



HAL
open science

**Compétitivité des légumineuses vis-à-vis des adventices :
traits impliqués dans la capture précoce de l'azote
minéral du sol et complémentarité des traits entre
espèces dans des associations légumineuse-non
légumineuse**

Elana Dayoub

► **To cite this version:**

Elana Dayoub. Compétitivité des légumineuses vis-à-vis des adventices : traits impliqués dans la capture précoce de l'azote minéral du sol et complémentarité des traits entre espèces dans des associations légumineuse-non légumineuse. Sciences agricoles. Université d'Angers, 2017. Français. NNT : 2017ANGE0031 . tel-01730275

HAL Id: tel-01730275

<https://theses.hal.science/tel-01730275>

Submitted on 13 Mar 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Thèse de Doctorat

Elana DAYOUB

*Mémoire présenté en vue de l'obtention du
grade de Docteur de l'Université d'Angers
sous le sceau de l'Université Bretagne Loire*

École doctorale : Végétal, environnement, nutrition, agroalimentaire, mer (VENAM)

Discipline : Biologie des organismes- Biotechnologie animales, végétales et microbiennes

Spécialité : Sciences agronomiques

Unité de recherche : LEVA (Légumineuses, Ecophysiologie Végétale, Agroécologie) – Groupe ESA

Soutenue le 06 Juin 2017

Thèse N°: 77961

Compétitivité des légumineuses vis-à-vis des adventices : traits impliqués dans la capture précoce de l'azote minéral du sol et complémentarité des traits entre espèces dans des associations légumineuse-non légumineuse

JURY

Rapporteurs : **Nathalie COLBACH**, Directeur de recherche, INRA, AgroSup Dijon, Université de Bourgogne
Gérard DUC, Directeur de recherche, INRA, AgroSup Dijon, Université de Bourgogne

Examineurs : **Anne-Sophie VOISIN**, Chargé de recherche, INRA, Dijon
Anis LIMAMI, Professeur des Universités, Université d'Angers

Directeur de Thèse : **Guénaëlle CORRE-HELLOU**, Enseignant-Chercheur, Ecole Supérieure d'Agricultures, Angers

Co-directeur de Thèse : **Steven J. SHIRTLIFFE**, Professeur des Universités, University of Saskatchewan, Canada

L'auteur du présent document vous autorise à le partager, reproduire, distribuer et communiquer selon



les conditions suivantes :

- Vous devez le citer en l'attribuant de la manière indiquée par l'auteur (mais pas d'une manière qui suggérerait qu'il approuve votre utilisation de l'œuvre).
- Vous n'avez pas le droit d'utiliser ce document à des fins commerciales.

Avant de vous présenter les résultats de ma thèse, je voudrais remercier toutes les personnes qui ont participé à la réalisation de ce travail.

Je tiens tout d'abord à remercier Guénaëlle Corre-Hellou pour m'avoir proposé ce sujet intéressant de thèse, pour m'avoir encouragée et pour avoir supervisé ce travail jusqu'au dernier moment. J'ai eu beaucoup de chance de travailler avec vous et j'ai beaucoup appris. Merci pour votre présence, pour la confiance que vous m'avez donnée, et pour tout le reste...

Je tiens aussi à remercier Steve Shirtliffe, malgré la distance qui sépare la France et le Canada, avec qui nous avons réussi à échanger des idées et qui m'a beaucoup aidée pour les corrections d'anglais.

Je tiens à remercier les membres du jury : Nathalie Colbach, Gérard Duc, Anne-Sophie Voisin et Anis Limami d'avoir accepté d'évaluer et examiner mes travaux de thèse.

Je tiens à remercier les membres du comité de pilotage de la thèse : Safia Medien, Muriel Valantin-Morison, Delphine Moreau et Stéphane Cordeau pour avoir enrichi ce travail avec des idées intéressantes et pour m'avoir aidée à bien orienter les objectifs et les hypothèses de la thèse.

Je remercie particulièrement les co-encadrants de ma thèse : Christophe Naudin et Guillaume Piva pour leurs contributions que j'ai beaucoup appréciées, dans toutes les étapes de ce travail. Merci pour les corrections, les propositions et les conseils.

Un grand merci à Joëlle Fustec pour m'avoir accueillie au sein du LEVA, depuis mon stage de Master 2 et ensuite jusqu'à la réalisation de ma thèse, et pour m'avoir permise de travailler dans de très bonnes conditions.

Les expérimentations en serre et au champ n'auraient pas pu être menées à bien sans l'aide et l'attention de l'équipe technique du LEVA. Donc, je souhaite adresser un grand merci à Vincent Oury, Julien Poret, Antonin Leret, Alexandre Belouzard et Yohan Simon ; pour leur aide, leurs conseils et la bonne ambiance de travail. J'ai beaucoup apprécié votre contribution à la réalisation de ce travail.

Un grand merci de cœur à Solange Renaud, pour son aide à tous les niveaux. Merci pour la présence, les conseils et l'attention. J'ai eu beaucoup de chance de travailler avec toi.

REMERCIEMENTS

Je n'oublie pas Hélène Bobille, Martine Mauline, Baptiste Drut et Nicolas Carton, mes collègues de travail. Merci pour les agréables pauses café et les échanges.

J'ai également une pensée pour tous les autres membres du LEVA ainsi que pour le personnel de l'ESA.

Un grand merci à tous les stagiaires (Frank Emmanuel, Mickaël et Rachel) qui ont participé activement et dans la bonne humeur à la réalisation de certaines expérimentations.

Ce travail a été financé par le gouvernement syrien et le CASDAR n°5376 (Compte d'Affectation Spéciale pour le Développement Agricole et Rural, ministère français de l'agriculture). Merci pour le financement qui m'a permis de réaliser ma thèse dans les meilleures conditions.

Un grand merci à mes amis Mariam, Nivine, Mariel, Rima, Bushra, Maram, Séverine, Wajd et Jeannette, pour votre présence, votre attention, et pour vos encouragements permanents.

Je souhaite remercier ma famille, ma mère, mon père, Hala et Hanan qui sont loin mais aussi proches. C'est grâce à vous que je suis arrivée là. Merci d'avoir cru en moi.

Cette thèse a fait l'objet des valorisations suivantes :

Articles

DAYOUB E., NAUDIN C., PIVA G., SHIRTLIFFE S. J., FUSTEC J., CORRE-HELLOU G. (2017) Traits affecting early season nitrogen uptake in nine legume species. *Heliyon*, 2017. 3(2): p. Article e00244, <http://dx.doi.org/10.1016/j.heliyon.2017.e00244>.

DAYOUB E., NAUDIN C., PIVA G., SHIRTLIFFE S. J., FUSTEC J., CORRE-HELLOU G. (2017) Improving weed suppression and crop productivity of oilseed rape when intercropped with legume species by increasing trait diversity. Submitted to *Field crops research*, March 3th 2017.

Communications orales

DAYOUB E., NAUDIN C., PIVA G., SHIRTLIFFE S. J., CORRE-HELLOU G. Competitiveness of different legumes against weeds and interactions within cropping systems. Workshop: Weed control with less reliance on herbicides (Designing resilient cropping systems), 26th-28th May 2014, LEVA, Groupe ESA, Angers, France.

DAYOUB E., NAUDIN C., PIVA G., CORRE-HELLOU G. Compétitivité de différentes légumineuses vis-à-vis des adventices en relation avec leurs traits fonctionnels au cours de la croissance précoce. 7^{ème} édition de la journée des doctorants de la SFR QUASAV, 27th Novembre 2014, Angers, France.

Posters

DAYOUB E., NAUDIN C., PIVA G., SHIRTLIFFE S. J., FUSTEC J., CORRE-HELLOU G. (2015) Effect of various innovative cropping systems including legumes on weed community and weed biomass. 17th European Weed Research Society Symposium "Weed management in changing environments", 23th-26th June 2015, Montpellier, France.

DAYOUB E., NAUDIN C., PIVA G., CORRE-HELLOU G (2014) Competitiveness of legumes species against weeds in relation with their functional traits during the early growth. 4^{ème} journées scientifiques de l'école doctorale VENAM, 4 et 5 Décembre, 2014, Angers, France.

DAYOUB E., NAUDIN C., PIVA G., CORRE-HELLOU G Effet sur les adventices de différents systèmes de culture innovants insérant des plantes de services au colza ou au maïs en Pays de la Loire. Colloque national: Système de culture innovante : concevoir, former, accompagner de la route du RMT, 17 mai 2016, Paris, France.

Sommaire

| | |
|---|-----------|
| 1. INTRODUCTION – SYNTHÈSE BIBLIOGRAPHIQUE ET PROBLÉMATIQUE | 1 |
| SYNTHÈSE BIBLIOGRAPHIQUE | 3 |
| 1.1. TRANSITION VERS UNE GESTION PLUS DURABLE DES ADVENTICES DANS DES SYSTÈMES DE CULTURE MOINS DÉPENDANTS DES HERBICIDES..... | 3 |
| 1.2. MIEUX CONNAÎTRE LA COMPÉTITIVITÉ DES CULTURES VIS-A-VIS DES ADVENTICES..... | 6 |
| 1.3. TRAITS FONCTIONNELS IMPLIQUÉS DANS LA COMPÉTITIVITÉ VIS-A-VIS DES ADVENTICES | 7 |
| 1.4. PHASE PRÉCOCE : PÉRIODE CLÉ | 8 |
| 1.5. STRATÉGIE D'ACQUISITION D'AZOTE ET COMPÉTITIVITÉ DES LÉGUMINEUSES VIS-A-VIS DES ADVENTICES..... | 9 |
| 1.6. RÔLES VARIÉS DES LÉGUMINEUSES DANS LES SYSTÈMES DE CULTURE EN FONCTION DE LEUR MODE D'INSERTION..... | 10 |
| 1.7. MODIFICATION DE LA FLORE ADVENTICE EN RÉPONSE À L'INSERTION DE LÉGUMINEUSE DANS LES SYSTÈMES DE CULTURE 14 | |
| PROBLÉMATIQUE ET QUESTIONS DE RECHERCHE | 15 |
| 2. DÉMARCHE DE LA THÈSE : | 20 |
| 3. ORGANISATION DU MANUSCRIT..... | 29 |
| 4. CHAPITRE 1. TRAITS AFFECTING EARLY SEASON NITROGEN UPTAKE IN NINE LEGUME SPECIES | 32 |
| ABSTRACT..... | 34 |
| 4.1. INTRODUCTION..... | 35 |
| 4.2. MATERIALS AND METHODS..... | 36 |
| 4.3. RESULTS..... | 41 |
| 4.4. DISCUSSION | 50 |
| 5. CHAPITRE 2. RESPONSE OF BLACK NIGHTSHADE TO CHICKPEA, FABA BEAN AND COMMON VETCH DURING EARLY GROWTH | 54 |
| ABSTRACT..... | 56 |
| 5.1. INTRODUCTION..... | 57 |
| 5.2. MATERIALS AND METHODS..... | 59 |

| | | |
|-----------|--|------------|
| 5.3. | RESULTS AND DISCUSSION | 63 |
| 6. | CHAPITRE 3. IMPROVING WEED SUPPRESSION AND CROP PRODUCTIVITY OF OILSEED RAPE WHEN INTERCROPPED WITH LEGUME SPECIES BY INCREASING TRAIT DIVERSITY | 76 |
| | ABSTRACT | 78 |
| 6.1. | INTRODUCTION | 80 |
| 6.2. | MATERIAL AND METHODS | 82 |
| 6.3. | RESULTS | 87 |
| 6.4. | DISCUSSION | 98 |
| 6.5. | CONCLUSIONS | 102 |
| 7. | CHAPITRE 4. WEED SUPPRESSION AND CHANGES IN SPECIES COMPOSITION OF WEED COMMUNITIES OCCURRING IN NO-TILLAGE SEED MAIZE WITH LIVING OR DEAD MULCHES..... | 104 |
| | ABSTRACT | 106 |
| 7.1. | INTRODUCTION | 108 |
| 7.2. | MATERIALS AND METHODS..... | 110 |
| 7.3. | RESULTS | 115 |
| 7.4. | DISCUSSION | 132 |
| 8. | DISCUSSION GENERALE ET PERSPECTIVES DE LA THESE..... | 138 |
| 8.1. | COMPETITIVITE DE DIFFERENTES ESPECES DES LEGUMINEUSES VIS-A-VIS DES ADVENTICES EN LIEN AVEC LEURS TRAITS FONCTIONNELS PENDANT LA PHASE DE CROISSANCE PRECOCE | 140 |
| 8.2. | L'INTERET DE CETTE APPROCHE ET SES APPLICATIONS A L'ECHELLE DE LA PARCELLE..... | 144 |
| 8.3. | IMPACT DE L'INSERTION D'UN COUVERT DE LEGUMINEUSES COMME COUVERT DANS UNE CULTURE PRINCIPALE SUR LA REGULATION DES ADVENTICES ET LA COMPOSITION DE LA FLORE ADVENTICES..... | 145 |
| 8.4. | LE ROLE CLE DES LEGUMINEUSES DANS LES SYSTEMES DE CULTURE..... | 153 |
| 8.5. | LE CHOIX DES ESPECES EN FONCTION DE LEURS TRAITS | 154 |
| 8.6. | COMPLEXITE DE LA GESTION DE SYSTEME DE CULTURE | 155 |
| 8.7. | PERSPECTIVES DE RECHERCHE | 156 |
| 9. | REFERENCES BIBLIOGRAPHIQUES..... | 158 |

TABLE DES ILLUSTRATIONS..... 172

FIGURES 174

TABLEAUX..... 178

1. Introduction – Synthèse bibliographique et problématique

Synthèse bibliographique

1.1. Transition vers une gestion plus durable des adventices dans des systèmes de culture moins dépendants des herbicides

L'évolution de l'agriculture en France depuis la seconde guerre mondiale est, dans un certain nombre de régions de grande culture et d'élevage, marquée fortement par la spécialisation des territoires et des exploitations agricoles. Cette spécialisation a progressivement conduit à une simplification des assolements accompagnée d'un usage accru des intrants et d'une standardisation des itinéraires techniques (Duc et al., 2010; Evans, 1998; Schott et al., 2010) dans un souci d'améliorer les rendements et la lutte contre les bio-agresseurs des cultures. Au sein des systèmes de culture actuels, la gestion des adventices repose quasi exclusivement sur l'application d'herbicides. Cette place prépondérante est liée à l'étendue de la gamme d'herbicides disponibles, de leur efficacité sur la quasi-totalité des espèces adventices et de leur facilité d'utilisation. Si l'on regarde les pratiques agricoles de protection phytosanitaire appliquées actuellement aux grandes cultures on note que les herbicides occupent 40% du total d'IFT (indice de fréquence de traitement) moyen toutes cultures confondues (Valantin-Morison et al., 2008). La maîtrise de l'enherbement est un facteur sanitaire problématique pour tous les types de cultures.

Toutefois, la durabilité des systèmes reposant sur l'utilisation d'herbicides comme premier moyen de gestion des adventices est sérieusement remise en question aujourd'hui pour des raisons économiques, techniques et environnementales.

Des phénomènes de résistance des adventices aux herbicides se sont développés (Heap, 2014). De nouveaux cas d'adventices résistantes à une ou plusieurs familles d'herbicides se révèlent chaque année (Fig. 1.1, 1.2). Des herbicides ont perdu leur efficacité et donnent des résultats aléatoires ou insuffisants. Certaines adventices sont devenues difficiles à contrôler (Chauvel et al., 2004; Chauvel et al., 2001). Une spécialisation de la flore est observée en lien avec la standardisation des pratiques. Les rotations courtes, sans cultures de printemps, augmentent considérablement le risque de développement de populations résistantes. L'utilisation répétée d'un même mode d'action des herbicides augmente le risque de sélectionner des individus résistants à ce mode d'action. La recherche de nouvelles molécules avec de nouveaux modes d'action est très coûteuse. De plus la dépendance aux herbicides des systèmes de culture a des conséquences environnementales majeures. Les données de contamination des eaux superficielles et souterraines de l'Ifen, bien qu'hétérogènes et non représentatives sur tout le territoire font état d'une contamination quasi généralisée des eaux de surface et souterraines par les pesticides avec une prépondérance des herbicides (Valantin-Morison et al., 2008). La pression sociétale sur ce sujet se traduit par des changements de réglementation, des

interdictions des matières actives considérées comme les plus polluantes. Dans ce contexte, les agriculteurs ont globalement une gamme de solutions techniques plus limitée et plus coûteuse.

Les politiques agricoles (ex. réformes de la PAC) et/ou le Grenelle de l'Environnement incitent aussi à repenser les pratiques agricoles actuelles vers une agriculture moins dépendante de la lutte chimique.

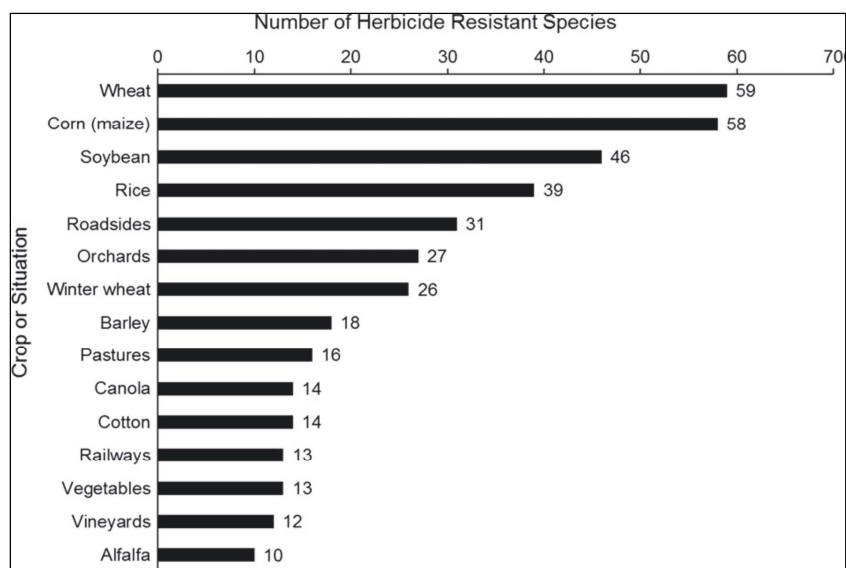


Figure 1.1. Le nombre d'espèces adventices résistantes aux herbicides dans différentes cultures, d'après Heap (2014)

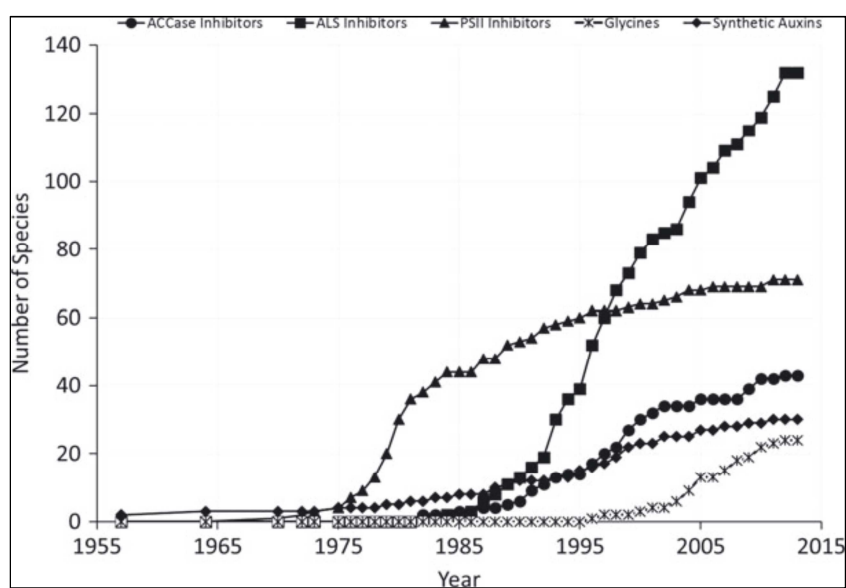


Figure 1.2. Augmentation au cours du temps du nombre d'espèces adventices résistantes à plusieurs classes d'herbicides. D'après Heap (2014)

De nombreux travaux cherchent aujourd'hui à concevoir des systèmes de culture moins dépendants des intrants tout en visant à maintenir la productivité des cultures et en respectant l'environnement et en assurant la viabilité économique. La protection intégrée pour la lutte contre les adventices se développe et repose sur des combinaisons de techniques culturales aux effets complémentaires en valorisant les régulations physiques et biologiques pour maîtriser les adventices en agissant à différentes étapes de leur cycle de vie (Munier-Jolain et al., 2008).

