggregate stability, but did not directly investigate the soil microbiological processes that may be involved. Advancement in our understanding of the biotic mechanisms by which soil aggregates are stabilised following digestate amendment will require adequate understanding of microbial community dynamics as well. As already discussed in chapter 1, our current understanding of soil biological changes in relation to amendment with different digestates in different soil contexts is still limited (Karimi et al., 2022). However, evidence points towards the effects of digestate application on soil biology being relatively small, over the short and the long term (Coelho, 2019; Johansen et al., 2013). When compared to other classical organic fertilisers/amendments such as animal manures, plant wastes and composts, digestate application leads to weaker responses in global microbial activity (Karimi et al., 2022). Although the results have not been reported fully in this thesis manuscript, qPCR and detailed sequencing analysis of fungal, bacterial, and archaeal DNA in the soils samples taken from incubation of digestates after 182 days were carried out as part of a Masters Student internship project. The results showed little significant

differences between the control soil and soils amended with digestates, even given the very high dose of digestate applied ( $6 \text{ g}_C \text{ kg}_{\text{dry soil}}^{-1}$ ), which would reinforce the conclusions from the other studies.

Even assuming that some changes in microbial populations do occur over the short-term following digestate amendment, for the moment it is not exactly known which microbial taxonomic group (fungi or bacteria) is most involved in aggregate stabilisation in certain soil contexts, although evidence points towards filamentous fungi being principally responsible for stabilising macro-aggregates in most cases (Lehmann et al., 2017). What is becoming apparent from research covering numerous types of organic soil amendments and their impact on soil structure is that increasing overall soil microbial biomass is favourable to soil structural stability (Chenu et al., 2011), and that increased soil microbial diversity appears to be an important factor in both soil aggregation and stabilisation (Lehmann et al., 2017). It is possible that future research into the effects of digestate amendment on combined soil microbial diversity and abundance will one day provide useful insight into understanding the predominant biotic mechanisms underlying the processes of aggregate stabilisation.

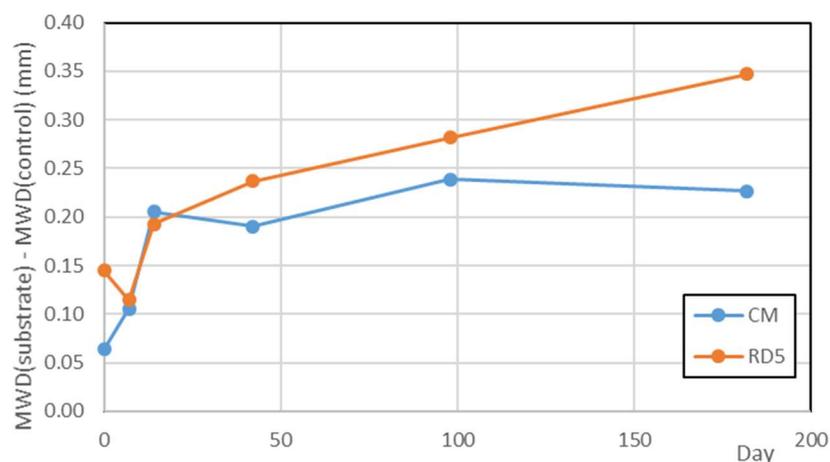
## 7.2.2 Advancement of our understanding of the agricultural impact of digestates on soil structural stability in varying contexts

### 7.2.2.1 Soil amendment with digestate vs. untreated organic residue

One key debate around the mobilisation of farm organic residues (crop residues, cover crops, manures and slurries) for biogas production from AD is what effect the replacement of their use in soil application by the resulting digestates will have. A number of studies, have compared between the agricultural use of digestates and their substrates in terms of their use as fertiliser, their impact on SOC or GHG emissions (discussed in chapter 1), or their effect on soil microbiology (see previous section). However, the sparseness of existing studies means that there is still the need for comparison of the impact of digestates vs. substrates (classic organic amendments), or digestates vs. mineral fertilisation, on soil aggregate stability over both the short- and long-term in various contexts. Indeed, the results

of the current thesis highlight this need, simply in view of the variety of possible outcomes originating from the choice of the type of digestate alone.

While we would not be able to provide a conclusive answer, it is possible to comment on comparison of digestates with the two undigested residues tested - a maize straw (MS) and a cow manure (CM) (see chapter 3). As short-term soil aggregate stability dynamics are determined by labile carbon forms more than recalcitrant carbon forms, all digestates had predictably lower impact on soil AS than a readily decomposed maize straw (which had a total C mineralisation over 50%) over the 6-month incubation period. Béghin-Tanneau (2020), over the course of his thesis work, compared between the effects on soil AS of a similar carbon dose of undigested and digested maize silage and found a similar result.



*Fig. 7.5 Comparison of the AS dynamic in soil following amendment with cow manure (CM) and a raw dry digestate of cow manure (RD5)*

On the other hand, many of the digestates tested in our soil incubation trials actually initiated a comparable or higher reaction in soil AS than CM, notably a digestate issue from dry digestion of cow manure (RD5) (see Fig. 7.5.). In certain cases, the digestate may still contain large amounts of readily biodegradable C leading to a strong microbial response following amendment to soil (Albuquerque et al., 2012b). However, this comparison only applies in the case of soil amendment with an identical quantity of organic carbon, and does not account for the organic carbon lost over the course of AD, or

the limitations to N-rich digestate application rates as per the nitrates directive. Our findings are in no way intended to compare the use of digestate over other organic amendments, but rather to provide the understanding required in their correct management concerning maintenance of soil physical health. Studies taking into account the realistic differences in application rates between digested and undigested substrates would provide perhaps a more useful response to this comparison.

#### 7.2.2.2 The wider impacts of AD on soil structural stability and the integration with an agro-ecological transition

This thesis work provides a number of insights into potential ways to improve utilisation of digestates in agriculture for the maintenance of soil structure, but it was not within the scope of the current thesis project to investigate the impact of digestate application on other aspects of soil (chemical and biological) fertility. The value of digestates as an organic fertiliser, and their potential for replacing imported mineral fertilisers, given the urgent need for alternative nutrient sources, is of perhaps greater current interest and importance to the agricultural sector than their organic amendment value. Given the numerous interactions involved in soil fertility, there is certainly a need for a comprehensive study into the broader impacts of AD installations in an agricultural context on soil fertility, including physical health. For example, the beneficial effects of digestate fertilisation on plant growth, and the simultaneous benefits of plant root systems and plant residues to soil structure, should be considered in any wider study of digestate impact on soil physical health. It has been claimed that the most significant impact of digestate application on soil physical properties may be due to changes in cropping regimes (Möller, 2015; Paolini et al., 2018), such as the introduction of cover crops destined for AD. Evaluation of the effects of AD installations on the cycles of C and N at a farm-scale has provided useful information (Moinard, 2021), revealing both positive impacts in terms of overall C storage, and negative impacts in terms of N loss through emissions. Integration of analyses of soil structure into similar farm-scale studies may serve to answer an important questions around the risks and benefits of widespread mobilisation of on-farm AD plants to soil physical health.

Beyond the questions around what impact AD has on the current farming landscape is the question of how it may be integrated with future agricultural practices and, in particular, what place it has in the drive towards regenerative, agro-ecological systems. The use of digestates as fertilizer and soil amendment could be integrated positively with other recommended management practices for protection of soil structure, such as diversified crop rotations, or reduced tillage (Jensen et al., 2019). For example, the adoption of minimal tillage practices coupled with digestate application through injection (in order to reduce ammonia emissions through volatilisation) would lead to higher concentration of digestate near the soil surface. This may then concentrate positive impacts on soil AS at the soil surface where the effects of disaggregation by water are most apparent. The removal of cover crops for biogas production if followed by the return of the produced digestate does not appear to negatively affect soil AS or earthworm populations relative to direct incorporation of the cover crop (Frøseth et al., 2014). Comparison between the long-term effects of biodynamic organic farms operating with or without digestate application show little difference in terms of SOC and microbial populations (Wentzel et al., 2015). However, the authors of this same research acknowledge that the other practices used to promote soil health that are typical to biodynamic farms, such as the use of highly diversified crop rotations, had a far greater influence on the soil than the type of organic fertilisation used. In our opinion, the valorisation of digestates from AD alongside adoption of agro-ecological practices is entirely possible, but requires a considerable amount of further research in order to determine the potential benefits and trade-offs involved.

This thesis work has highlighted a number of knowledge gaps and research needs for developing a more complete understanding of digestates and their impact on soils in an agricultural context. Going forward, our methods and findings may be adapted, and used to form part of a greater body of information around digestate impact on the soil. In light of the rapidly developing AD sector, and the growing need for better soil management, a comprehensive typology should be prioritised, combining data on the effects of digestates on carbon storage, nutrient supply, aggregate stability and other

physical properties, based on a large number of digestates and comprising results from trials in a number of agricultural contexts.

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## 9 Appendices

○ Appendix 1 – Properties of soils

*Table. 9.7 Physico-chemical properties of the soils tested during the thesis project*

Soil name		EFELE	KERGUE	NAIZIN	MUEL	GEVEZE
Chapter where soil is studied		3,4,6	6	6	2	5
Taxonomy		Luvisol-redoxisol	Brunisol	Humic cambisol	Brunisol	Luvisol
Clay (0 - 0,002 mm)	g kg <sup>-1</sup>	15	17	27	19	15
Silt (0,002 - 0,05 mm)	g kg <sup>-1</sup>	69	42	51	60	77
Sand (0,05 - 2 mm)	g kg <sup>-1</sup>	16	41	22	21	8
Organic matter	g kg <sup>-1</sup>	15	32	48	23	21
TN	g kg <sup>-1</sup>	1.0	1.7	2.7	1.3	1.3
CaCO <sub>3</sub>	g kg <sup>-1</sup>	<1	<1	<1	<1	<1
TOC	g kg <sup>-1</sup>	8.7	18.7	28.0	13.2	12.3
C-N ratio	-	8.4	10.9	10.5	10.1	9.5
pH (water)	-	6.9	5.6	6.3	6.0	6.2
P (Olsen)	g kg <sup>-1</sup>	0.1	0.1	0.0	0.0	0.0
CEC Metson	cmol+ kg <sup>-1</sup>	6.4	9.3	10.9	6.7	7.0
Cu	mg kg <sup>-1</sup>	6.2	4.0	3.8	3.2	2.9
Fe	mg kg <sup>-1</sup>	284	158	105	230	141
Mn	mg kg <sup>-1</sup>	50	31	16	61	50
Zn	mg kg <sup>-1</sup>	6.3	2.5	2.3	1.7	3.9
Al	g 100g <sup>-1</sup>	0.1	0.3	0.3	0.1	0.1
Fe	g 100g <sup>-1</sup>	0.4	0.5	0.6	0.5	0.4
Si	g 100g <sup>-1</sup>	0.0	0.0	0.0	0.0	0.0
Al	g 100g <sup>-1</sup>	9.3	8.2	9.2	5.0	4.2
Ca	g 100g <sup>-1</sup>	1.4	1.9	1.5	0.2	0.3
Fe	g 100g <sup>-1</sup>	5.6	5.3	5.7	2.9	1.7
Mg	g 100g <sup>-1</sup>	0.7	1.1	0.8	0.3	0.3
Mn	mg kg <sup>-1</sup>	1258	1190	1235	409	591
P	g 100g <sup>-1</sup>	0.2	0.2	0.2	0.1	0.1
K	g 100g <sup>-1</sup>	2.3	2.4	2.2	1.6	1.5
Na	g 100g <sup>-1</sup>	1.7	1.8	1.8	0.6	0.8