Plusieurs techniques alternatives à effet partiel peuvent être mobilisées et combinées : diversification temporelle dans la rotation, densité de semis, écartements entre rangs, choix d'espèces et variétés compétitives, diversification spatiale (associations de cultures, semis sous couvert), désherbage mécanique (Blackshaw et al., 2006; Lemerle et al., 2001; Sarrantonio and Gallandt, 2003). Ces techniques peuvent agir sur les adventices de différentes façons à différents moments de leur cycle : réduire le stock semencier, esquiver les périodes préférentielles de levées des adventices, perturber la levée en modifiant le milieu, détruire les plantules levées, réduire la croissance des adventices pour réduire la compétition sur la culture et donc les pertes de rendement, et enfin restreindre la production semencière et la ré-alimentation du stock.

1.2. Mieux connaître la compétitivité des cultures vis-à-vis des adventices

Accroître la compétitivité des cultures est une composante importante dans la conception de systèmes de culture moins dépendants des herbicides (Blackshaw et al., 2007; Lindquist and Mortensen, 1998; Zerner et al., 2008). Il s'agit de favoriser une capture et une utilisation des ressources du milieu importantes et rapides par la culture semée laissant ainsi moins de ressources pour les adventices. Les références sont toutefois peu nombreuses sur la caractérisation de la compétitivité d'une diversité d'espèces vis-à-vis des adventices en raison (i) du faible nombre d'espèces dans les assolements et le faible investissement en recherche sur les espèces jugées plus mineures, (ii) de la faible connaissance des caractéristiques déterminantes des espèces à l'origine d'écarts de compétitivité, (iii), de la faible prise en compte de la compétitivité vis-à-vis des adventices dans les processus de sélection des variétés, (iv) et de l'évaluation des espèces ou variétés qui se fait le plus souvent en situations peu ou pas enherbées.

Par ailleurs la compétitivité d'une espèce et son rôle dans la gestion des adventices dépend aussi du système de culture dans lequel elle est insérée. Une même espèce peut être présente dans une rotation en culture principale, en culture intermédiaire, en plante de service associée à une autre espèce... En fonction de ce mode d'insertion, leurs objectifs dédiés et leur rôle de cette espèce vis-à-vis de la gestion des adventices peuvent varier.

Une meilleure connaissance de ce qui détermine des différences de compétitivité entre espèces cultivées vis-à-vis des adventices et en tenir compte pour un agencement des espèces dans le temps et

dans l'espace permettant de réduire la croissance des adventices est une approche aujourd'hui à développer.

Les légumineuses sont considérées comme des espèces clés à insérer dans les systèmes de culture pour accroître leur durabilité notamment grâce à leur aptitude à fixer l'azote de l'air (Sarrantonio and Gallandt, 2003); en revanche, elles sont réputées peu compétitives vis-à-vis des adventices (Campiglia et al., 2010; Kour et al., 2013; Kruidhof et al., 2008; McDonald, 2003), ce qui peut freiner leur insertion dans des systèmes moins dépendants des herbicides. Néanmoins nous manquons de connaissances sur ce qui explique la faible compétitivité des légumineuses vis-à-vis des adventices et sur d'éventuelles différences entre espèces. D'autre part, dans le cadre de la conception de systèmes de cultures plus durables moins dépendants des herbicides, il est intéressant d'étudier l'insertion des légumineuses dans le temps comme dans l'espace au sein des systèmes de culture. Ainsi, il est intéressant de travailler à comment agencer des légumineuses et des non-légumineuses en tenant compte de leur compétitivité respective vis-à-vis des adventices.

1.3. Traits fonctionnels impliqués dans la compétitivité vis-à-vis des adventices

Selon Violle et al. (2007), un trait est toute caractéristique morphologique, physiologique ou phénologique mesurable à l'échelle de la plante individuelle, de la cellule ou l'organe, sans qu'il soit fait référence aux facteurs du milieu ou à aucun autre niveau d'organisation (population, communauté, écosystème). Certains auteurs ont défendu l'intérêt d'utiliser les caractéristiques morphologiques et physiologiques pour mesurer la compétitivité de différentes espèces cultivées (Lemerle et al., 2001). Un trait contribue à la compétitivité de la culture s'il informe de la capacité à utiliser rapidement des ressources disponibles et à augmenter sa croissance comparativement aux espèces avec lesquelles elle est en compétition (Donald, 1968). Certains auteurs considèrent la compétitivité comme la combinaison de la capacité à réduire les adventices et la capacité à tolérer la présence d'adventices (Gibson et al., 2003; Goldberg, 1990). Cette compétitivité globale est dépendante d'autres traits tels que la hauteur, la surface foliaire, la structure du couvert et la précocité, que plusieurs auteurs ont étudié pour différentes espèces. La compétitivité ne peut être attribuée qu'à un seul trait mais plutôt à une combinaison de plusieurs caractéristiques de la plante (Asif et al., 2014).

La mise en évidence des traits principaux influençant la compétition culture-adventice n'est pas évidente car la compétition est influencée non seulement par les caractéristiques des espèces mais aussi par les pratiques culturales et l'environnement (Booth and Swanton, 2002; Gunton et al., 2011; Weiher et al., 1999). Par ailleurs la compétition se situe à l'échelle de la population et du peuplement et ne peut s'étudier seulement à l'échelle de la plante individuelle.

Au sein des céréales, plusieurs auteurs ont mis en évidence des différences de compétitivité entre génotypes (Lemerle et al., 2001; Lemerle et al., 1995; Lemerle et al., 1996; Reeves and Brooke, 1977)

Des comparaisons entre légumineuses ont été aussi réalisées (Den Hollander et al., 2007; McDonald, 2003; Uchino et al., 2011).

Des traits aériens relatifs à la compétition pour la lumière tels que la hauteur, la surface foliaire, la fermeture rapide du couvert ont été souvent considérés déterminants dans la compétition culture-adventice (Beckie et al., 2008; Pakeman et al., 2015). Toutefois, dans plusieurs travaux, les traits aériens n'expliquent qu'une faible part de la capacité à réduire les adventices, notamment chez les légumineuses : chez le pois (*Pisum sativum* L.) (McDonald, 2003), le pois-chiche (*Cicer arietinum* L.) (Radicetti et al., 2012) ou la lentille (*Lens culinaris* L.) (Tepe et al., 2005).

En comparaison des traits aériens, les traits racinaires ont reçu moins d'attention dans la compétition culture-adventice. Cependant des études montrent que la compétition racinaire en particulier pour l'azote peut être plus forte que la compétition pour la lumière (Casper and Jackson, 1997; Satorre and Snaydon, 1992). Des traits tels que la densité racinaire, la vitesse d'élongation en profondeur et en latéral peuvent influencer la compétition intra et interspécifique (Fargione and Tilman, 2006; Stevanato et al., 2011). Les différences de stratégies d'acquisition de l'azote peuvent jouer un rôle déterminant dans les différences de compétitivité entre espèces et génotypes mais sont souvent peu étudiées.

La caractérisation des traits relatifs à la compétitivité vis-à-vis des adventices peut permettre de mieux analyser la variabilité entre espèces en termes de stratégie d'acquisition et d'utilisation des ressources. L'approche par les traits peut aussi aider à identifier les complémentarités possibles entre espèces associées pour accroître la compétitivité d'un couvert.

1.4. Phase précoce : période clé

La croissance précoce, qui va de la levée jusqu'à l'apparition de la compétition entre plantes, est une phase clé qui détermine l'état de chacune des espèces au moment de l'entrée en compétition (Fayaud et al., 2014) et déterminerait leur capacité pour l'acquisition ultérieure des ressources du milieu. Une espèce ayant un avantage pour accéder aux ressources dès les premiers stades peut voir son avantage compétitif s'accroître dans la suite du cycle. Plusieurs facteurs peuvent influencer la croissance des plantules dans cette phase précoce. La masse de semences, le type de levée (hypogée ou épigée) et la disponibilité en azote dans le sol sont des facteurs influençant la croissance des plantules pendant la phase précoce (Fayaud et al., 2014). La masse de semence est un trait déterminant pour expliquer les différences entre les espèces et les variétés au niveau de la biomasse aérienne des plantules dès la levée (Dürr and Boiffin, 1995; Fayaud et al., 2014; Gardarin et al., 2010; Gross, 1984; Tamet et al., 1996).

La compétition culture-adventice peut s'installer tôt dans le cycle. Même si peu de travaux s'intéressent aux stades très précoces, plusieurs auteurs insistent sur l'importance de la maîtrise des adventices dès les premiers stades de la culture et aussi aux premiers stades de développement des

adventices. Croster & Masiunas (1998) montrent que la compétition interspécifique entre le pois (*Pisum sativum* L.) et la morelle noire de l'est (*Solanum ptycanthum* Dunal) semée en même temps que le pois se met en place très tôt, ce que nécessite un contrôle précoce des adventices pendant les deux premières semaines de culture. Hall et al (1992) indiquent chez le maïs la nécessité de contrôler les adventices entre le stade 3 à 14 feuilles, période critique pendant laquelle un fort enherbement peut impacter grandement le rendement.

Bien que la croissance précoce soit jugée cruciale dans la mise en place de la compétition entre culture-adventices, les traits aériens et racinaires impliqués dans la capacité à capter tôt les ressources sont rarement étudiés. Des différences entre espèces ou génotypes de stratégies de croissance et d'acquisition précoce de l'azote (N) pourraient toutefois être déterminantes.

1.5. Stratégie d'acquisition d'azote et compétitivité des légumineuses vis-à-vis des adventices

Les légumineuses sont réputées moins compétitives pour capter l'azote minéral du sol que d'autres espèces. En laissant davantage d'N disponible pour les adventices, cette faible capacité de capture d'N pourrait expliquer en partie leur faible compétitivité vis-à-vis des adventices. Plusieurs espèces d'adventices consomment de larges quantités d'N (Hans and Johnson, 2002; Qasem, 1992). De plus la croissance de beaucoup d'adventices est améliorée par une augmentation de la disponibilité en N minéral du sol (Blackshaw et al., 2003; Henson and Jordan, 1982; Supasilapa et al., 1992).

Les traits explicatifs de la faible compétitivité des légumineuses pour capter l'N minéral du sol en début de cycle et l'identification d'une variabilité entre espèces ont été jusqu'ici peu explorés. Les légumineuses peuvent en début de cycle reposer sur trois sources d'azote : l'azote de la semence, l'azote du sol et dans une moindre mesure l'azote atmosphérique (N₂) à partir de la mise en place de l'appareil fixateur de son fonctionnement. La mise en place des nodules et leur activité se met en place progressivement. Dans le premier de mois culture, cette source d'azote contribue peu à l'accumulation d'azote de la culture. Des différences de démarrage de la fixation peuvent toutefois exister entre espèces en relation avec la masse de la semence, le type de la levée, et la vitesse de vidange des réserves (Dobert and Blevins, 1993; Sprent and Thomas, 1984; Tang et al., 1999). Par ailleurs, les légumineuses peuvent reposer beaucoup sur l'azote contenu dans leur semence pendant les premiers stades (durant le premier mois après la levée) en raison de la masse élevée de la semence de certaines espèces et leur richesse en azote (Herdina and Silsbury, 1990; Voisin et al., 2005). Ceci peut contribuer à une faible utilisation d'azote exogène chez les légumineuses en début de cycle comparativement à d'autres espèces non fixatrices d'azote atmosphérique. La contribution relative de chaque source d'N peut néanmoins varier entre espèces de légumineuse pendant la croissance précoce et en fonction de la disponibilité initiale en N.

Les légumineuses sont connues aussi pour avoir des enracinements moins développés que les graminées. Des travaux montrent que la vitesse de progression des racines en profondeur est plus faible chez des légumineuses que chez des céréales (Corre-Hellou et al., 2007; Thorup-Kristensen, 2001). La densité racinaire atteint seulement la moitié de celle des céréales (Greenwood et al., 1982; Hamblin and Tennant, 1987; Hamblin and Hamblin, 1985). Des différences d'enracinement entre légumineuses ont déjà été mises en évidence mais peu de travaux concernent les stades précoces.

1.6. Rôles variés des légumineuses dans les systèmes de culture en fonction de leur mode d'insertion

La compétitivité des légumineuses et leur rôle dans la gestion des adventices dépendent aussi du système de culture dans lequel elles sont insérées. Elles peuvent permettre de diversifier les systèmes de culture dans le temps via leur insertion dans une rotation. La diversification temporelle et ses effets sur les adventices sont bien connus du fait qu'elle peut rompre le cycle des adventices, induire une modification de la flore (éventuellement moins nuisible) et limiter le risque d'apparition de résistance.. La rotation des cultures est bien intégrée dans les processus de décision des agriculteurs concernant la gestion des adventices (Macé et al., 2007). Les légumineuses peuvent être utilisées aussi pour diversifier les systèmes de culture dans l'espace via des associations d'espèces cultivées ensemble sur la même parcelle. L'association de cultures est définie comme la culture simultanée de deux espèces ou plus sur une même surface pendant un temps significatif de leur cycle (Willey, 1979). Différents modes de gestion des adventices via les associations végétales peuvent être distingués. Dans nos travaux (Fig.1.3), nous nous intéresserons à des associations d'espèces correspondant aux cas ci-dessous.

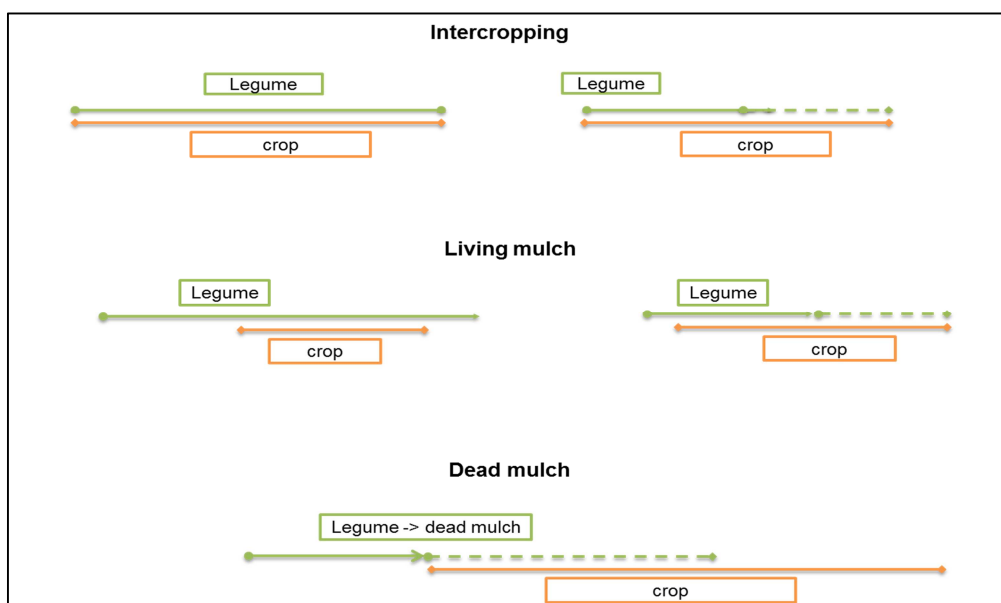


Figure 1.3. Modes d'insertion des légumineuses dans les systèmes de culture étudiés dans le cadre de la thèse

Un premier cas concerne l'association d'espèces complémentaires pour l'utilisation des ressources. Les deux espèces sont semées en même temps. Il s'agit ici le plus souvent d'associations substitutives. Chaque espèce dans l'association est semée à un certain pourcentage de la densité en culture pure. La somme de ces densités relatives est de 100% (Andrews and Kassam, 1976). L'objectif est de récolter le plus souvent les deux espèces cultivées. Dans certains cas, seule la culture jugée principale est récoltée et la deuxième est une plante compagne non récoltée, qui peut être détruite en cours de cycle (Cadoux et al., 2015; Gharineh and Telavat, 2009; Lorin et al., 2015).

La complémentarité des espèces associées peut mener à une meilleure capture et utilisation des ressources (lumière, nutriments) (Justes et al., 2014; Louarn et al., 2010). Ainsi, les associations peuvent être plus efficaces que les cultures pures pour utiliser des ressources qui se retrouvent non utilisables par les adventices, affectant ainsi leur croissance. Cette meilleure utilisation des ressources au profit des espèces cultivées peut se produire si les composantes de l'association ont des pics de demande asynchrones, s'exerçant dans des espaces différents (aérien et souterrain), ou utilisent différentes formes d'une même ressource. Dit autrement, c'est la complémentarité des espèces associées pour l'utilisation des ressources qui doit être privilégié. Ainsi, des différences phénologiques entre espèces peuvent leur permettre d'utiliser les ressources à des périodes différentes (Ofori and Stern, 1987; Willey, 1979). Des différences d'architecture aérienne entre plantes peuvent permettre aux espèces d'occuper l'espace plus efficacement et ainsi augmenter l'interception du rayonnement. Plusieurs travaux soulignent l'importance de la complémentarité pour l'utilisation de la lumière pour expliquer l'effet positif des cultures associées pour la lutte contre les adventices (Corre-Hellou et al., 2011; Hauggaard-Nielsen et al., 2001b). Les mélanges d'espèces les plus efficaces sont ceux qui interceptent le plus de rayonnement au début du cycle (Liebman and Dyck, 1993). Par ailleurs, des espèces qui ont des systèmes racinaires complémentaires (plus ou moins profonds) (Berendse, 1979) peuvent exploiter plus efficacement les ressources.