○ Appendix 2 - Supplementary information for article in chapter 4

Table. 9.8 Estimated mean of digestate components (characteristics) ( $g\ kg^{-1}_{dry\ soil}$ ) added to the soil prior to incubation trials, as well as the soil parameters measured over the course of the incubation

Mod	RD1a	RD1b	RD2	RD3	RD4	RD5	LP2	LP4	LP7	SP2	SP3	CS4	CS5	CS6	CS7	MAX	MIN
TS	17.32	17.71	14.53	19.09	18.02	14.95	16.25	20.87	16.69	12.98	13.64	16.05	16.65	15.25	21.29	21.29	12.98
VS	10.76	10.34	10.01	11.45	11.07	10.97	10.01	10.83	11.00	10.69	11.40	11.06	10.65	10.50	11.03	11.45	10.01
DA	6.60	7.39	4.61	7.79	7.07	3.95	6.18	10.19	5.98	2.30	2.34	5.08	5.90	4.77	10.38	10.38	2.30
TC	6.00	6.00	6.00	6.00	6.00	5.33	6.00	6.00	6.00	6.00	6.00	6.00	6.06	6.00	6.00	6.06	5.33
IC	0.07	0.06	0.08	0.06	0.07	0.08	0.10	0.09	0.01	0.07	0.02	0.05	0.10	0.08	0.04	0.04	0.01
TOC	5.93	5.94	5.92	5.94	5.93	5.25	5.90	5.91	5.99	5.93	5.98	5.95	5.95	5.92	5.96	5.99	5.25
TKN	0.42	0.44	0.46	0.61	0.52	0.38	0.58	0.61	0.96	0.32	0.25	0.32	0.44	0.34	0.55	0.96	0.25
SPOM_%COT	8.63	8.55	6.03	6.18	4.83	6.50	11.53	11.64	11.14	2.39	1.44	2.37	5.65	5.02	1.42	11.64	1.42
REOM_%COT	1.90	2.04	4.79	2.94	5.08	3.21	6.26	8.70	8.19	3.16	2.03	4.49	4.11	1.66	3.60	8.70	1.66
SEOM_%COT	14.97	15.01	14.38	15.35	20.38	16.58	17.97	25.59	19.27	11.03	13.16	16.43	12.92	14.51	15.77	25.59	11.03
PEOM_%COT	28.18	34.36	23.79	31.89	27.35	49.53	18.77	14.88	21.40	32.46	41.18	39.59	35.69	37.66	21.64	49.53	14.88
NEOM_%COT	46.32	40.04	51.00	43.64	42.36	24.18	45.46	39.19	40.00	50.96	42.19	37.13	41.63	41.15	57.57	57.57	24.18
SPOM_%TKN	17.42	17.44	13.32	9.19	9.68	15.96	18.77	18.20	14.29	11.24	6.99	15.47	15.09	12.24	9.14	18.77	6.99
REOM_%TKN	5.69	5.91	8.42	5.52	9.18	9.46	9.06	11.53	13.10	9.25	8.67	13.18	10.60	4.64	7.82	13.18	4.64
SEOM_%TKN	26.80	26.45	19.17	23.67	24.14	30.18	19.57	27.10	27.51	17.82	26.19	32.93	24.36	24.75	29.42	32.93	17.82
PEOM_%TKN	27.07	27.15	25.03	27.85	24.35	17.40	22.60	15.89	18.78	22.76	29.52	12.09	23.08	21.53	12.69	29.52	12.09
NEOM_%TKN	23.02	23.06	34.06	33.77	32.65	26.99	30.00	27.28	26.32	38.92	28.63	26.32	26.88	36.84	40.93	40.93	23.02
SPOM_CN	6.97	6.56	5.88	6.55	5.68	5.65	6.29	6.18	4.84	4.01	4.88	2.85	5.03	7.17	1.69	7.17	1.69