Dans le cas particulier de l'association légumineuse-non légumineuse, la complémentarité principale se situe dans l'utilisation de deux sources d'azote : l'azote du sol et l'azote atmosphérique. Bien que les légumineuses et non légumineuses utilisent l'azote minéral du sol, la légumineuse a aussi la possibilité de fixer l'azote atmosphérique permettant aux deux espèces de se compléter pour une acquisition optimale de l'azote disponible dans le milieu.

Des exemples d'associations (intercropping) d'espèces complémentaires pour les ressources ont été rassemblés dans le tableau 1.1. Les situations présentées sont des associations dans lesquelles la non-légumineuse est la culture principale et la légumineuse est récoltée ou non. Les résultats montrent l'intérêt de ces associations pour réduire la biomasse d'adventices par rapport à la culture pure non-légumineuse.

Tableau 1.1 Le pourcentage de réduction de la biomasse des adventices par différents types d'association légumineuse-non-légumineuses. Exemples issus de la littérature.

| System | Location | Legume | Crop | Percentage weed biomass reduction (%) * | Reference |
|----------------------|--|--|--|---|--------------------------------|
| Intercropping | India | Chickpea (<i>Cicer arietinum</i> L.) | Wheat (<i>Triticum aestivum</i> L.) | 70 | Banik et al. (2006) |
| | Canada | Soybean (<i>Glycine max</i> L.) | Maize (<i>Zea mays</i> L.) | 87 | Carruthers et al. (1998) |
| | | Lupin (<i>Lupinus albus</i> L.) | | 6 | |
| | Germany | Winter Pea (<i>Pisum sativum</i> L.) | Triticale (<i>Triticale hexaploide</i> Lart.) | 31 | Gronle (2014) |
| | Canada | Field pea (<i>P. sativum</i> L.) | Wheat (<i>T.aestivum</i> L.) | 29 | Szumigalski & Van Acker (2005) |
| | France | Oilseed rape (<i>Brassica napus</i> L.) | Faba bean (<i>Vicia faba</i> L.) | 75 | Lorin et al . (2015) |
| | Iran | Oilseed rape (<i>B. napus</i> L.) | Faba bean (<i>V. faba</i> L.) | 78 | Gharineh and Moosavi (2010) |
| Living mulch | USA | Subterranean Clover (<i>Trifolium subterraneum</i> L.) | Maize (<i>Z. mays</i> L.) | 37 | Enache and Ilnicki (1990) |
| | Canada | Red clover (<i>T. pratense</i> L.) | Wheat (<i>T. aestivum</i> L.) | 18 | Pridham & Entz (2008) |
| | | Hairy vetch (<i>Vicia villosa</i> Roth.) | Wheat (<i>T. aestivum</i> L.) | 34 | |
| | Denmark | White clover (<i>T. repens</i> L.) | Wheat (<i>T. aestivum</i> L.) | 50 | Thorsted et al.(2006) |
| Dead mulch | Canada | Yellow sweet clover (<i>Melilotus officinalis</i> (L.) Lam) | Pea (<i>Pisum sativum</i> L.) | 88 | Blackshaw et al. (2001) |
| | | | Flax (<i>Linum usitatissimum</i> L.) | | |
| | Italy | Hairy vetch (<i>V. villosa</i> Roth.) | Tomato | 26 | Campiglia et al. (2010) |
| | | Subterranean Clover (<i>T.subterraneum</i> L.) | (<i>Lycopersicon esculentum</i> Mill.) | 32 | |
| USA | Hairy vetch (<i>V. villosa</i> Roth.) | Maize (<i>Z. mays</i> L.) | 44 | Reddy and koger (2004) | |

- * pourcentage de réduction de la biomasse des adventices par rapport un témoin (sans couvert pour le mulch vivant ou mort et la non-légumineuse pure pour la catégorie intercropping).

- Les modalités d'associations et les témoins ont été conduits de manière similaire.

Un deuxième cas concerne l'insertion d'un couvert (le plus souvent non récolté) avant l'implantation de la culture principale et maintenu sous forme d'un mulch vivant ou mort pendant la croissance de la culture principale. Il s'agit d'un dispositif le plus souvent additif : la culture principale est semée à la même densité qu'en culture pure et le couvert est rajouté à cette culture principale augmentant la densité de plantes. Rajouter une plante de couverture implantée avant le semis de la culture principale peut réduire l'infestation par les adventices (De Tourdonnet et al., 2008). Si la plante de couverture se développe avant les adventices, la présence d'un couvert végétal peut créer des conditions défavorables à la germination. En effet, plusieurs facteurs importants pour la germination des adventices (lumière, proportion d'infra-rouge, amplitude thermique) seront moins favorables sous un couvert vivant (Teasdale and Daughtry, 1993). Plusieurs travaux ont montré que certains couverts sécrétaient des substances allélopathiques inhibant la germination des adventices (Bhowmik, 2003; Ercoli et al., 2007; Hill et al., 2007; Xuan et al., 2003). De plus, si le couvert est suffisamment développé, il peut aussi constituer une barrière physique à la levée des adventices. Enfin, la présence d'un couvert peut aussi favoriser la prédation des semences d'adventices par la faune (Meiss et al., 2010). Dans certains cas, le mulch est détruit avant le semis de la culture principale ou pendant le cycle de la culture (par le gel, mécaniquement ou chimiquement). Les résidus du couvert réduisent la transmission de la lumière au sol et forment aussi une barrière physique empêchant la germination et la levée des adventices (Teasdale, 1993; Teasdale and Mohler, 2000). D'autres mécanismes peuvent influencer la germination des adventices dans le cas de résidus détruits avant le semis : l'incorporation des résidus peut modifier les conditions favorables pour la germination des adventices en créant un environnement favorable pour les pathogènes des semences d'adventices ou en obligeant les semences à entrer en dormance (Dyck and Liebman, 1994).

Par une occupation de l'espace habituellement laissé libre entre les rangs de la culture principale, un couvert vivant accroît la compétition pour la lumière mais aussi pour les nutriments au détriment de la croissance des adventices. Plusieurs travaux montrent que l'effet bénéfique sur la réduction de la biomasse d'adventices est corrélé à la biomasse du couvert (Hiltbrunner et al., 2007b; Ross et al., 2001; Sheaffer et al., 2002). Toutefois, la croissance de la plante de service doit être contrôlé afin de ne pas trop impacter la croissance de la culture principale.

L'utilisation de ces couverts est plus particulièrement intéressante dans le cas d'espèces principales lentes à s'installer. C'est notamment le cas des espèces à petites graines et/ou des espèces à feuilles érigées ayant des caractéristiques ne favorisant pas une occupation rapide de l'espace (Liebman and Staver, 2001).

Quelques exemples d'insertion de mulch vivant ou mort dans une culture principale sont présentés dans le tableau 1.1 et leurs effets sur le pourcentage de réduction de la biomasse d'adventices par rapport à la culture principale pure sans couvert.

1.7. Modification de la flore adventice en réponse à l'insertion de légumineuse dans les systèmes de culture

Peu de travaux ont étudié l'effet de l'insertion de légumineuses dans les systèmes de culture sur la composition de la flore adventice en particulier dans le cas d'associations légumineuse-non légumineuse. Les espèces associées peuvent présenter des traits contrastés; ainsi les légumineuses ont souvent une production de biomasse et une faible capacité à utiliser l'N du sol par rapport à des graminées. Certaines adventices ont quant à elles une forte capacité de capture d'N minéral du sol et leurs croissances répondent positivement à une disponibilité accrue d'N dans le sol. La composition de la flore adventice pourrait être modifiée en fonction de la capacité des couverts insérés à une culture principale à réduire ou au contraire à accroître l'azote disponible.

La flore adventice est influencée aussi fortement par la diversité des pratiques culturales telles que la période de semis, l'alternance des programmes de désherbage, le fauchage et la gestion de la fertilisation (Barberi and Lo Cascio, 2001; Cardina et al., 2002).

La gestion de la diversité de la flore adventice peut être importante sur le long-terme. Une flore plus diversifiée avec moins des espèces dominantes (très compétitives) peut contribuer à une gestion plus efficace des adventices et à réduire le risque d'impact sur le rendement sur le long-terme. De tels effets ont été relatés dans des essais long-terme comparant différents système de culture intégrant du maïs, du soja et du blé en rotation (Ferrero et al., 2017).

Problématique et questions de recherche

Ce travail s'inscrit dans un cadre général qui porte sur les relations entre l'acquisition d'azote et la compétitivité vis-à-vis des adventices chez les légumineuses en culture seule ou associée à une non-légumineuse. Les légumineuses sont surtout étudiées pour leur capacité à fixer l'azote atmosphérique et à en restituer. De plus, Elles sont connues pour être peu compétitives vis-à-vis des adventices. Ces deux types de fonctions relatives à la gestion de l'azote et la gestion des adventices sont toutefois étudiés le plus souvent séparément alors qu'ils peuvent être liés. L'identification de ces liens permettrait de mieux agir sur ces fonctions par le choix des espèces, des pratiques ou la façon d'insérer ces légumineuses dans les systèmes de culture plus économes à la fois en azote et en herbicides. Les questions de recherche traitées se situent à différentes échelles spatiales et temporelles (Fig. 1.4) :

Dans un premier volet de la thèse nous étudions la question suivante :

- 1- Quelle est la variabilité entre espèces de légumineuses de capacité de capture de l'azote minéral du sol et quelle sont les traits à l'origine de cette variabilité?**

Cette question se focalise sur la mise en place de la compétition (adventice-légumineuse) et se situe à l'échelle de la plante, des interactions plante-plante et pendant les stades précoces. Elle a été traitée via des expérimentations en conditions contrôlées en rhizotrons.

Dans un deuxième volet de la thèse nous étudions cette deuxième question :

- 2- Est-ce que la combinaison d'espèces légumineuse et non-légumineuse aux traits complémentaires (notamment pour l'azote) est un levier pertinent pour gérer les adventices en étudiant l'impact sur leur croissance et la composition de la flore?**

Cette question a été traitée au champ à l'échelle du peuplement avec trois composantes (légumineuse, non-légumineuse et adventices) pendant le cycle de culture ou la succession de cultures.

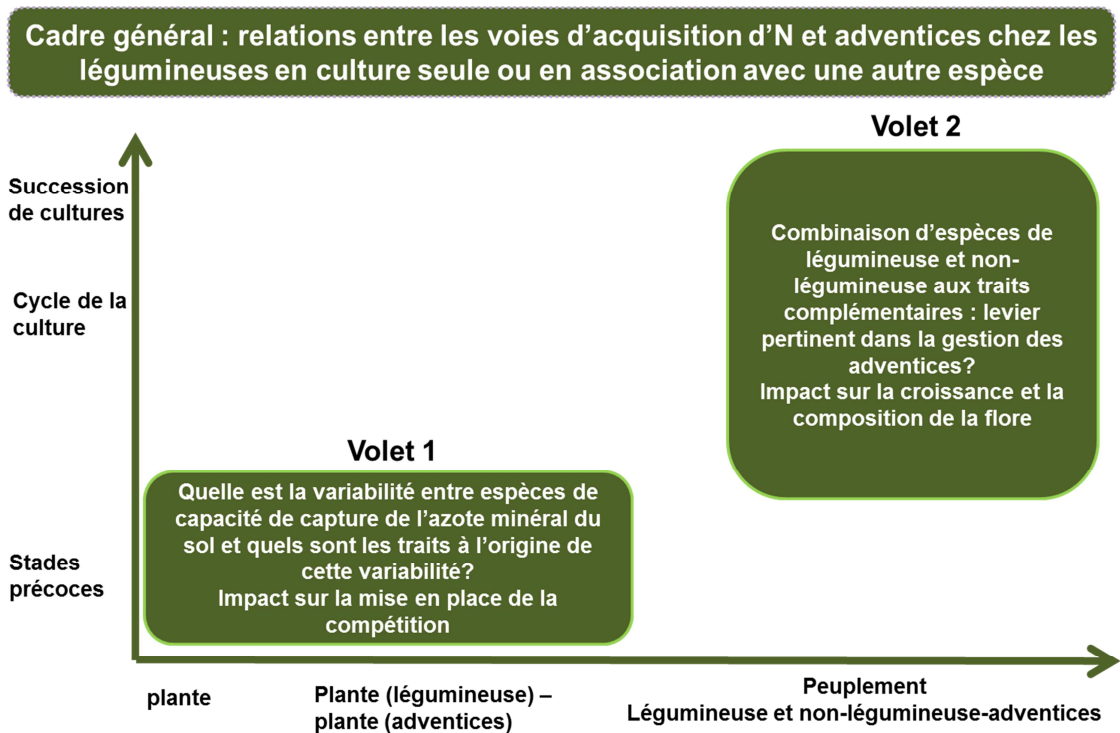


Figure 1.4 Les questions de recherche abordées concernant les deux volets de la thèse

Volet1 : Approche écophysiological de la compétitivité de différentes légumineuses sur les adventices pendant la phase de croissance précoce en relation avec l'acquisition d'azote (Fig. 1.5)

1.5)

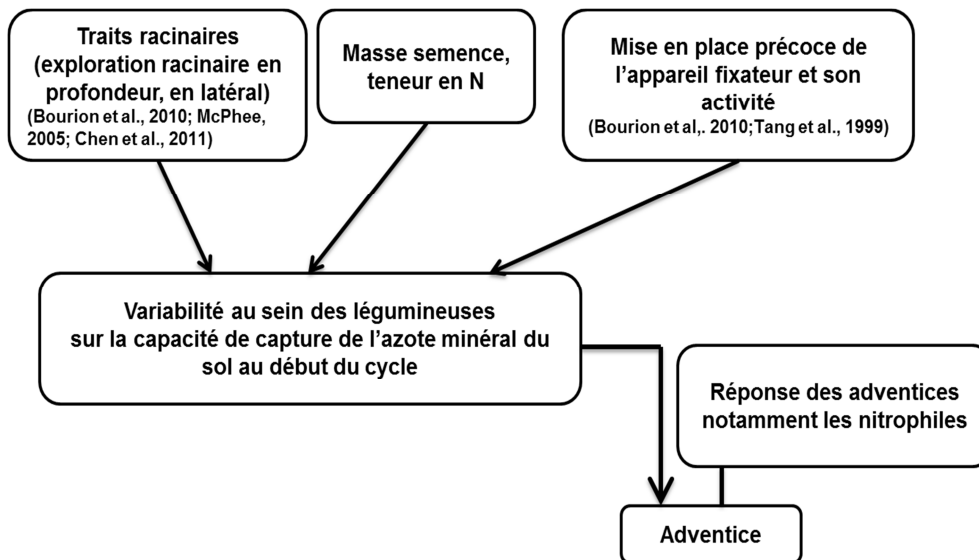


Figure 1.5. Questions et hypothèses du volet 1 : compétitivité de différentes légumineuses sur les adventices pendant la phase de croissance précoce en relation avec l'acquisition d'azote

Ce travail cherche à caractériser la variabilité au sein des légumineuses et à identifier les traits principaux permettant de classer les espèces de légumineuses selon leur stratégie d'acquisition d'azote en début de cycle et leur compétitivité pour capter l'azote minéral du sol. Ce travail vise aussi à

déterminer les conséquences de cette variabilité sur la croissance et l'acquisition en azote d'une adventice nitrophile associée à différentes légumineuses et dans le cas de deux niveaux de disponibilité en azote minéral du sol.

Hypothèse 1 :

Les légumineuses sont connues pour être peu compétitives vis-à-vis des adventices (Kruidhof et al., 2008; McDonald, 2003) en lien avec une faible capacité à prélever de l'azote du sol (Corre-Hellou et al., 2011; Thorup-Kristensen, 1994). Mais nous faisons l'hypothèse qu'il existe une variabilité entre espèces dès le début du cycle en relation avec des traits à priori clé : 1) Des traits racinaires tels que la vitesse de progression des racines en profondeur ou en latéral qui déterminent l'accès rapide aux ressources. 2) Des traits caractérisant les semences dont la masse semence l'N de la semence, réserves importantes qui peuvent être mobilisées. Plusieurs travaux ont montré une forte corrélation entre la biomasse de la plantule et la masse de la semence (Dürr and Boiffin, 1995; Fayaud et al., 2014; Tamet et al., 1996). 3) Des traits relatifs à la mise en place de l'appareil fixateur (nombre et masse des nodules, activité des nodules). En effet, une espèce démarrant rapidement leur fixation de N₂ reposera moins sur l'N du sol pour assurer ses besoins au début de cycle.

Par conséquent, la croissance et l'accumulation d'azote d'une adventice peut varier en fonction de la légumineuse avec laquelle elle est en présence, en particulier quand il s'agit d'une espèce adventice nitrophile (qui répond à la disponibilité de N dans le sol). Des travaux précédents ont également montré qu'il existe une variabilité entre génotypes au sein d'une espèce pour des traits racinaires (Bourion et al., 2010 ; Chen et al., 2011 ; Mc Phee, 2005). Nous nous intéressons ici davantage aux différences entre espèces sans considérer la variabilité intergénotypique ; nous cherchons à mettre en valeur les traits impliqués dans la croissance et l'acquisition précoce de l'azote ; les valeurs de ces traits peuvent effectivement varier à la fois entre espèces et entre variétés au sein d'une espèce.