REOM_CN	4.70	4.60	7.39	5.18	6.31	4.70	7.11	7.29	3.89	6.44	5.55	6.35	5.22	6.25	5.00	7.39	3.89
SEOM_CN	7.86	7.58	9.71	6.33	9.61	7.64	9.40	9.13	4.35	11.64	11.95	9.32	7.14	10.25	5.83	11.95	4.35
PEOM_CN	14.71	17.03	12.42	11.14	12.81	39.68	8.50	9.07	7.13	26.90	33.09	61.13	21.08	30.93	18.52	61.13	7.13
NEOM_CN	28.63	24.92	19.56	12.74	14.78	12.44	15.55	14.01	9.47	24.64	35.73	26.32	20.95	19.74	15.29	35.73	9.47
TK	0.89	1.05	0.69	1.29	0.60	0.64	0.96	1.09	0.84	0.20	0.31	0.33	0.93	0.87	0.21	1.29	0.20
Na	0.49	0.52	0.14	0.73	0.18	0.05	0.20	0.39	0.49	0.04	0.17	0.09	0.06	0.06	0.05	0.73	0.04
Ca	0.40	0.40	0.36	0.64	0.35	0.25	0.46	0.51	0.32	0.22	0.18	0.27	0.36	0.28	0.43	0.64	0.18
Fe	0.11	0.12	0.05	0.38	0.11	0.05	0.07	0.15	0.40	0.03	0.13	0.11	0.08	0.07	0.39	0.40	0.03
Mg	0.12	0.14	0.11	0.16	0.16	0.09	0.11	0.18	0.04	0.10	0.10	0.15	0.12	0.08	0.08	0.18	0.04
Al	0.11	0.13	0.06	0.04	0.11	0.07	0.09	0.16	0.22	0.02	0.01	0.09	0.10	0.08	0.31	0.31	0.01
Cu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
pH_0	7.76	7.89	7.40	8.10	7.57	7.09	7.42	8.15	7.50	6.92	7.18	7.26	7.27	7.18	7.02	8.15	6.92
cond_0	460	498	324	924	338	217	367	580	661	139	309	228	337	277	207	923.75	139.13
WDPT_0	3.85	4.24	3.22	2.25	2.82	2.97	3.44	3.12	4.25	2.32	1.83	1.93	4.57	2.05	1.38	4.57	1.38
WDPT_14	6.22	6.65	4.37	2.56	4.18	3.21	3.00	3.72	3.74	2.91	1.78	2.25	4.63	3.68	2.11	6.65	1.78
WDPT_98	8.08	5.66	5.34	3.72	3.33	3.76	3.81	3.52	6.77	5.01	2.68	2.23	5.68	4.03	2.31	8.08	2.23
dMWD_0	0.10	0.10	0.19	-0.04	0.04	0.14	0.30	0.28	0.19	0.06	0.04	0.16	0.13	0.10	0.05	0.30	-0.04
dMWD_7	0.15	0.14	0.16	0.61	0.50	0.11	0.19	0.22	0.03	0.09	0.16	0.17	0.09	0.08	0.24	0.61	0.03
dMWD_14	0.20	0.15	0.12	0.14	0.24	0.19	0.24	0.23	0.24	0.09	0.17	0.13	0.08	0.09	0.05	0.24	0.05
dMWD_42	0.21	0.16	0.21	0.03	0.03	0.24	0.21	0.19	0.32	0.12	0.02	0.11	0.11	0.01	0.03	0.32	0.01
dMWD_98	0.43	0.39	0.34	0.12	0.26	0.28	0.31	0.16	0.34	0.14	0.25	0.13	0.26	0.14	0.09	0.43	0.09
dMWD_182	0.26	0.23	0.51	0.23	0.50	0.35	0.32	0.68	0.78	0.20	0.32	0.15	0.35	0.16	0.23	0.78	0.15
AUC	55.02	48.04	57.64	25.91	48.10	48.94	50.95	54.22	74.76	26.09	35.75	24.01	39.68	19.15	20.13	74.76	19.15
dMWD_max	0.43	0.39	0.57	0.61	0.54	0.45	0.38	0.68	0.78	0.21	0.32	0.21	0.35	0.16	0.28	0.78	0.16