Volet 2 : Approche agronomique : Insertion de légumineuses comme plantes de services à une culture principale. Effets sur la régulation des adventices

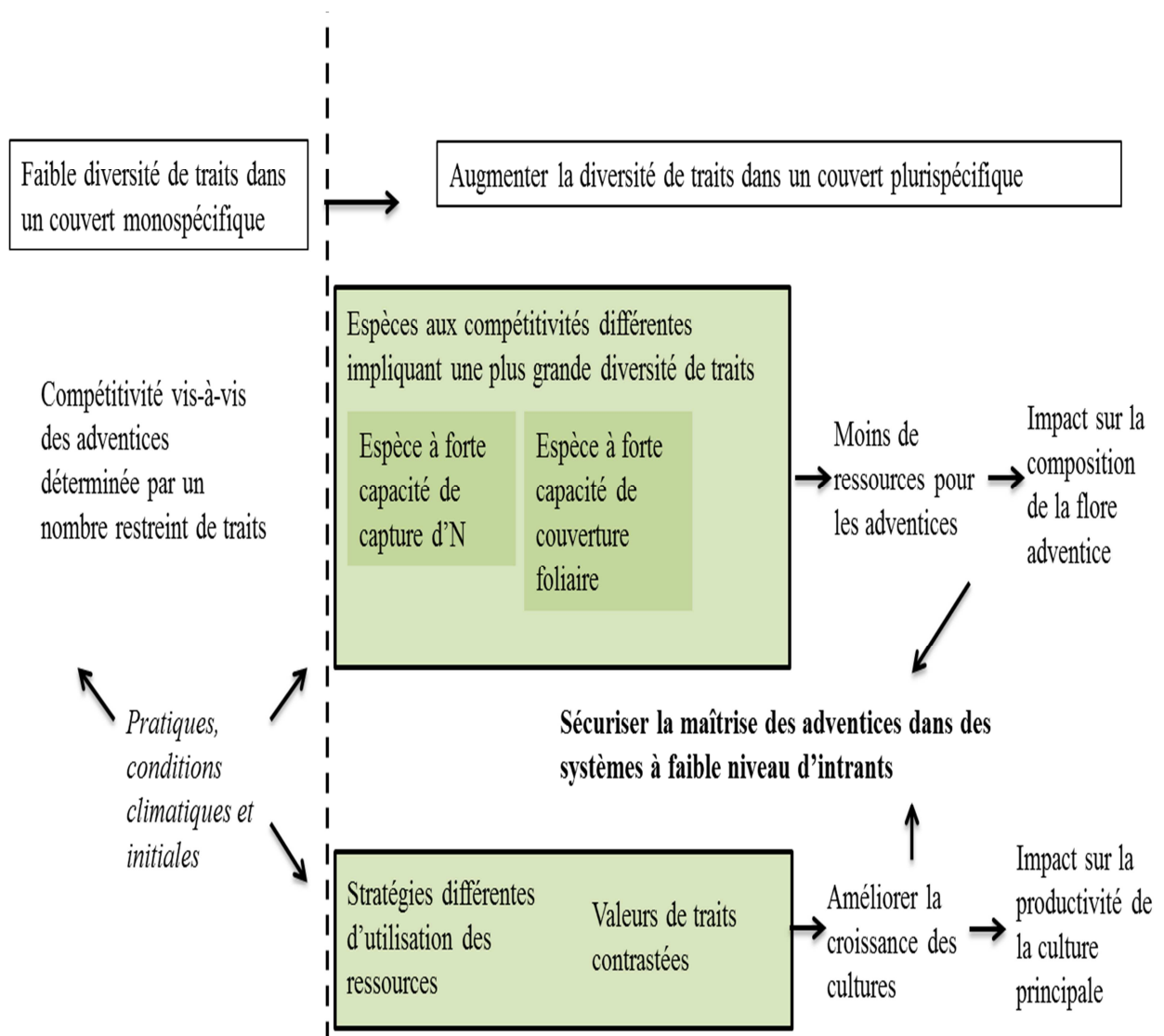


Figure 1.6. Questions et hypothèses du volet 2 : Insertion de légumineuses comme plantes de services à une culture principale. Effets sur la régulation des adventices

Ce deuxième volet se situe à des échelles temporelles (cycle de la culture annuelle ou deux cycles successifs) et spatiales (essais au champ à l'échelle du peuplement et du système de culture) très différentes de celles des expérimentations conduites dans le premier volet. Toutefois, nous nous intéressons aussi, dans ce deuxième volet, à la question de la compétitivité des légumineuses vis-à-vis des adventices en relation avec les caractéristiques des légumineuses et notamment leur capacité à capter l'azote minéral du sol. Toutefois, les légumineuses sont aussi étudiées en interaction avec le système de culture dans lequel elles sont insérées. Par ailleurs, dans ce volet, nous nous intéressons non seulement aux traits des espèces de légumineuse mais aussi aux traits des autres espèces cultivées auxquelles elles sont associées ainsi qu'aux traits de réponse des adventices.

Dans ces travaux, nous étudions des associations d'espèces combinant une espèce légumineuse et une espèce non-légumineuse. L'espèce non-légumineuse est la culture principale. L'espèce légumineuse est considérée plante de services et n'est pas récoltée.

Hypothèse 2 :

Concernant ce deuxième volet, nous faisons l'hypothèse que l'association d'une légumineuse à une non-légumineuse est un levier pertinent pour accroître la compétitivité vis-à-vis des adventices en augmentant la diversité des traits par rapport à un couvert monospécifique (Fig 1.6). Dans le cas d'une culture pure, la compétitivité vis-à-vis des adventices est déterminée par un nombre restreint de traits. Alors qu'une association pourrait permettre d'avoir sur la même parcelle des espèces aux compétitivités différentes impliquant une plus grande diversité de traits (espèce à forte capacité de capture d'N, espèce à forte capacité de couverture foliaire) laissant moins de ressources à la fois de la lumière et l'N aux adventices. La plus faible disponibilité en ressources peut impacter aussi la composition de la flore adventice

Par ailleurs dans l'association les espèces peuvent avoir des stratégies différentes d'utilisation des ressources (Par exemple, l'utilisation des sources d'N avec une légumineuse et une non- légumineuse); les espèces peuvent aussi avoir des valeurs contrastées sur certains traits (hauteur, surface foliaire, biomasse aérienne ...) permettant une occupation de l'espace et des ressources meilleures leur conférant une complémentarité conduisant à une amélioration de leur croissance que si elles étaient seules; cette amélioration de la croissance peut contribuer aussi à sécuriser la maîtrise des adventices tout en impactant la productivité de la culture principale, notamment si la complémentarité est plus forte que la compétition.

Nous étudions en premier lieu dans ce travail les effets des espèces et combinaisons d'espèces, néanmoins, ces effets peuvent être aussi dépendants des pratiques et des conditions climatiques et initiales.

2. Démarche de la thèse :

Ce Chapitre présente les principaux choix méthodologiques réalisés pour répondre aux questions des deux volets de la thèse.

Volet 1 : Approfondir la question de la compétitivité des légumineuses durant la phase de croissance précoce vis-à-vis des adventices, en lien avec la capacité de capture de l'azote minéral du sol. Pour cela, une approche écophysiological est privilégiée à travers l'identification de traits fonctionnels associés à la capture précoce de l'azote minéral du sol par les légumineuses.

Afin de répondre à cet objectif, deux expérimentations ont été réalisées en conditions contrôlées dans la serre du LEVA en 2014 (Fig. 2.1).

Expérimentation 1

Neuf espèces de légumineuse, l'arachide (*Arachis hypogaea* L.), le fenugrec (*Trigonella foenum-graecum* L.), la féverole (*Vicia faba* L.), la lentille (*Lens culinaris* L.), la luzerne (*Medicago sativa* L.), le pois (*Pisum sativum* L.), le pois chiche (*Cicer arietinum* L.), le soja (*Glycine max* L.) et la vesce commune (*Vicia. sativa* L.), ont été choisies afin d'obtenir des valeurs contrastés sur les traits à priori déterminants pour la capture d'azote minéral du sol. Elles ont été choisies aussi en fonction de leur intérêt dans la diversification de systèmes de culture actuels. Ces neuf espèces présentent une large gamme de masse initiale de semence (de 0.002 g pour la luzerne à 0.539 g pour la féverole) et de richesse initiale en azote (de 3.97 % pour le pois à 8.17% pour le soja). Par ailleurs, des travaux précédents comparant différentes légumineuses ont aussi été utilisés pour disposer de références sur différents traits : la biomasse aérienne et racinaire (Tang et al., 1999), l'exploration racinaire (Gregory, 1988; Hamblin and Hamblin, 1985; Thorup-Kristensen, 2001), le démarrage la fixation symbiotique de N₂ (Tang et al., 1999) et la capacité à capter l'azote du sol (Thorup-Kristensen, 1994; Thorup-Kristensen, 2001). Peu de références étaient toutefois disponibles sur la phase précoce. Deux espèces non fixatrices ont été ajoutées à cette expérimentation. Le blé (*Triticum aestivum* L.) et le colza (*Brassica napus* L.), deux espèces connus pour leur plus forte capacité à capter l'azote minéral du sol par rapport aux légumineuses (le colza ayant une plus forte capacité de capture que le blé (Moreau et al., 2013). Ces espèces nous ont servi de références pour comparer la capacité des espèces étudiées à capter l'azote du sol. Les espèces ont été cultivées dans un sol sableux en rhizotrons (une plante par rhizotron) afin d'assurer un suivi spatial et temporel de l'exploration racinaire pendant la phase de croissance précoce. Les rhizotrons sont transparents de taille 50 × 20 × 3 cm ; ils sont couverts avec des bâches en plastique noir et ils sont inclinés à 45° de sorte que les racines progressent le long de la vitre, facilitant leur suivi (Jamont et al., 2013). Les semences ont été calibrées pour réduire l'hétérogénéité de la masse de la semence au sein d'une espèce. Les semences ont été pré-germées sur papier filtre jusqu'à la sortie de la radicule. Ainsi, nous étudions la croissance précoce des différentes légumineuses à partir d'un même stade initial sans tenir compte des différences de vitesse de

germination qui peuvent exister entre les espèces. La croissance précoce est suivie pendant le premier mois de culture. La période d'un mois de culture a été choisie car (i) elle correspond à une phase où se mettent en place les capteurs aériens et racinaires plus ou moins rapidement selon les espèces (Fayaud et al., 2014), (ii) la compétition entre plantes est peu ou pas encore présente (Croster and Masiunas, 1998), (iii) et la fixation symbiotique n'est pas la source principale de l'azote accumulé dans la plante même si les plantes commencent la mise en place de leur appareil fixateur et que certaines démarrent même l'activité fixatrice de N_2 (Tang et al., 1999). Les légumineuses ont été inoculées avec les souches de *Rhizobium* spp. adaptées à chaque espèce. Les espèces ont été cultivées à deux niveaux contrastés d'azote: 7.01 et 35.97 mg NO_3^- N par kg de sol sec. Le niveau élevé d'azote devrait inhiber la formation des nodules et leur activité alors que le faible niveau d'azote ne devrait pas être limitant pour la mise en place de l'appareil fixateur et le démarrage de son activité (Tang et al., 1999). Les modalités (11 espèces (9 légumineuses et 2 non-légumineuses) \times 2 niveaux d'azote) sont réparties selon un dispositif en randomisation totale à 4 répétitions.



Figure 2.1. La disposition des rhizotrons dans la serre du LEVA pendant la première expérimentation

Expérimentation 2

Suite à l'expérimentation 1, trois espèces de légumineuses, la féverole (*Vicia faba* L.), le pois chiche (*Cicer arietinum* L.) et la vesce commune (*Vicia sativa* L.), ont été choisies en fonction de leurs traits contrastés pour la capture de l'azote du sol. Chaque espèce a été associée en rhizotrons à une adventice : la morelle noire (*Solanum nigrum* L.) en utilisant un dispositif additif (une légumineuse + une adventice). Les associations ont été comparées aux espèces seules. Le dispositif additif est le plus souvent utilisé dans les études sur la compétition culture adventice. Nous avons choisi la morelle noire en tant qu'adventice répondant positivement à l'azote disponible dans le sol (Bvenura and Afolayan, 2014; Khan et al., 1995; Opiyo, 2004). Cette expérimentation a été conduite selon un dispositif similaire à la première expérimentation.

Mesures et analyses

1- Evolution de l'enracinement en profondeur et en latéral

Puiseurs travaux précédents montrent l'intérêt de caractériser la dynamique d'enracinement en profondeur et en latéral, variables clés pour évaluer l'occupation rapide et large d'un volume de sol et la capture précoce de l'azote minéral du sol au début du cycle (Dunbabin, 2007). L'utilisation des rhizotrons nous a permis de déterminer la progression racinaire en profondeur et en latéral pour les légumineuses ainsi que pour l'adventice au cours des *expérimentations 1 et 2* et jusqu'au moment où les racines arrivent au fond des rhizotrons (Fig. 2.2). La méthode est décrite en détail dans les articles concernant les chapitres 1 et 2.

2- Biomasse aérienne et racinaire

La biomasse racinaire et aérienne des légumineuses et celle de l'adventice ont été déterminées à partir d'un prélèvement réalisé 35 jours après la germination des plantules. Les racines des légumineuses et de l'adventice ont été distinguées.

3- Nombre et biomasse des nodules

Afin de quantifier le nombre et la biomasse des nodules à la fin de l'expérimentation 1, les nodules ont été séparées des racines et comptées puis pesées (matière sèche) pour chaque espèce de légumineuse (Fig. 2.2).

4- Analyse de l'azote dans la plante

Les différents échantillons ont été broyés afin de mesurer la quantité de l'azote total accumulé et le ratio $^{15}\text{N}:^{14}\text{N}$ afin de déterminer les trois sources contribuant à l'azote total : l'azote de la semence, l'azote minéral prélevé du sol et l'azote atmosphérique (N_2). La quantité d'azote accumulé dans l'adventice a été aussi mesuré afin d'analyser la compétition pour l'azote du sol entre la légumineuse et l'adventice associées. Les méthodes et calculs sont détaillés dans les articles des chapitres 1 et 2.



Figure 2.2. Á gauche : photographie montrant le suivi racinaire chez le pois chiche cultivé en rhizotron. Au milieu : démoulage du rhizotron pour prélever le système racinaire en fin d'expérimentation. Á droite : nodules isolés pour leur comptage

Volet 2 : Etudier l'effet du mode d'insertion des légumineuses en tant que plantes de services associées à une espèce non-légumineuse sur la densité, la biomasse et la richesse spécifique des adventices.

Dans ce volet, deux expérimentations ont été mises en place en plein champ.

La première expérimentation consiste en l'insertion de légumineuses annuelles dans une culture de colza à l'échelle du cycle de culture de ce dernier. Ce type d'association est déjà pratiqué par des agriculteurs et recommandée par les organismes de conseil. Les objectifs recherchés par les agriculteurs sont principalement : i) améliorer le contrôle des adventices pendant l'automne-hiver et réduire ainsi le nombre de traitements herbicides, ii) ainsi que réduire la dose d'engrais N apporté sur le colza en profitant de l'N issu de la minéralisation des résidus de la légumineuse, le plus souvent détruite par le gel pendant l'hiver (Cadoux et al., 2015). Certains agriculteurs envisagent aussi ces associations en faisant cohabiter les deux espèces tout au long du cycle et en récoltant les deux composantes. Ces situations sont toutefois moins répandues.

La deuxième expérimentation a testé différents couverts de légumineuses pérennes en comparaison de graminées dans une culture de maïs semence à l'échelle pluriannuelle (étude conduite sur deux ans, deux cycles culturaux de maïs successifs). En production de semences, les itinéraires techniques sont souvent très standardisés et très consommateurs en intrants en particulier en herbicides pour répondre à des exigences fortes en terme de qualité de la production, notamment la pureté spécifique. Néanmoins des innovations sont explorées aussi dans ce secteur face à des préoccupations environnementales plus fortes et la moins grande disponibilité de produits phytosanitaires homologués.

Ces deux expérimentations permettent d'explorer l'insertion de légumineuses dans des systèmes très contrastés. La durée de l'étude est très différente. Pour la première expérimentation, elle correspond à la durée du cycle du colza (10 mois). Pour la deuxième, elle démarre avant le semis du maïs avec l'installation des couverts (6 mois) et deux cycles successifs deux maïs (5 mois x 2) entrecoupés par la période d'interculture entre eux (6 mois). D'autre part, les périodes de co-culture de la légumineuse et la non-légumineuse sont aussi variables. Elle correspond à la période allant du semis du colza à la sortie de l'hiver dans l'association colza-légumineuse dans le cas où le gel a détruit la légumineuse durant l'hiver ou au-delà quand la légumineuse n'a été que partiellement détruite. Elle correspond à la durée du cycle du maïs dans l'expérimentation 2, dans le cas d'un couvert maintenu vivant. Les pratiques culturales diffèrent aussi : aucune pratique culturale n'est réalisée pour modifier la croissance de la légumineuse dans l'expérimentation 1 alors que plusieurs pratiques sont mises en œuvre et de façon différenciée au cours des deux années pour maîtriser la compétition légumineuse-maïs. Dans les deux types d'expérimentation, on cherche à étudier l'intérêt des associations en

comparaison des cultures pures dans un contexte fort de pression d'adventices. Dans l'expérimentation 1, aucun désherbage n'est effectué sur l'ensemble de l'essai. Dans l'expérimentation 2, des traitements herbicides ont été utilisés pour réguler ou détruire le couvert et des interventions supplémentaires ont été appliquées dans certaines situations pour éviter une trop forte compétition des adventices sur le maïs.

Le colza et le maïs se caractérisent par leurs forts besoins en N pour garantir un niveau de biomasse et un niveau de rendement satisfaisants. Le colza est connu aussi pour avoir une très forte capacité à capter l'N minéral du sol. Ces cultures se caractérisent, toutes les deux aussi, par une installation assez lente du couvert aérien en début de cycle en lien notamment avec la faible masse de la semence chez le colza et une faible vitesse de mise en place de la surface foliaire et une faible croissance en hauteur chez les variétés de maïs utilisées en multiplication de semences. Ces deux espèces diffèrent aussi par leur plasticité : le colza est capable de présenter une architecture et une biomasse par plante variable en fonction de la densité de plantes et le niveau de compétition entre elles (Diepenbrock, 2000; Thurling, 1974). Le maïs a quant à lui moins de possibilité de compensations à différents moments du cycle. L'installation de cette culture est donc une phase primordiale pour la croissance et l'élaboration du rendement.

Les dispositifs expérimentaux diffèrent aussi : essai factoriel en station expérimentale pour l'expérimentation 1 en microparcelles et essai en bandes chez un agriculteur avec une adaptation des pratiques en cours d'expérimentation en fonction de l'évolution du système. Nous étudions deux facteurs principaux à savoir la nature du couvert et son maintien ou pas (mulch vivant ou mort). Nous sommes en revanche plus proche dans l'expérimentation 2 d'un essai système que d'un essai factoriel.

Expérimentation 1

Nous étudions l'impact du choix de la légumineuse en association avec du colza en comparaison du colza pur. Des modalités de légumineuses pures ont aussi été insérées pour caractériser les différences de traits des différentes espèces étudiées.

La vesce et la féverole ont été choisies pour leurs traits à priori contrastés concernant leur capacité à capter l'azote du sol. La féverole est connue aussi pour une croissance foliaire et végétative plus importante que d'autres légumineuses (Jensen et al., 2010). Les espèces et variétés choisies sont sensibles au gel.