Table. 9.9 Calculated complexity index for the SPOM fraction (CI\_SPOM) of extracted OM from digestates. Same letters next to values denote values that are not significantly different ( $p>0.05$ ).

Digestate	CI_SPOM	SD
LP2	0.838 a	0.208
RD3	0.856 a	0.067
RD2	0.674 ab	0.197
SP2	0.687 ab	0.048
CS4	0.636 abc	0.145
LP7	0.554 abc	0.075
RD1a	0.545 abc	0.087
RD1b	0.577 abc	0.150
RD4	0.625 abc	0.093
SP3	0.587 abc	0.170
CS6	0.440 bc	0.040
LP4	0.471 bc	0.047
RD5	0.413 bc	0.047
CS5	0.330 c	0.020
CS7	0.309 c	0.060

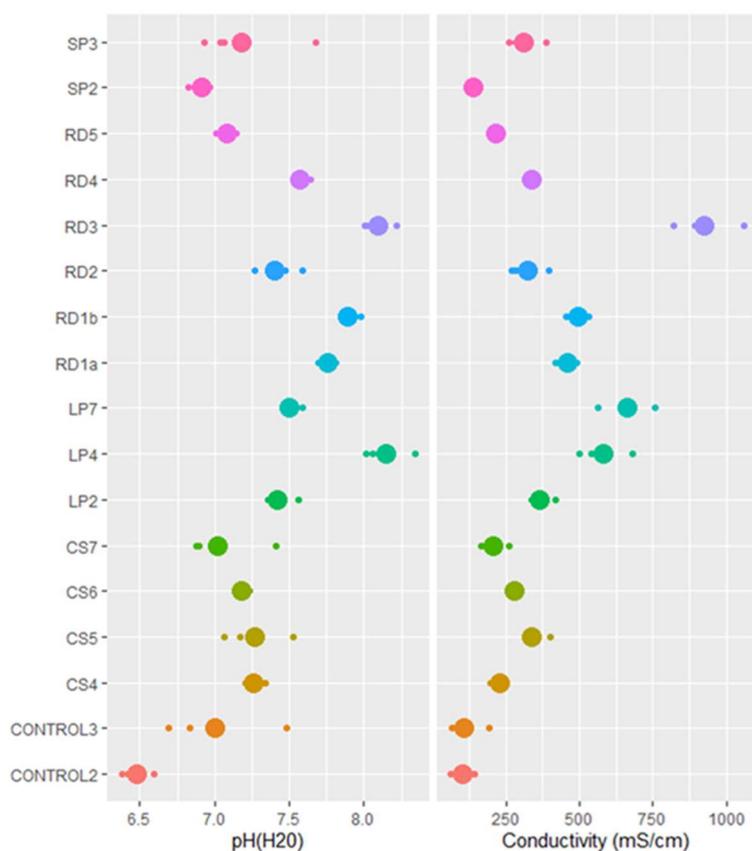


Fig. 9.20. Soil pH and conductivity following amendment with digestates (at day 0 of incubation trials). Measurement was taken on a 1:5 ratio mixture of soil and milliQ water. Large points represent the mean, small points represent each repetition.

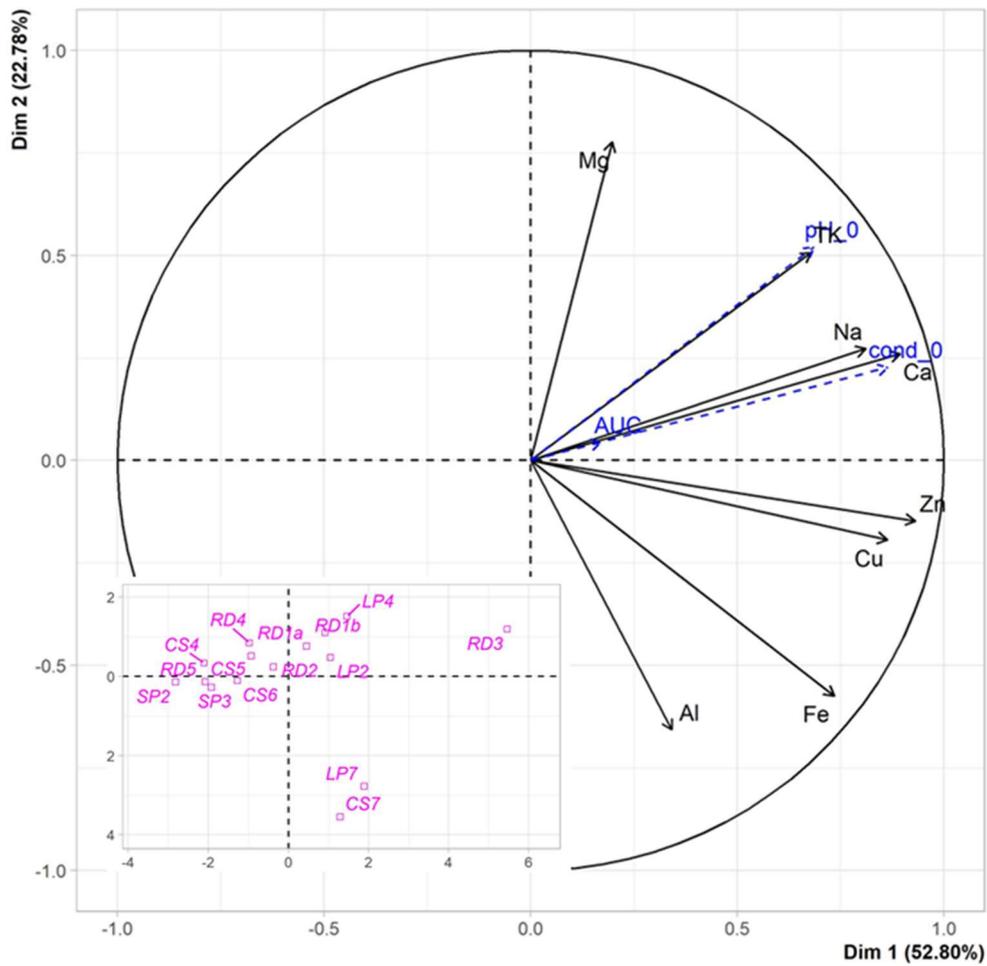


Fig. 9.21. PCA biplot of analysed total elemental composition of digestates, with measured soil pH and conductivity following amendment at day 0 of incubation trials as supplementary variables (blue dashed line). The AUC calculated as an indicator of overall impact on soil aggregate stability (AS) is also placed as a supplementary variable (blue dashed line). The plot of individuals is provided in the bottom left.

Table. 9.10 Full list of model parameters and calculated performance indicators returned by XLStat for PLS-R modelling

Y	X	n. X	n. Dim	Q <sup>2</sup>	R <sup>2</sup> Y	R <sup>2</sup>	
AUC	EXT_TOC,EXT_TN, CI_SPOM	11	2	0.54	0.69	0.71	
	EXT_TOC, EXT_TN	10	3	0.44	0.73	0.75	
			2	0.57	0.69	0.71	
	EXT_TOC	5	3	0.35	0.64	0.66	
			2	0.45	0.60	0.62	
	EXT_TN	5	3	0.55	0.73	0.75	
			2	0.64	0.72	0.74	
	SPOM_COT, REOM_COT, SPOM_NT, REOM_NT	4	3	0.50	0.67	0.69	
	EXT_TOC, CI_SPOM	6	2	0.41	0.60	0.62	
	EXT_TN, CI_SPOM	6	2	0.56	0.71	0.73	
	EXT_TN, SPOM_TOC, CI_SPOM	7	2	0.58	0.70	0.72	
	EXT_TN, RAND1, RAND2	7	2	0.53	0.72	0.74	
	EXT_TN, Cond_0	6	4	0.44	0.78	0.80	
			3	0.57	0.76	0.78	
	EXT_TN, pH_0	6	3	0.50	-	0.75	
			6	2	0.59	0.72	0.75
	EXT_TOC, TKN	6	3	0.39	0.65	0.67	
			2	0.50	0.62	0.64	
	EXT_TN, WDPT_0	6	3	0.51	0.75	0.77	
	EXT_TN, cond_0, pH_0, WDPT_0	8	3	0.54	0.78	0.81	
EXT_TN, Na, K, Ca, Mg, Fe, Al, Zn, Cu	13	3	0.51	0.77	0.79		
EXT_TN, Zn	6	3	0.66	0.79	0.81		
EXT_TN, TK, Fe, Na	8	4	0.52	0.82	0.85		
MWD_0	EXT_TOC, EXT_TN	10	2	0.47	0.67	-	
	EXT_TOC	5	1	0.32	0.39	0.45	
	EXT_TN	5	2	0.48	0.66	-	
MWD_182	EXT_TOC	5	1	0.55	0.61	0.63	
	EXT_TN	5	1	0.53	0.57	0.66	

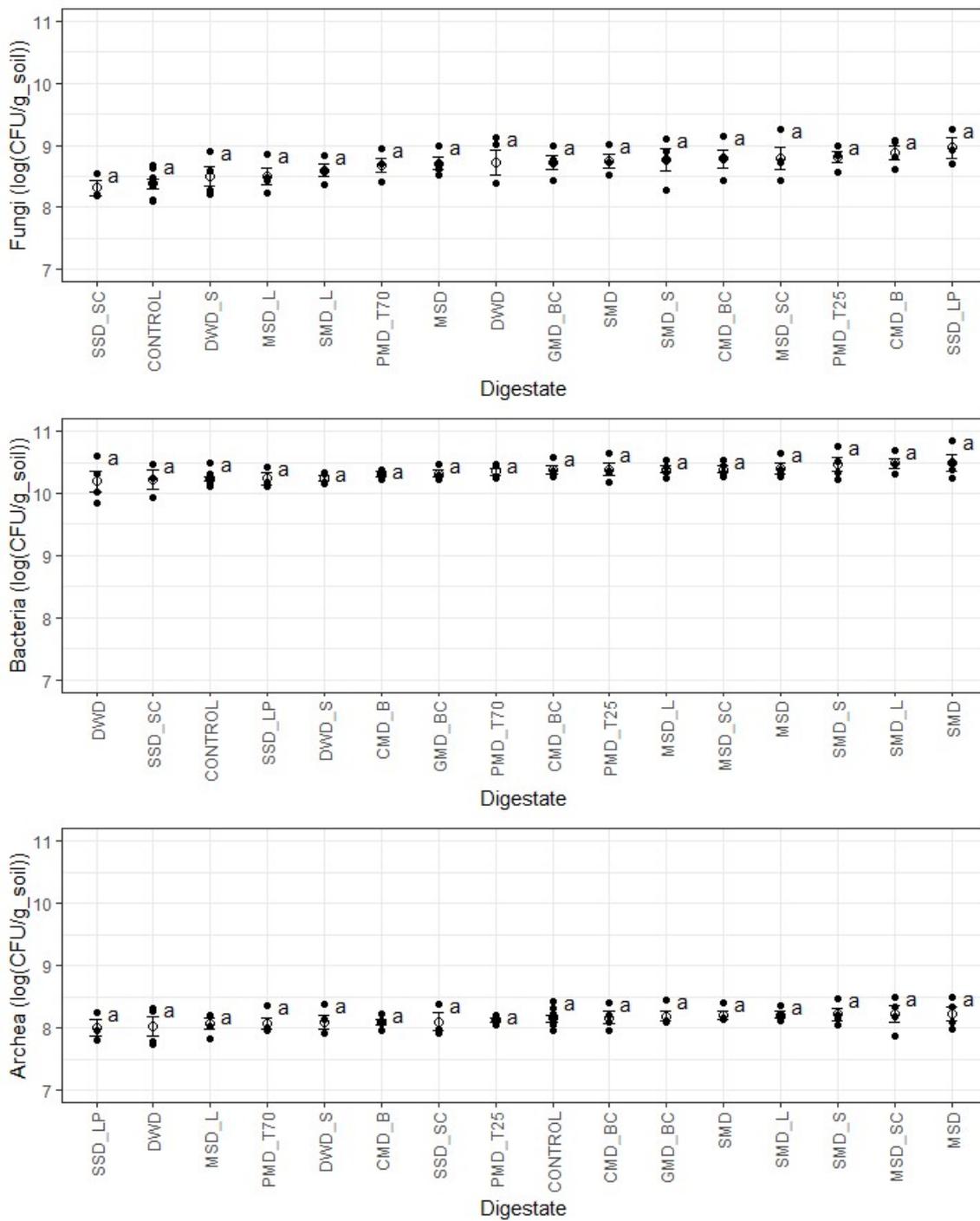


Fig. 9.22 Boxplots of gene abundance for soil microbial communities determined by qPCR

o Appendix 3 - Supplementary information for article in chapter 5:  
Plan of field trial and precision of sampling points

A portion of the experimental site was divided into a randomised block design, composing three blocks of three treatments oriented along the east-west axis, in order to block any effect from the previously observed heterogeneity in soil conditions across this axis. Care was taken to place the site at a reasonable distance from field boundaries with large trees (minimum distance 20-30m) and hedges (10-15m).