L'expérimentation a été répétée deux fois en 2012/2013 et 2015/2016 ans sur deux parcelles différentes de la station expérimentale de la FNAMS (Fédération Nationale des Agriculteurs Multiplicateurs de Semences) à Brain-sur-l'Authion, Maine-et-Loire. Les modalités sont réparties selon un dispositif en bloc à quatre répétitions (Fig. 2.3). Les associations ont été étudiées avec un dispositif substitutif. Le colza et la légumineuse sont semés à 50% de la densité en culture pure. Ces associations sont le plus souvent étudiées selon un dispositif additif (Cadoux et al., 2015; Lorin et al., 2015). Nous avons choisi de cultiver les cultures avec un dispositif substitutif avec l'objectif de maximiser la complémentarité interspécifique entre le colza et la légumineuse pour à la fois maîtriser les adventices sans pénaliser le colza. Le choix de ce dispositif est aussi guidé par l'idée de valoriser la forte plasticité du colza et sa capacité de compensation face à une plus faible densité de semis.

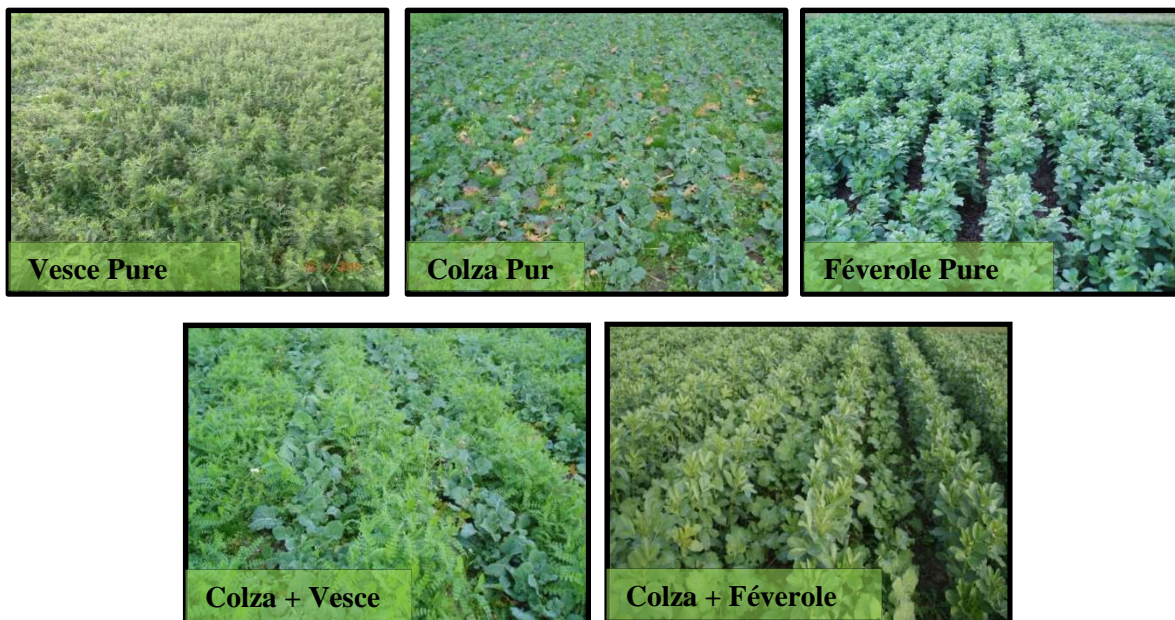


Figure 2.3. Photos présentant les 5 modalités de l'essai (colza-légumineuse) au champ

Mesures et analyses

Dans cette expérimentation, la compétitivité vis-à-vis des adventices des différentes espèces et leur association est évaluée à deux périodes clés : à l'entrée de l'hiver (décembre) et la sortie de l'hiver (février), c'est-à-dire au début et à la fin de la période de co-culture des deux espèces avant la destruction prévue des légumineuses par le gel. L'impact sur les adventices a été mesuré via des mesures de biomasse et de densité d'adventices. La biomasse d'adventices est considérée une variable pertinente quand on étudie la compétition culture-adventices (Lins and Boerboom, 2002). Les adventices ont été comptés par espèce à chaque prélèvement afin d'évaluer la composition de la flore adventice. Aux mêmes dates, des caractéristiques des espèces à priori déterminantes pour expliquer des différences de compétitivité vis-à-vis des adventices ont été mesurées : la hauteur, la densité, la biomasse aérienne, la surface foliaire et capacité à capter l'N du sol. Le rendement du colza a été déterminé à maturité.

Expérimentation 2

Nous étudions ici l'impact de l'insertion de légumineuses (luzerne et trèfle blanc) en association avec du maïs semence en comparaison d'un maïs conduit sans couvert. Deux espèces de couvert non-légumineuses (ray-grass et avoine) ainsi qu'une association graminée-légumineuse (ray-grass/trèfle blanc) ont aussi été étudiées et comparées aux légumineuses. Les couverts sont des espèces pérennes permettant de les maintenir sur plusieurs années en couvert permanent, excepté l'avoine qui est une, espèce annuelle. L'avoine a été retenue en raison de sa forte compétitivité en début de cycle et d'effets allélopathiques (Belz, 2007; Weston, 1996) contribuant un contrôle élevé des adventices.

Cet essai a été conduit sur une parcelle pour la production du maïs semence chez un agriculteur au lieu-dit « Le Voisinay » à Saint-Mathurin-sur-Loire près d'Angers. Le choix des modalités s'est fait en concertation avec l'agriculteur qui était à l'origine de l'essai. Il souhaitait tester l'insertion de couverts pour pallier à des difficultés de gestion de certaines adventices dominantes (liseron des champs : *Convolvulus arvensis* L.) et des difficultés à maintenir un rendement élevé sur le long-terme sur des parcelles en monoculture de maïs semence.






Les couverts ont été semés en novembre 2012. Au moment de semis du maïs en mai 2013, le couvert a été géré de deux façons différentes afin de créer deux systèmes différents (Fig. 2.4). Dans le premier système, les différents couverts ont été maintenus (mulch vivant) mais ont été fauchés au moment du semis pour ralentir leur croissance alors que dans le deuxième système, le couvert (couvert mort) a été détruit via l'utilisation de glyphosate. En deuxième année de l'essai (2014), un travail de strip-till (technique de travail du sol localisé sur les rangs) a été réalisé sur les rangs du maïs afin de réduire la compétition avec les couverts.

Mesures et analyses

Les couverts ont été caractérisés pendant la phase d'installation avant le semis du maïs par les mesures suivantes : la hauteur du couvert, la biomasse aérienne et l'azote minéral dans le sol. L'effet de cette phase d'installation sur la biomasse et la densité d'adventices a été étudié. La biomasse aérienne des couverts, du maïs et des adventices ont été mesurées à différentes dates pour évaluer la compétition entre les trois composantes à plusieurs stades clés du cycle du maïs. Le niveau d'N minéral du sol a été aussi mesuré à plusieurs stades. De plus, les adventices ont été comptées par espèce à chaque prélèvement afin d'évaluer l'évolution de la composition de la flore adventice en fonction du système et du couvert.

Les mesures et analyses sont détaillées dans les chapitres 3 et 4.

Table 2.1. Les plantes de couverture étudiées dans l'expérimentation conduite au champ du maïs semence

| | Espèce | Variété | Type d vie | Densité de semis (kg ha ⁻¹) |
|---|--|----------------|------------|---|
|  | Lucerne cultivée <i>Medicago sativa</i> L. | Cannelle | Pérenne | 12 |
|  | Trèfle blanc <i>Trifolium repens</i> L. | Alice | Pérenne | 20 |
|  | Ray-grass anglais <i>Lolium perenne</i> L. | Aberavon | Pérenne | 20 |
|  | Avoine <i>Avena sativa</i> L. | Une de mai | Annuel | 80 |
|  | Mixture (Ray-grass/Trèfle blanc) | Aberavon/Alice | | 10-10 |

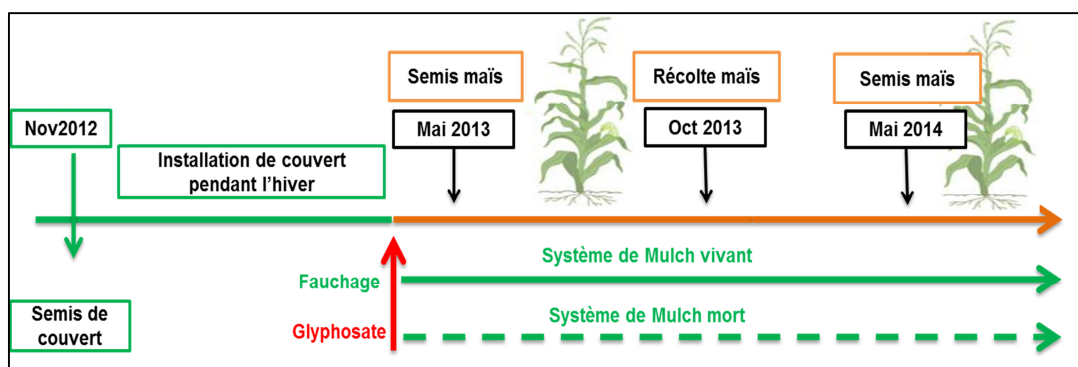


Figure 2.4. Illustration des systèmes étudiés dans l'essai en maïs semence

3. Organisation du manuscrit

La présentation des travaux est organisée en 4 chapitres :

Les chapitres 1 et 2 concernent le premier volet de la thèse et **les chapitre 3 et 4** concernent le deuxième volet.

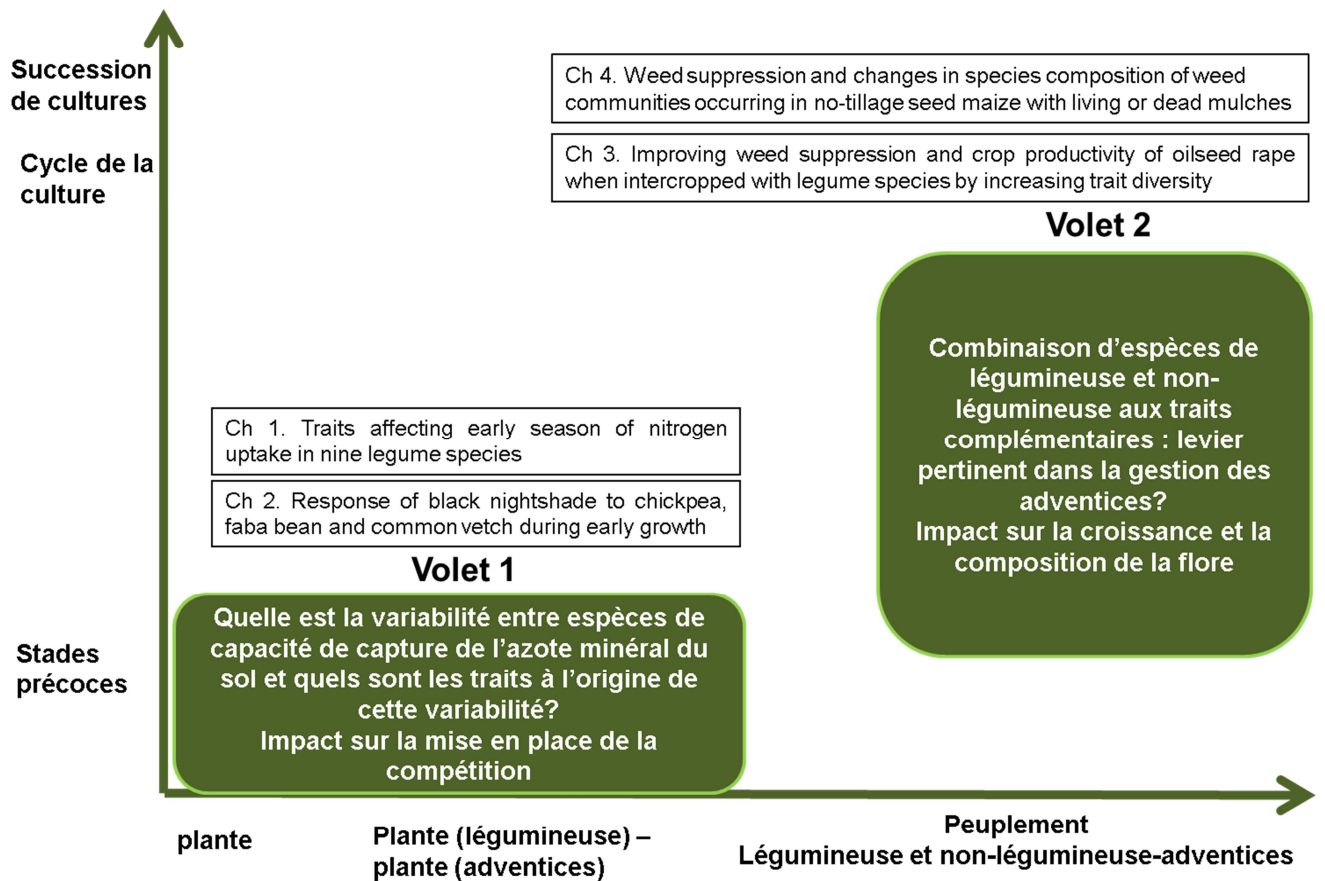
Ces quatre chapitres correspondent à quatre articles.

Le chapitre 1 est intitulé *Traits affecting early season nitrogen uptake in nine legume species*. Il se focalise sur la variabilité entre espèces de la capture de l'azote en tout début de cycle et identifie des traits clés qui permettent de classer les espèces selon leur potentielle compétitivité pour l'azote du sol.

Le chapitre 2 est intitulé *Response of black nightshade to chickpea, faba bean and common vetch during early growth*. Cette partie étudie la réponse d'une adventice nitrophile, la morelle à la présence de légumineuses. Trois légumineuses aux traits contrastés (d'après les résultats du chapitre 1) sont étudiés.

Le chapitre 3 est intitulé *Improving weed suppression and crop productivity of oilseed rape when intercropped with legume species by increasing trait diversity*. Cet article étudie l'effet sur les adventices de l'association d'une légumineuse à une culture de colza. Les contrastes entre les composantes associées en terme de compétitivité vis-à-vis des adventices sont étudiés. L'intérêt d'associer des espèces aux traits contrastés est exploré.

Le chapitre 4 est intitulé *Weed suppression and changes in species composition of weed communities occurring in no-tillage seed maize with living or dead mulches*. Cet article étudie l'effet sur les adventices de l'insertion de couvert dans une culture de maïs en production de semences. Des comparaisons entre espèces de couvert sont présentées en lien avec la dynamique de croissance et la dynamique des flux azotés et en interaction avec les pratiques culturales. L'impact sur la composition de la flore adventices est présenté.



Dans le chapitre 1, nous allons étudier la variabilité de la capacité à capter l'azote minéral du sol pour une gamme d'espèces de légumineuse en identifiant les traits majeurs impliqués dans cette capacité à la phase de croissance précoce. Ce chapitre repose sur une expérimentation conduite en conditions contrôlées. A l'issue de cette expérimentation, nous avons identifié les traits principaux impliqués dans la capacité des espèces de légumineuse à capter l'azote du sol permettant une classification..

4. Chapitre 1. Traits affecting early season nitrogen uptake in nine legume species

Ce chapitre correspond à un article publié dans la revue *Helyion*

Dayoub, E., Naudin, C., Piva, G., Shirtliffe, S.J., Fustec, J., Corre-Hellou, G., 2017. Traits affecting early season nitrogen uptake in nine legume species. *Helyion* 3, Article e00244, <http://dx.doi.org/00210.01016/j.helyion.02017.e00244>.

Traits affecting early season nitrogen uptake in nine legume species

Elana Dayoub¹, Christophe Naudin¹, Guillaume Piva¹, Steven J. Shirtliffe², Joëlle Fustec¹ and Guénaëlle Corre-Hellou^{1*}

¹ USC LEVA, INRA, Ecole Supérieure d'Agricultures, Uni. Bretagne Loire, SFR 4207 QUASAV, 55 rue Rabelais, 49007, Angers, France

² Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada

Abstract

Legume crops are known to have low soil N uptake early in their life cycle, which can weaken their ability to compete with other species, such as weeds or other crops in intercropping systems. However, there is limited knowledge on the main traits involved in soil N uptake during early growth and for a range of species. The objective of this research was to identify the main traits explaining the variability among legume species in soil N uptake and to study the effect of the soil mineral N supply on the legume strategy for the use of available N sources during early growth. Nine legume species were grown in rhizotrons with or without N supply. Root expansion, shoot and root biomass, nodule establishment, N₂ fixation and mineral soil N uptake were measured. A large interspecific variability was observed for all traits affecting soil N uptake. Root lateral expansion and early biomass in relation to seed mass were the major traits influencing soil N uptake regardless of the level of soil N availability. Fenugreek, lentil, alfalfa, and common vetch could be considered weak competitors for soil N due to their low plant biomass and low lateral root expansion. Conversely, peanut, pea, chickpea and soybean had a greater soil N uptake. Faba bean was separated from other species having a higher nodule biomass, a higher N₂ fixation and a lower seed reserve depletion. Faba bean was able to simultaneously fix N₂ and take up soil N. This work has identified traits of seed mass, shoot and root biomass, root lateral expansion, N₂ fixation and seed reserve depletion that allowing classification of legume species regarding their soil N uptake ability during early growth.

Keywords Fabaceae; Nitrogen; Biological nitrogen fixation; soil N; root growth; Crop establishment

4.1. Introduction

Legumes are known to be less efficient at recovering soil inorganic N during the growing season compared to non-legume crops including those in the Poaceae or Brassicaceae family (Thorup-Kristensen, 1994). This low soil N uptake may be a key factor explaining their low ability to reduce N leaching as catch crops (Thorup-Kristensen, 2001), their low ability to compete against weeds (Corre-Hellou et al., 2011) and compete with non-legumes when intercropped (Jensen, 1996; Naudin et al., 2010).

Early plant growth is a crucial phase for the establishment of relative competitive ability with other species (weeds or crops) (Bellostas et al., 2003; Fayaud et al., 2014). Small differences in early-season seedling growth can result in large differences in size and resources capture ability later in the season (Andersen et al., 2007). Therefore plant strategies resulting in greater early season crop growth and N acquisition can be critical for determining competitive interactions between crops and other species. During early growth legumes species are expected to have low soil N uptake. Nevertheless, key factors explaining this early low soil N uptake ability of legumes are not known. During early growth, legumes may rely mainly on soil N uptake similar to non-legume species because legumes have not had time to initiate nodulation and develop effective nodules infected with nitrogen fixing bacteria. Moreover, grain legumes can rely also on seed N reserve for a significant time due to the large seeds with a relatively high nitr

ogen content (Herdina and Silsbury, 1990). This can result in low use of external N sources.