The three treatments comprised differing rates of digestate application:

- No digestate (D0) – ‘0 Dose’
- 25 m<sup>3</sup>/Ha digestate (D25) – ‘1 Dose’
- 50 m<sup>3</sup>/Ha digestate (D50) – ‘2 Dose’

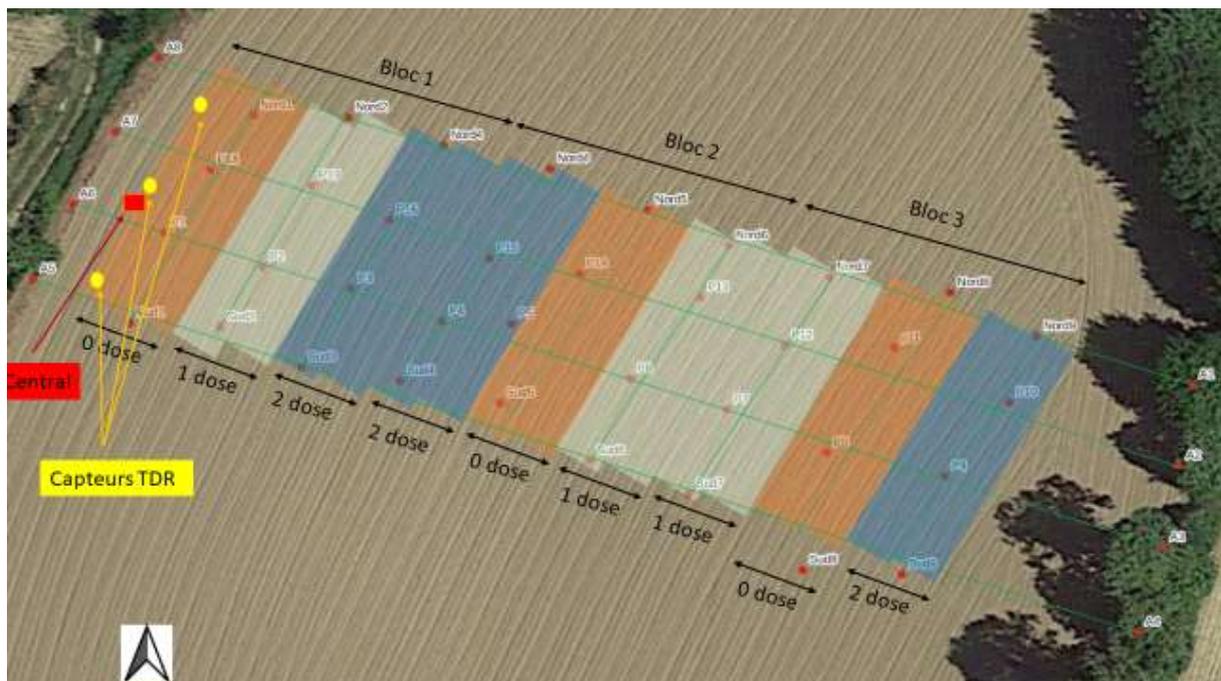


Fig. 9.23 Satellite image of field trial site with superimposed layout of the complete randomised block design used.

Three fixed soil humidity, conductivity and temperature sensors were installed at the edge of the parcel, in one block that received no digestate 3 weeks after the date of commencement of the trial.

The sensors were buried at 10cm below the soil surface, and took measurements at half hour intervals. The volumetric humidity was verified by taking regular soil samples using a cylinder of fixed size and drying for 48 hours at 105°C.

Soil sampling from all blocks was carried out the day before digestate application (using an approximate positioning of the envisaged experimental block arrangement), and at several dates after (see fig). The north-south boundaries and the centreline for each zone within the site was plotted by GPS, and two samples taken from approximately 3 m either side of this centreline at the northern and southern ends of each zone, respectively (see figure). Variability in the accuracy of the GPS (+/-3 m on average) on the day of sampling means some samples may have been taken from outside the intended area.