Few studies have examined the variability among legume species in early season soil N uptake (Hamblin and Hamblin, 1985). Early season soil N uptake may differ among legume species because of differences in root expansion, early plant growth and symbiotic N₂ fixation (nodules establishment and activity).

Although the ability of roots to quickly and effectively occupy the soil volume during crop establishment can be a major trait related to the competitiveness for soil N, species differences in this trait are not well documented (Jamont et al., 2013). A greater root occupancy of the soil volume should allow for greater N uptake, resulting in less available N for other species (Dunbabin, 2007). Thus, the root depth penetration and lateral expansion may be key traits affecting the competitive ability of legumes for soil N during early growth. Moreover, species that have a greater initial growth are often more competitive for soil resources at the beginning of the crop life cycle (Andersen et al., 2007).

During the early growth phase, legumes may access three different sources of N: the seed reserve, soil N, and in a lower extent, atmospheric N₂. In similar agronomic conditions, the relative contributions of each of these sources to the plant growth may differ across species. The seed mass can explain differences among species in the level of shoot biomass during early growth (Fayaud et al., 2014; Gross, 1984). A species able to rely on biological N₂ fixation for N nutrition earlier than others may

have reduced N uptake from the soil. Nodule establishment and activity can differ across species according to seed size (Doberst and Blevins, 1993), type of emergence (hypogeal or epigeal) (Sprent and Thomas, 1984) and depletion of seed N reserves, all which interact with the mineral soil N availability (Voisin et al., 2010).

Although some studies have shown differences among legume crops in root development (Hamblin and Hamblin, 1985) and N₂ fixation (Herridge et al., 2008; Saia et al., 2016), there have been few legume species studied, and the simultaneous analysis of different traits, correlations between several traits, and consequences on soil N uptake during early growth have not been investigated.

The objectives of this research were i) to identify the main traits explaining the variability among legume species in soil N uptake during early growth and ii) to study the effect of the soil mineral N supply on the legume strategy for the use of available N sources. A greenhouse experiment was undertaken to monitor the early growth stages of nine legumes species sown in rhizotrons either at a low or high level of soil mineral N.

4.2. Materials and Methods

4.2.1. Plant material and experimental design

Nine divergent legume species (Table 4.1) were selected for evaluation based on their expected contrasting traits related to early growth and N acquisition. Peanut (*Arachis hypogaea* L.), fenugreek (*Trigonella foenum-graecum* L.), faba bean (*Vicia faba* L.), winter lentil (*Lens culinaris* L.), alfalfa (*Medicago sativa* L.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L.) and common vetch (*Vicia sativa* L.) were grown with or without mineral nitrogen in a factorial completely randomized design experiment. The experiment was conducted under natural light in a greenhouse from March 27th to April 30th, 2014 in western France (Angers; 47.5°N, 0.6°W). The mean temperature and humidity during the experiment were 20.1 ± SD 0.9 °C and 41.8 ± SD 10.3%, respectively. This experiment was carried out using transparent rhizotrons (Jamont et al., 2013) with inner dimensions of 50 X 20 X 3 cm that were covered with black polyethylene plastic sheeting. The rhizotrons were inclined at 45° so that the roots would grow towards the underside. Each rhizotron was filled with 4.1 kg of a mixture of sandy fresh soil (25%) and dry sand (75%). The soil was sieved (7 mm) prior to being homogeneously mixed with dry sand. The sand was previously washed and dried at 105 °C for 48 h. The final composition of the soil sand mixture was 2.9% clay, 6% loam, 90.6% sand and 0.4% organic matter, with 7 mg NO₃⁻ N kg⁻¹ dry soil and the pH of the mixture was 6.9. The phosphorus (P Olsen) and potassium (K) contents were 30.3 and 78.7 mg kg⁻¹ of dry soil, respectively. 150 ml of a N-free nutrient solution was added to each rhizotron at the following concentrations (mmol l⁻¹): 1.24 KCl, 0.34 KH₂PO₄, 0.24 MgSO₄ 7H₂O, 0.18 CaCl₂ and 0.04 FeCl₃, with a controlled pH of 6.8. In half of the rhizotrons, the soil was supplemented with a solution of

KNO₃ to reach a concentration of 36 mg NO₃⁻ N kg⁻¹ dry soil. The nutrient solutions were mixed with the soil before filling the rhizotrons.

The legume seeds were weighed and selected to reduce the variation in the individual seed mass to less than ± 3.5% (except alfalfa, with a variation of ± 7%, because of the high seed dry matter (DM) heterogeneity). The seeds were first germinated on filter paper until the radicle emerged from the seed (BBCH stage 05) (JKI, 2010), and one seed was sown per rhizotron before being inoculated with an appropriate strain of rhizobia (Table 4.1) at an estimated rate of 10⁸ cells per plant to allow for N₂ fixation (Naudin et al., 2011); for each legume species, the seeds were sown in four rhizotrons without N (0N treatment) and four rhizotrons with a N supply (N treatment). After the seedling emergence, a 1-cm thick layer of sand (100 g) was added to the surface of the rhizotron to limit water evaporation from the soil. During the experiment, the soil humidity was kept at field capacity.

Common wheat (*Triticum aestivum* L, cv Renan) and oilseed rape (*Brassica napus* L., cv Boheme), known for their contrasting abilities to acquire N (Moreau et al., 2013) , were grown as controls to compare the ability of legume species with non-legume species to deplete soil N.

Plants of each legume species were also grown simultaneously in N-free sand to determine the ¹⁵N abundance of plants relying only on N₂ fixation (β value) and thus estimate the N₂ fixation using the natural abundance method (Amarger et al., 1979). The pots were supplied once per week with 100 ml of N-free nutrient solution with a composition of (mmol l⁻¹) 0.54 KCl, 0.17 KH₂PO₄, 0.12 MgSO₄ 7H₂O, 0.09 CaCl₂, and 0.02 FeCl₃ with a controlled pH of 6.8 and maintained at field capacity.

Table 4.1. List of studied species

| Latin name | Common name | Variety | Emergence type | Initial seed dry matter (g) (isdm) | Seed N content (%sN) | Phenological Stages at 35 DAG ³ | Rhizobium strain |
|-------------------------------------|---------------|---------------|----------------|------------------------------------|----------------------|--|---|
| <i>Medicago sativa</i> L. | Alfalfa | Cannelle | Epigeal | 0.002 | 6.68 | 13 | <i>Sinorhizobium meliloti</i> (2011) ² |
| <i>Trigonella foenum-graecum</i> L. | Fenugreek | Wild genotype | Epigeal | 0.012 | 5.44 | 14 | <i>Sinorhizobium</i> sp. (MSDJ3538) ² |
| <i>Lens culinaris</i> L. | Winter lentil | ANICIA | Hypogeal | 0.024 | 4.68 | 19 | <i>Rh.leguminosarum</i> bv <i>viciae</i> (P221) ² |
| <i>Vicia sativa</i> L. | Common vetch | Nacre | Hypogeal | 0.051 | 5.19 | 39 | <i>Rh.leguminosarum</i> bv <i>viciae</i> (P221) ² |
| <i>Glycine max</i> L. | Soybean | Protina | Epigeal | 0.177 | 8.17 | 13 | <i>Bradyrhizobium japonicum</i> (G49) ² |
| <i>Pisum sativum</i> L. | Pea | Standal | Hypogeal | 0.273 | 3.97 | 18/37 | <i>Rh.leguminosarum</i> bv <i>viciae</i> (P221) ² |
| <i>Cicer arietinum</i> L. | Chickpea | Principé | Hypogeal | 0.364 | 4.16 | 23 | <i>Mesorhizobium ciceri</i> (UMPCa7) ¹ |
| <i>Arachis hypogaea</i> L. | Peanut | TE3 | Epigeal | 0.407 | 5.69 | 15/22 | <i>Bradyrhizobium tropici</i> (CB756) ¹ |
| <i>Vicia faba</i> L. | Faba bean | Divine | Hypogeal | 0.539 | 4.97 | 16 | <i>Rhizobium leguminosarum</i> bv <i>viciae</i> (FH34) ² |

Sources of Rhizobium strains:

¹INRA, UMR Eco&Sols (Montpellier SupAgro – CIRAD – INRA – IRD), France

²UMR 1347 - Agroecologie, Pôle MERS, Microbiologie Environnementale et Risque Sanitaire, Center INRA, Dijon, France

³According to BBCH scale of (JKI, 2010) at sampling date (35 days after seed germination)

4.2.2. Sampling and data collection

The root depth and lateral expansion were monitored using a grid of 5 mm × 5 mm squares printed on a transparent plastic sheet fixed on the underside of the rhizotron. Three times a week for five weeks, the root presence in the grid squares was marked to denote vertical and horizontal root distribution.

The plants were harvested 35 days after germination (DAG) while still in early growth phase. At 35 days, legumes should rely mainly on seed N and soil N. A little nitrogenase activity was reported to be evident in grain legumes approximately 20 days after sowing at 20°C (Herdina and Silsbury, 1990), depending on the mineral N level. The root systems were removed from the rhizotron and washed with demineralized water. The roots and nodules were sorted, and the nodules were placed on a white sheet to be photographed with a Canon EOS 350D digital camera (lens: SIGMA 50 mm F2.8 DG). The pictures were analysed with ImageJ version 1.40 g (National Institutes of Health, USA; <http://rsb.info.nih.gov/ij/>) to obtain the total number of nodules. The seed residues were collected to quantify the remaining seed DM. Plants grown in N-free sand were harvested at the same time and sorted into shoot and root samples. After oven-drying (48 h at 70°C), the dry matter of the roots, shoots, nodules and seed residues were determined. All of the samples were ground to fine powder for total N and ¹⁵N:¹⁴N ratio measurements in the original seeds, nodulated roots, shoots, and seed residues using a CHN analyser (EA3000, Euro Vector, Milan, Italy) and a mass spectrometer (IsoPrime, Elementar, Hanau, Germany).

At each sowing and harvest date, soil samples were collected for all species (legumes, wheat and oilseed rape), and the soil inorganic N content was measured using a segmented flow analysis (SKALAR SA3000), which enables the determination of the nitrate content by KCl extraction according to the international standard ISO 14256-2.

4.2.3. Calculations and statistics

The part of the plant N derived from the seed was calculated using the amount of N from the seed (QN seed) consumed during the experiment by each plantlet. QN seed is the difference between the seed N content at sowing and that at harvest. Exogenous N is the difference between the N accumulated in the plant and the QN seed. The relative contributions of the exogenous sources of N (air and soil) were then determined.

The part of the plant exogenous N derived from the air (%Ndfa) was estimated using the ^{15}N natural abundance method (Amarger et al., 1979). Common wheat was used as a non-fixing reference crop (equation 1),

$$\%Ndfa=100 \times [(\delta^{15}\text{N}_{\text{wheat}}-\delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{wheat}} - \beta)] \quad [1]$$

where $\delta^{15}\text{N}_{\text{legume}}$ and $\delta^{15}\text{N}_{\text{wheat}}$ express the natural ^{15}N abundances of legume and of wheat, respectively. β is the isotopic fractionation factor measured from each species of legumes grown in N-free sand.

The $\delta^{15}\text{N}$ of the whole crop was calculated using equation 2:

$$\delta^{15}\text{N}_{\text{whole crop}}=[(\delta^{15}\text{N}_{\text{shoot}} \times \text{shoot DW}) + (\delta^{15}\text{N}_{\text{root}} \times \text{root DW})] / (\text{shootDW} + \text{rootDW}) \quad [2]$$

The values of $\delta^{15}\text{N}$ for legumes and common wheat were corrected considering the N derived from the seed as another source of N for the plant (Jensen et al., 1985), (equation 3):

$$\delta^{15}\text{N}_{\text{corrected}}=[(\delta^{15}\text{N}_{\text{crop}} - (\text{QN}_{\text{seed}} / \text{QN}_{\text{crop}}) \times \delta^{15}\text{N}_{\text{seed}})] / [(1-(\text{QN}_{\text{seed}} / \text{QN}_{\text{crop}}))] \quad [3]$$

where $\delta^{15}\text{N}_{\text{seed}}$ expresses the natural ^{15}N abundance of the original seeds.

The contribution of the soil N was calculated as the difference between the exogenous N and the N derived from air.

The rates of vertical and lateral root expansion (mm day^{-1}) were calculated by linear regression analysis. The effect of species on the shoot and root dry matter, root depth penetration and lateral expansion rates, nodule establishment, N acquisition and remaining soil N was tested by a one-way analysis of variance (type III sum of squares; $\alpha=0.05$). The normality of the residues and homoscedasticity were tested using Pearson's and Levene's tests, respectively ($\alpha=0.05$). The data were log transformed using $\text{Ln}(x)$ for the root, shoot DM, plant DM, root:shoot ratio and soil N uptake and $\text{log}(x)$ for the nodule number. Means were compared using Tukey's HSD test (Honest Significant Differences; $\alpha =0.05$) to determine whether a main effect was significant. All statistical analyses were performed using R commander package in R software, version 3.1.2 (RCore, 2012).

Principal component analysis (PCA) was used to analyse the correlations between the seed, shoot and root traits and N accumulation. PCA was implemented using the package FactoMineR (Lê et al., 2008) in R software. Each original variable was standardized to have equal weights in the multivariate analysis. The principal components (PC) were derived by calculating independent axes to explain the total variance in the dataset. An ascending hierarchical clustering algorithm was also applied to the data using the same standardized variables as in the PCA. The function hclust was implemented using the Euclidian distance to calculate the dissimilarity matrix among species using Ward's minimum variance method as the clustering algorithm. This type of analysis provides information on the relative distance among clusters and species. A separate PCA analysis was performed for each N treatment.

4.3. Results

4.3.1. Growth and N acquisition at a low mineral N level

Shoot and root biomass – In the 0N treatment, the biomass varied among species ($p < 0.0001$), with a coefficient of variation higher than 0.7 (Table 4.2). The shoot biomass varied from 0.008 (alfalfa) to 0.47 (faba bean) g per plant ($p < 0.0001$), and the root:shoot ratio ranged between 0.40 (pea) and 0.87 (alfalfa) ($p < 0.0001$). For all root parameters (root depth penetration rate, lateral expansion rate and root biomass), there were significant differences among legumes (Table 4.2 and 4.3, $p < 0.0001$). The rate of depth penetration of the taproot (linear regressions, average $R^2=0.96$) varied from 7.1 in alfalfa to 22.6 mm day⁻¹ in soybean. The rate of the root lateral expansion (linear regressions, average $R^2=0.86$) varied from 1.3 in fenugreek to 10.4 mm day⁻¹ in chickpea. The ranking of species differed according to the root parameters, and different types of root exploration were observed. Lentil and alfalfa both had slow lateral and vertical root expansions, whereas soybean and chickpea had rapid lateral and vertical root expansions. Common vetch had a rapid vertical root penetration but a medium lateral root expansion. Fenugreek had a medium rate of vertical root penetration and a very slow lateral root expansion. A greater variability was observed for lateral expansion (CV=0.55) than depth penetration (CV=0.35). For species in which the rate of depth penetration is greater than 15 mm day⁻¹ (peanut, faba bean, pea, chickpea, common vetch, soybean), the lateral expansion varied from 5.2 (common vetch) to 10.4 (chickpea) mm day⁻¹.

Nodule number and biomass – In the 0N treatments, all species began to produce nodules but not all of them showed developed functioning nodules. There were large differences in the number of nodules among species ($p < 0.0001$, Table 4. 3). The greatest number of nodules was observed in faba bean and common vetch, with 48.8 and 60.5 nodules per plant, respectively, whereas nodule number was very low for alfalfa (1.5), fenugreek (4.3) and chickpea (4.3). The mean biomass per nodule also varied very significantly ($p < 0.0001$). For example, the common vetch and faba bean roots both had numerous nodules, but the mean biomass per nodule was low in the common vetch (0.07 mg nodule⁻¹), whereas it was high in faba bean (0.36 mg nodule⁻¹).

Table 4.2. Shoot and root dry matter, root: shoot ratio and initial seed dry matter for nine legume species grown without N supply. Values are means (n=4, except for fenugreek, where n=3). CV: coefficient of variation. *P* values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$). ***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively. Means on the same line with different letters are significantly different ($P<0.05$)

| | Alfalfa | Fenugreek | Lentil | Common vetch | Soybean | Pea | Chickpea | Peanut | Faba bean | CV | <i>P</i> value |
|--|----------------|----------------|----------------|----------------|-----------------|----------------|----------------|-----------------|-----------------|------|----------------------------|
| Shoot dry matter (Sdm) (g pl ⁻¹) | 0.008 d | 0.038 c | 0.046 c | 0.159 b | 0.413 a | 0.406 a | 0.412 a | 0.398 a | 0.427 a | 0.73 | 1.02×10^{-15} *** |
| Root dry matter (Rdm) (g pl ⁻¹) | 0.007 e | 0.024 d | 0.028 d | 0.065 c | 0.219 ab | 0.166 b | 0.331 a | 0.277 ab | 0.292 ab | 0.82 | 2.04×10^{-15} *** |
| Root:shoot ratio (R:S) | 0.87 a | 0.63 bc | 0.62 bc | 0.41 c | 0.53 bc | 0.40 c | 0.80 ab | 0.69 ab | 0.68 ab | 0.25 | 8.00×10^{-06} *** |
| Initial seed dry matter (isdm) (g) | 0.002 | 0.012 | 0.024 | 0.051 | 0.177 | 0.273 | 0.364 | 0.407 | 0.539 | | |

Table 4.3. Root exploration and nodules establishment for nine legume species grown without N supply. Values are means (n=4, except for fenugreek, where n=3). CV: coefficient of variation. *P* values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$). ***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively. Means on the same line with different letters are significantly different ($P<0.05$)

| | Alfalfa | Fenugreek | Lentil | Common vetch | Soybean | Pea | Chickpea | Peanut | Faba bean | CV | <i>P</i> value |
|---|---------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|------|----------------------------|
| Root lateral expansion rate (Rlr) (mm day ⁻¹) | 1.8 d | 1.3 d | 3.5 cd | 5.2 bcd | 8.5 ab | 8.2 abc | 10.4 a | 10.1 a | 6.9 abc | 0.55 | 6.59×10^{-07} *** |
| Root depth penetration rate (Rdr) (mm day ⁻¹) | 7.1 c | 12.8 bc | 8.2 c | 21.0 a | 22.6 a | 18.6 ab | 20.4 ab | 16.5 ab | 18.5 ab | 0.35 | 1.56×10^{-06} *** |
| Nodule number (Nnb) (per plant) | 1.5 c | 4.3 bc | 21.5 ab | 60.5 a | 8.3 abc | 17.3 ab | 4.3 bc | 43.5 a | 48.8 a | 0.95 | 2.07×10^{-05} *** |
| Nodules dry matter (Ndm) (mg pl ⁻¹) | 0.03 b | 0.62 b | 0.60 b | 4.42 b | 1.84 b | 0.76 b | 0.28 b | 10.13 ab | 17.71 a | 1.50 | 7.98×10^{-05} *** |

N accumulation in the plant and N sources - Without a mineral N supply, the N accumulation differed largely among legume species ($p < 0.0001$, Fig. 4.1a). Faba bean accumulated much more N in the shoots and roots than any other legume species (approximately 36 mg pl^{-1}), whereas the least N accumulated were observed in fenugreek, alfalfa and lentil with less than 3 mg pl^{-1} .

For most species, more than 50% of the plant N was derived from the seed (peanut, faba bean, lentil, chickpea, pea and soybean), whereas in alfalfa, only 6% of the plant N was derived from the seeds (Fig. 4.1b). Faba bean, alfalfa, fenugreek, soybean and common vetch began to fix atmospheric N_2 ; in these species, the percentage of N derived from the atmosphere in plant N ranged from approximately 6% (common vetch) to 20% (faba bean). The other species, peanut, chickpea, lentil and pea still relied on only the seed N and mineral soil N. Although the nodules had been initiated, biological N_2 fixation was not effective yet.

Mineral soil N depletion by all legumes was lower than that of the non-fixing control plants, wheat and oilseed rape. However, the amount of nitrate remaining in the soil after 35 DAG varied between legume species from a high of 5.07 and 7.94 mg kg^{-1} of dry soil for soybean and fenugreek, respectively (Fig. 4.1c) compared to a low of 3.46 and 1.10 mg kg^{-1} for wheat and oilseed rape, respectively (data not shown).

Relationships between seed traits, early growth and N acquisition – The seed, shoot and root traits, N accumulation, soil N uptake and remaining soil N of species grown without N supply were included in a principal component analysis (Fig. 4.2a). The first two PCs comprised 72% of the total variability. The first component (PC1, 55% of the variability) consisted mostly of the level of soil N uptake in relation to the different shoot and root traits, while seventeen percent of the variation, accounted for by PC2, was mainly due to differences in N_2 fixation. The biomass of the plant, shoot, root, and initial seed were also correlated, and the remaining mineral soil N was negatively correlated with the plant biomass. The soil N uptake was also correlated to the lateral and vertical expansion, but not with the ability of a species to initiate biological N_2 fixation. The nodule growth and activity were higher for species having a low seed reserve depletion.

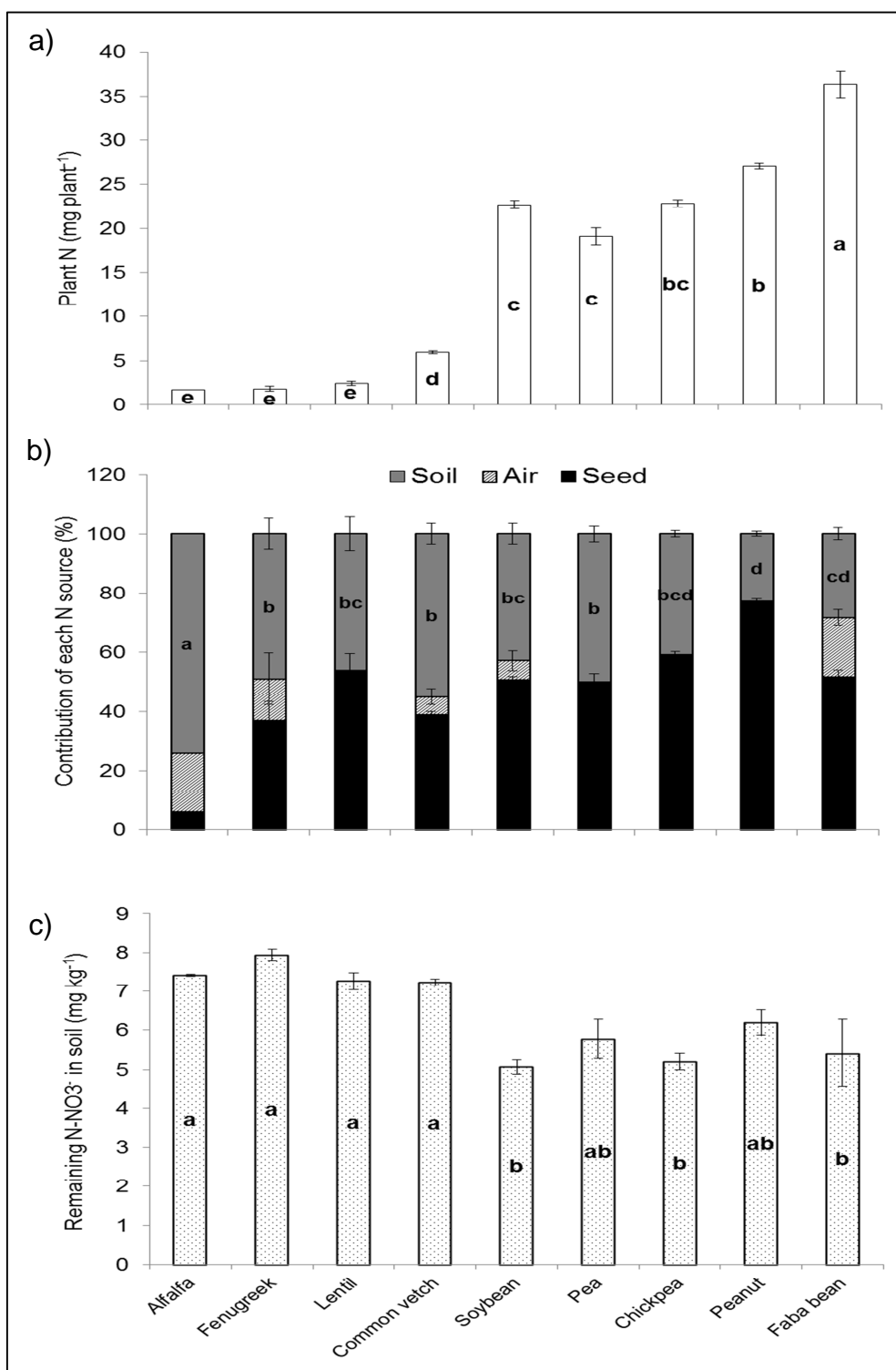


Figure 4.1. Plant N (a), the percentage of N derived from seed, air and soil (b) in the total plant N and remaining N-NO₃⁻ in soil (c) for nine legume species grown without N supply. Values are means ($n=4$, except for fenugreek, where $n=3$) \pm SE (standard error). Means with different letters are significantly different ($P<0.05$).

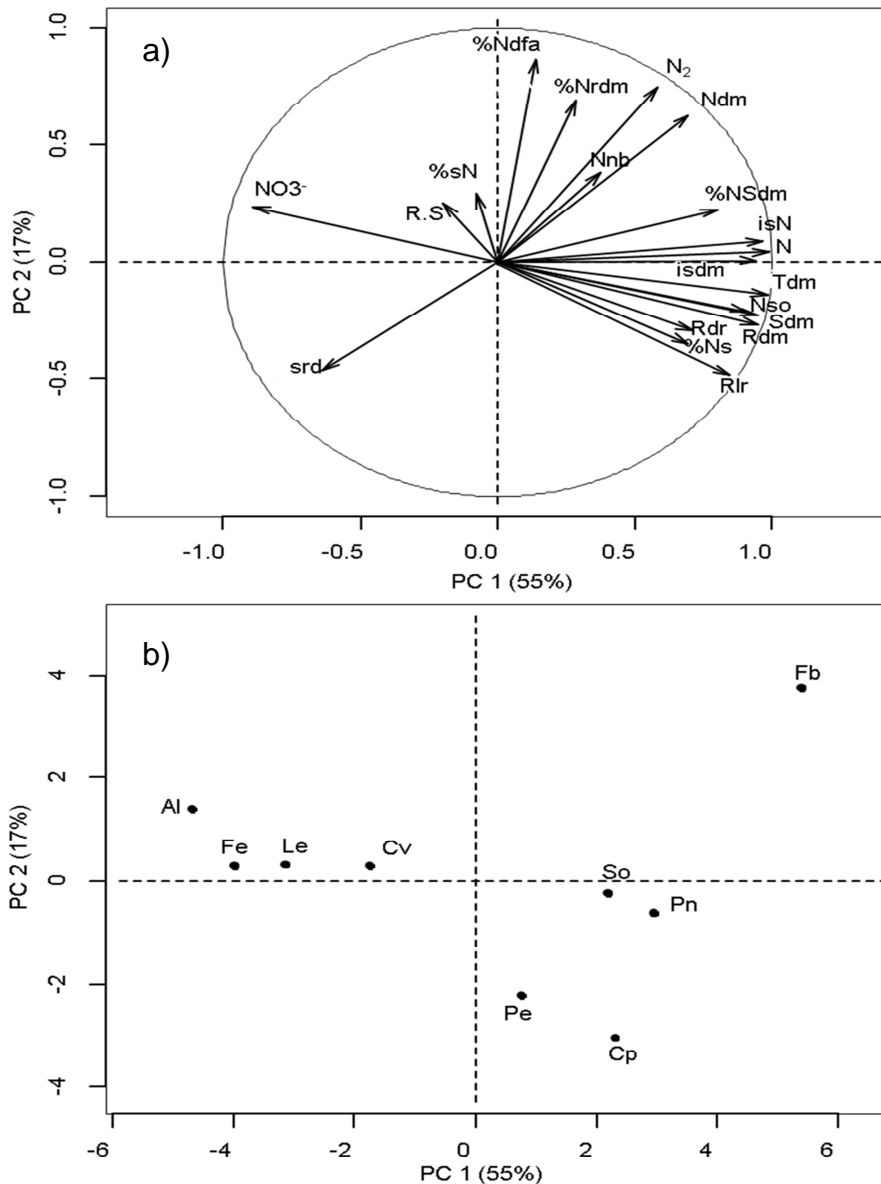


Figure 4.2. a Factor loadings for the variables measured on nine legume species grown under low soil N availability, for the first two axes of the principal component (PC) analysis. The percentage of the total variance explained by the first two principal components is shown in parentheses. **b** Projections of the different species on the first two axes of the principal component analysis. **Variables:** Shoot dry matter (Sdm), root dry matter (Rdm), total dry matter (Tdm), root:shoot ratio (R.S), initial seed dry matter (isdm), initial seed N (isN), N content in seeds (%sN), seed reserve depletion (srd), root lateral expansion rate (Rlr), root depth penetration rate (Rdr), nodule number (Nnb), nodule dry matter (Ndm), plant N (N), percentage of plant N derived from seeds (%Ns), N₂ fixed (N₂), percentage of exogenous N derived from air (%Ndfa), soil N uptake (Nso), N content in shoot dry matter (%NSdm), N content in root dry matter (%Nrdm), remaining N-NO₃⁻ in soil (NO₃⁻). Species: Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb).

Identification of groups of species - The PCA analysis separated plant species with a low or high soil N uptake ability (Fig. 4.2b). With the highest ability to deplete soil N combined with the highest biological N₂ fixation, faba bean shows a particular profile. Three relatively homogeneous groups of species were determined by hierarchical clustering analysis (Fig. 4.3a). The first cluster includes fenugreek, lentil, alfalfa and vetch, the four species characterized by the lowest soil N uptake, seed mass and shoot and root biomass and lateral root expansion. The second cluster included peanut, pea, chickpea, and soybean, four species characterized by a higher soil N uptake, shoot biomass and lateral root expansion. Faba bean was again separated from the others (Cluster 3), having a higher nodule biomass, a higher N₂ fixation and a lower seed reserve depletion than the other species.

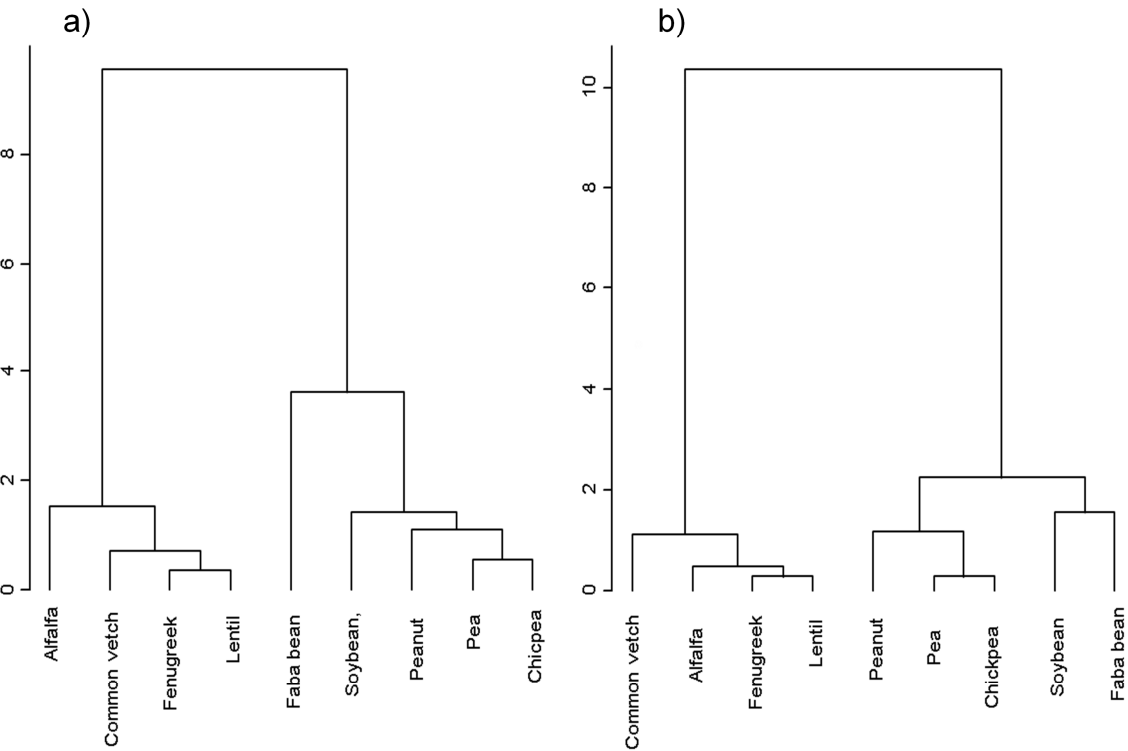


Figure 4.3. Dendrograms of relationships among species, calculated using Ward’s hierarchical clustering of species, which accounts for the distance matrix comparing all variables (used in PCA analysis) of each species grown under low N availability (a) or high soil N availability (b).

4.3.2. Growth and N acquisition at a high mineral N level

In the rhizotrons supplied with mineral N (N treatment), a large variation among species was also observed in the growth of shoots and roots (Fig. 4.4). The total biomass did not differ between the two levels of mineral N ($p > 0.05$), except in lentil. However, the root:shoot ratio was significantly lower in the N treatment than in the 0N for faba bean (-32%), chickpea (-27%) and soybean (-23%). The N supply did not significantly change the vertical root penetration rate ($p > 0.05$). A higher N availability modified the lateral root expansion only in soybean by reducing it by 34%. With a mineral N supply, the N accumulation in plants increased on average by 37% compared to the 0N treatment, with a large contribution of soil N to total plant N. Nodule number and biomass were largely reduced by the N supply for all species. Nodules were less affected by the mineral N supply in soybean and faba bean than in alfalfa, chickpea and pea, which did not initiate any nodules. The remaining soil N at 35 DAG, in the N treatment, varied across legume species from approximately 25 to 31 mg kg⁻¹ of dry soil, in the soybean and vetch treatments, respectively. The remaining soil N for soybean, peanut, chickpea, pea and faba bean were similar to those measured for wheat (24.4), and all legumes had a lower soil N depletion than that of oilseed rape (18.5).

A second principal component analysis (Fig. 4.5a) was performed to investigate whether the correlations between variables are modified with a higher soil N availability. The first two PCs comprised 79% of the total variability. The first component (PC1, 65% variation) consisted mostly of the level of soil N uptake in relation to the shoot and root biomass, initial seed mass and lateral root expansion, and PC2 (14% variation) accounted primarily for vertical root penetration, root:shoot ratio and number of nodules. Similar to the 0N treatment, in the N treatment, soil N uptake was mainly correlated with the total DM, shoot DM, root DM, initial seed mass and the lateral root expansion. The soil N uptake was less correlated to the lateral root expansion than to the depth root penetration. The lateral root expansion and vertical root penetration were less correlated in the N treatment than in the 0N (Fig. 4.2a, Fig. 4.5a).

Under high soil N availability, the dispersion (Fig. 4.5b) and the classification of the species within the groups identified in 0N were slightly modified (Fig. 4.3a, b). The first cluster included the same species as in 0N (fenugreek, lentil, alfalfa, vetch) and corresponded to the legume species having low soil N uptake ability. The second cluster included three species, peanut, pea, and chickpea, was mainly characterized by a high lateral root expansion. In the N treatments, faba bean and soybean are grouped into a cluster, with nodule number and biomass higher than those in the other species combined with a small seed reserve depletion. Soybean was more similar to faba bean than to peanut, pea and chickpea in the N treatment, because of reduced lateral root expansion and a lower effect of N on nodule establishment than in other species.