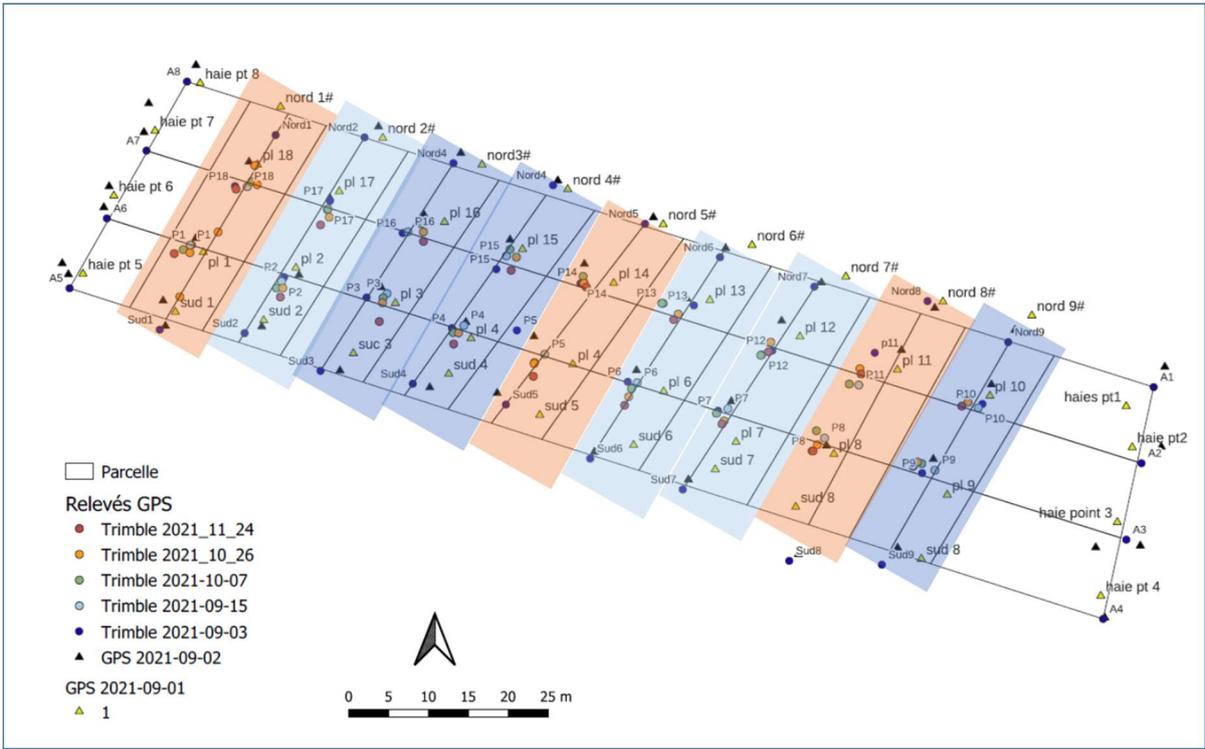


Fig. 9.24 GPS map of points where soil was sampled at each date during the field trial with an approximate plan of the location of each of the blocks of the complete randomised block layout.

**Title :** Study and prediction of the impact of anaerobic digestion process parameters on the composition of digestates, and their effect on the structural stability of soils

**Keywords :** Aggregate stability, digestate, anaerobic digestion, soil organic amendment

**Abstract :** Demand for renewable energy is driving rapid growth in the agricultural anaerobic digestion (AD) sector. Digestates, by-products from AD, are used as organic fertilisers and soil amendments. Yet, our understanding of the effects of the wide array of digestates produced on soil biological, chemical and physical processes is limited. Our objective was to investigate the impact of digestate application on soil physical health, through study of the aggregate stability (AS), a key indicator of soil resistance to crust formation and erosion. Laboratory testing of digestates from agricultural AD sites revealed that their short-term impact on soil AS may vary considerably. A proposed typology of impact categorises the likely effect of different digestates according to AD process parameters, as an aid to agronomic decision-making and AD plant conception. We determined digestate characteristics linked to changes in soil AS, and

considered the mechanisms involved, including direct interactions between soil and mineral or organic components, and indirect stimulation of microorganisms by “bioaccessible” organic matter. While the short-term effects of digestate on silt soil AS in field trials were limited compared to climate-induced variations, there were no negative effects. Digestate application may even lead to a very gradual improvement in AS of silt loam soils over time. However, certain mineral-rich digestates can induce transient negative effects on AS in other soil contexts. These findings provide insight into the short-term impacts of digestate on soil processes leading to aggregate stabilisation. Nevertheless, going forward, an integrated approach accounting for many issues surrounding digestate use in agriculture is required to achieve sustainable soil management.

**Titre :** Etude et prédiction de l'impact des paramètres de digestion anaérobie sur la composition des digestats et leur effet sur la stabilité structurale des sols

**Mots clés :** Stabilité structurale, digestat, méthanisation, amendement organique du sol

**Résumé :** La croissance de la digestion anaérobie en Europe conduit à une production importante de digestats. L'objectif de cette thèse était d'améliorer la compréhension de l'impact de l'application de ces derniers sur la fertilité physique du sol, grâce à l'analyse de la dynamique à court terme de la stabilité des agrégats du sol (SA), un indicateur clé de la résistance structurelle du sol à la formation de la croûte et à l'érosion. Les analyses réalisées sur des digestats prélevés ont montré que leurs impacts à court terme sur la SA du sol peuvent varier considérablement. Une première typologie d'impact a été élaborée, catégorisant l'effet des différents digestats selon les modes de production utilisés. Nous avons également pu identifier des corrélations entre les caractéristiques du digestat et leurs effets sur la SA du sol. Ce qui a permis d'identifier l'implication des mécanismes suivants : i) interactions directes entre le sol et les composants minéraux et organiques du

-digestat, ii) stimulation indirecte des processus microbiologiques par la matière organique du digestat conduisant à la stabilisation des agrégats. Une expérimentation au champ a montré que, si les effets du digestat sur la SA d'un sol limoneux sont limités par rapport aux variations induites par le climat, leur application peut néanmoins compenser les impacts négatifs transitoires d'un travail du sol. Nos résultats indiquent que l'incorporation de digestat peut même conduire à un impact positif progressif mais global sur la SA des sols limoneux à moyen terme. Cependant, en fonction des propriétés du sol et du contexte agricole, il convient de veiller à évaluer au préalable la qualité des digestats destinés à l'épandage, car certaines caractéristiques telles que des teneurs élevées en minéraux peuvent entraîner des effets négatifs transitoires sur la SA du sol.