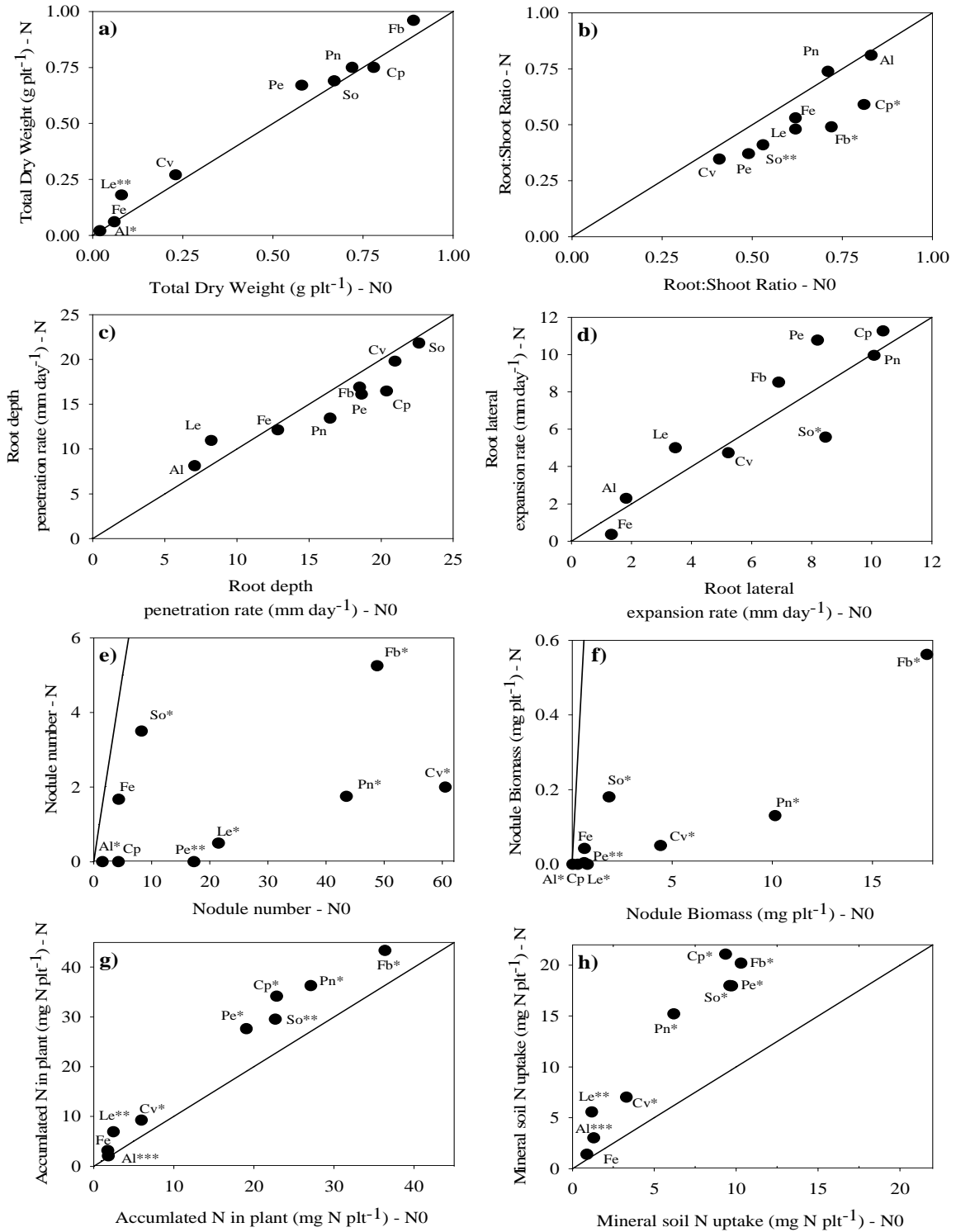


Figure 4.4. Dry matter (a) and root:shoot ratio (b), root depth penetration (c) and lateral root expansion (d), nodule number (e) nodule dry matter (f), plant N (g), soil N uptake (h) with (N) and without N supply (0N) for nine legume species: Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb). ***, **, * indicate significant differences between the two levels of soil N availability at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively.

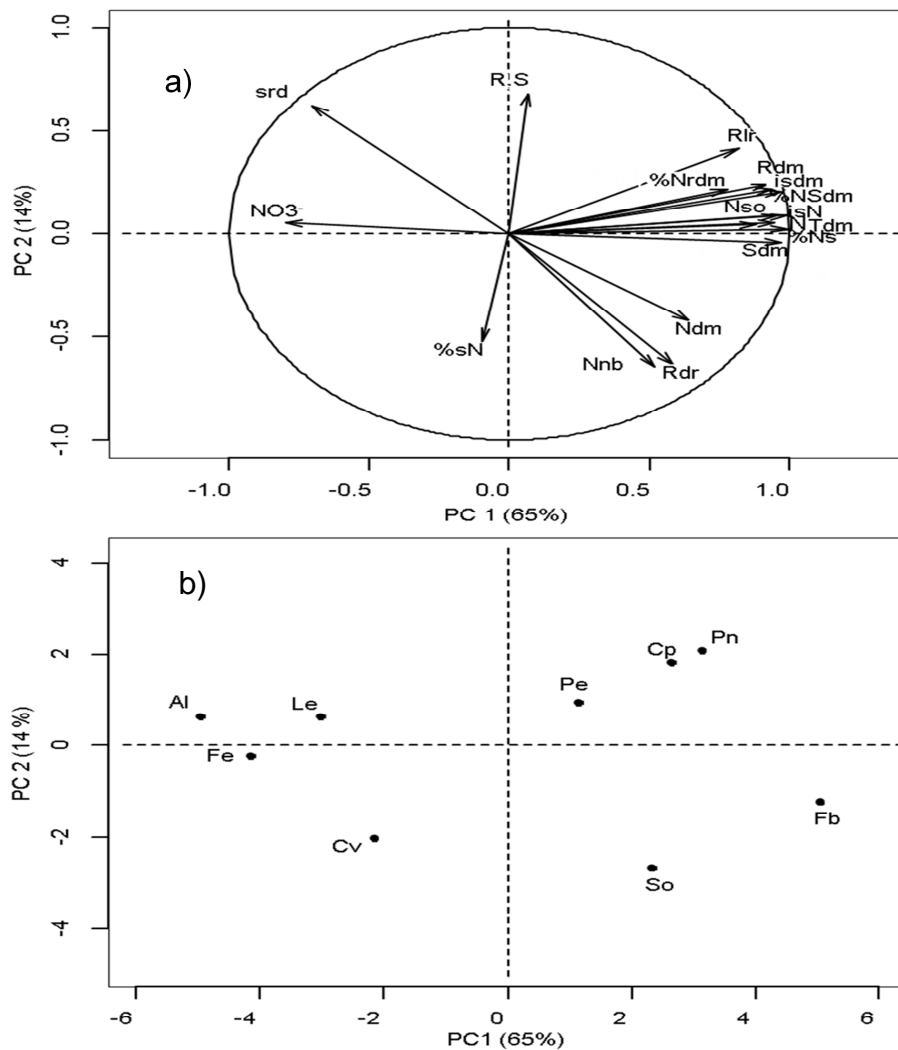


Figure 4.5. a Factor loadings for the variables measured on nine legume species grown under high soil N availability for the first two axes of the principal component (PC) analysis. The percentage of the total variance explained by the first two principal components is shown in parentheses. **b** Projection of the different species on the first two axes of the principal component analysis. **Variables:** Shoot dry matter (Sdm), root dry matter (Rdm), total dry matter (Tdm), root:shoot ratio (R.S), initial seed dry matter (isdm), initial seed N (isN), N content in seeds (%sN), seed reserve depletion (srd), root lateral expansion rate (Rlr), root depth penetration rate (Rdr), nodule number (Nnb), nodule dry matter (Ndm), plant N (N), percentage of plant N derived from seeds (%Ns), Soil N uptake (Nso), N content in shoot dry matter (%NSdm), N content in root dry matter (%Nrdm), remaining N-NO₃⁻ in soil (NO₃⁻). **Species:** Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb)

4.4. Discussion

During the early growth observed in this experiment, the soil N uptake was low for all legume species compared to non-legumes due to the high dependency of the plant on the seed N and to low N demand at this stage. A large proportion of the nitrogen accumulated in the shoots and roots came from the seed (up to 77%). Large and N-rich legume seeds contain a substantial amount of N and are potentially able to sustain seedling growth for a significant part of the plant cycle (Herdina and Silsbury, 1990). This particular trait of legumes probably contributes to the late utilization of exogenous N (mineral soil N and N₂ from biological N₂ fixation) for growth compared to that of non-legume species. In our experiment, because of the high contribution of the seed N to the total plant N, the plants could satisfy their N demand during early growth even in the 0N treatment, where the mineral N availability was low. Consistently, we did not find any significant difference in the total plant N between the N and 0N treatments, except for lentil.

Although legume species showed a low soil N uptake ability, a large variability among them was observed for all investigated traits, with consequences on the remaining soil N, regardless of the level of soil N availability. In the early growth phase, consistent with Fayaud et al. (2014), large differences among species were correlated with the seed mass. The growth and N acquisition were better correlated with the initial seed mass than with the initial seed N. However, legumes with a seed mass greater than 0.17 g such as soybean, pea, chickpea, peanut and faba bean had similar shoot biomass but displayed contrasting values for the root traits. Legumes often have a slow root depth penetration compared to of non-legumes (Bellostas et al., 2003; Corre-Hellou et al., 2007; Thorup-Kristensen, 2001). We found large differences both in the depth and lateral root development among legume species. Furthermore soil N uptake was correlated with the root lateral expansion rate. In the early growth phase, the root lateral expansion appeared to be a more important trait than the root depth penetration for the soil N uptake. Branch roots may allow exploring the soil better than taproot during early stages. Moreover, in our experiment, the soil N availability did not modify the root depth penetration as observed in previous studies (Corre-Hellou and Crozat, 2005) but could modify the root lateral expansion. The root:shoot ratio tended to be reduced with a high soil N availability, as observed in other studies (Corre-Hellou and Crozat, 2005; Hamblin and Hamblin, 1985). This ratio was high and differed among species; however, in our study, it was not a key trait involved in the soil N uptake. The nodule biomass per plant differed across species and was not correlated with the seed mass. Within each species, the relationships between the seed size and nodule biomass per plant have been observed in different lima bean (*Phaseolus lunatus* L.) cultivars (Dobert and Blevins, 1993), in some clover species (Evers, 1982), and in three varieties of soybean (*Glycine max* L.) (Smith and Ellis, 1980). Other authors have also demonstrated differences in the onset of nodule initiation among species due to the different distributions between above- and below-ground organs with hypogeal or

epigeal emergence (Sprent and Thomas, 1984). The type of emergence did not explain the differences in nodulation and N₂ fixation during the early growth stage in our study. All species had reduced nodule establishment and N₂ fixation at high levels of soil N. However, faba bean and soybean were able to establish nodules even under high soil N availability. Other studies have demonstrated that faba bean maintains high levels of nitrogen fixation at high levels of soil N availability compared to other legumes which are more sensitive to available soil N (Harper and Gibson, 1984; Turpin et al., 2002). We demonstrated that the ability to initiate a larger number of nodules early and to start N₂ fixation was not correlated with soil N uptake under the early growth stages. The root exploration (mainly lateral expansion) and early biomass in relation to the seed mass were the major traits relative to N uptake, whatever the level of soil N availability.

The cluster analysis classified the species into three groups. Peanut, pea, chickpea and soybean, with a rapid early shoot growth and a high lateral root expansion, had a better soil N uptake. These characteristics may result in a better competitive ability with neighbouring plants (crops and weeds) for soil N at the beginning of the crop's life cycle. Conversely, fenugreek, lentil, alfalfa, and vetch could be considered weakly competitive legumes for soil N due to their small seed mass, small plant biomass and low lateral root expansion. At the beginning of the crop cycle, they may leave a larger part of the available N for weeds or another associated crop. Among the species studied, faba bean had several unique traits. Faba bean used only 66% of the seed reserves in this early phase. Furthermore, it exhibited an ability to fix atmospheric nitrogen and simultaneously to take up soil nitrate at a much greater rate than the other species studied. Indeed, faba bean tended to benefit from different sources of nitrogen for its root and shoot growth. Hence, a strong competition against other associated species for both light and N may be predicted in faba bean in this early growth phase. The classification of species may differ according to the soil N availability depending on the specific response of each trait, but we observed only slight differences. Larger differences between species in response to the N availability might occur during the later stages.

Fenugreek and vetch are mainly used for forage or cover crops in the time period between two main crops or as living mulch within a main crop. Their slow establishment and low ability for soil N uptake at the beginning of the crop cycle may affect their yield when harvested and their ability to play expected roles such as the reduction of N leaching and increased cover allowing weed control. Whatever the type of use (forage or grain), legumes species can be intercropped with non-legume species to increase soil N uptake, the competitive ability of the cover against weeds and to reduce N losses during the beginning of the crop cycle and after (Corre-Hellou et al., 2011; Hauggaard-Nielsen et al., 2009; Hiltbrunner et al., 2007b). Mixing legumes with different levels of competitive abilities can also be a relevant strategy (Mikić et al., 2015).

Cool-season legumes and warm-season legumes have rarely been compared under same growth conditions. Nevertheless, warm-season legumes are also relevant species for temperate cropping

systems used as frost-sensitive cover crops in a mixture with other main crops such as wheat or oilseed rape.

Further experiments are required in field conditions to assess whether differences in the soil N uptake ability are also observed and maintained at later stages and to see how the shoot and root traits are influenced by various soil and climate conditions and interspecific competition. Such an analysis can be extended to identify differences across genotypes within a species. Some root traits may differ significantly across cultivars as shown, for example, for chickpea (Kashiwagi et al., 2006), lentil (Sarker et al., 2005) and lupin (Mohler et al., 2012). The interspecific variability during the early stages documented here has identified traits involved in early N acquisition allowing for the classification of species among legumes with different strategies and competitiveness for N. These findings will help us to better understand and manage interspecific competition for soil N between legumes and other species (crops and weeds) at the beginning of the crop's life cycle.

Funding statement

This work was funded by CASDAR n°5376 (Compte d'Affectation Spéciale pour le Développement Agricole et Rural, French Ministry of Agriculture) and the Syrian Ministry of Foreign Affairs.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We gratefully acknowledge the technical staff of LEVA (Légumineuses, Ecophysiologie Végétale, Agroécologie) for their excellent technical assistance. We are most grateful to the PLATIN' (Plateau d'Isotopie de Normandie) core facility for all element and isotope analysis used in this study.

Dans le chapitre 2, à partir des résultats obtenus dans le chapitre 1 concernant la variabilité inter spécifique d'une gamme d'espèces de légumineuse, nous avons choisis trois espèces de légumineuse ayant des traits contrastés pour leur capacité de capture de l'azote du sol. Ce chapitre repose sur une expérimentation conduite en conditions contrôlées en rhizotrons. Chaque espèce de légumineuse a été associée à une adventice selon un dispositif additif afin d'évaluer la réponse d'une adventice nitrophile aux différentes espèces de légumineuse pendant la phase de croissance précoce.

5. Chapitre 2. Response of black nightshade to chickpea, faba bean and common vetch during early growth

Ce chapitre correspond à un article en préparation pour la soumission dans le journal Weed science

Response of black nightshade to chickpea, faba bean and common vetch during early growth

Elana Dayoub¹, Christophe Naudin¹, Guillaume Piva¹, Steven J. Shirtliffe², Joëlle Fustec¹ and Guénaëlle Corre-Hellou^{1*}

¹ USC LEVA, INRA, Ecole Supérieure d'Agricultures, Univ. Bretagne Loire, SFR 4207 QUASAV, 55 rue Rabelais, 49100 Angers, France

² Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada

Abstract

A better knowledge on the response of weeds to legume species during early growth is required to better understand weed-crop competition establishment related to species functional traits and to therefore enhance crop competitiveness against weeds. An experiment in controlled conditions using rhizotrons was carried out to determine the competitive ability of three legume species; chickpea, faba bean and common vetch when grown in combination with black nightshade (1:1) with or without N mineral supply. Studied species were also grown in pure stand, one plant per rhizotron. Aboveground and belowground competition between weed and legume was investigated during early growth. Interspecific variability was observed between the three legume species concerning early growth and competitive ability for mineral soil N. Black nightshade responded early and differently as a function of legume species. Chickpea entailed the greatest reduction in black nightshade total biomass (-77%) and N accumulation (-85%) compared to pure stand. Conversely, faba bean and common vetch facilitated weed growth and increased its root depth penetration at approximately 73% compared to pure stand. Regardless N supply, chickpea was a good competitor while faba bean and common vetch provided an environment favourable for the black nightshade growth.

Nomenclature: Black nightshade, *Solanum nigrum* L. SOLNI; chickpea, *Cicer arietinum* L.; faba bean, *Vicia faba* L.; common vetch, *Vicia sativa* L.

Key words: *Solanum nigrum* L., legumes species, early growth, soil nitrogen uptake, root growth.

5.1. Introduction

Grain legumes are seen as key rotation crops in many parts of the world with rotational and nitrogen benefits for the following crop. However, grain legumes are often considered poor competitors against weeds compared to other species such as cereals (Evers, 1982; Lemerle et al., 1995; McDonald, 2003; Wall et al., 1991; Whish et al., 2002). Nevertheless, the inter-specific variability concerning the competitiveness against weeds has rarely been studied.

Excellent weed control is critical to avoid high yield losses, but is difficult to achieve (Harker et al., 2001; Townley-Smith and Wright, 1994). Early herbicide application is often recommended (Harker et al., 2001). Recently, attention has shifted to increasing the integration of non-chemical methods of weed control into the current farming systems due to an increase in herbicide resistant weed species (Heap, 2014). Thus, enhancing crop competitive ability with weeds is now a crucial component of weed management (Blackshaw et al., 2007; Lindquist and Mortensen, 1998; Mohler, 2001; Zerner et al., 2008). In cereal crops, significant inter-specific variation in competitiveness against weeds has been demonstrated (Lemerle et al., 2001; Lemerle et al., 1995; Lemerle et al., 1996; Reeves and Brooke, 1977) but less attention has been paid to grain legumes.

Several studies have worked on crop-weed competition to identify the main functional traits involved in the competitiveness against weeds. Crop traits relating to competition for light such as height, leaf area, early growth, canopy closure are often considered determinant in crop-weed competition (Beckie et al., 2008; Pakeman et al., 2015). Such aboveground traits explained partly differences in weed suppression ability among pea (McDonald, 2003), chickpea (Radicetti et al., 2012) or lentil cultivars (Tepe et al., 2005). In comparison with aboveground canopy measurements, belowground traits have received relatively little attention in crop-weed interactions. However, studies have indicated that root competition can be more important than competition for light, particularly for nitrogen (Casper and Jackson, 1997; Satorre and Snaydon, 1992). Traits such as root length density, root elongation rate, and total root length are determinants of competitive outcomes (Fargione and Tilman, 2006; Stevanato et al., 2011). The ability to take up N from the soil can play a certain role in determining the different competitive ability against weeds among species and genotypes.

Although fast early growth is considered crucial in crop-weed competition (Andersen et al., 2007; Bellostas et al., 2003; Fayaud et al., 2014), examining aboveground and belowground traits during the first stages has been rarely achieved. Small advantages in early season seedling growth can translate into large differences in size and resources capture ability later in the season (Andersen et al., 2007). Therefore early strategies of crop growth and N acquisition can be critical for determining competitive interactions between weeds and crops in later stages.

Grain legumes have a low ability to uptake soil N (Dayoub et al., 2017; Thorup-Kristensen, 1994). By leaving a large amount of soil N available for weeds, this low soil N uptake can contribute to their low

competitive ability against weeds. Many weeds consume large quantities of N (Hans and Johnson, 2002; Qasem, 1992). Additionally, growth of many weed species is enhanced by higher soil N availability (Blackshaw et al., 2003; Henson and Jordan, 1982; Supasilapa et al., 1992). During early growth, legumes may rely mainly on soil N uptake as other non-legume species because the low contribution of N₂ fixation to N demand due to the delayed nodule establishment. However, grain legumes can rely also on seed N reserve for a significant time due to the large seeds rich in N which can lead to a low use of exogenous N sources (Dayoub et al., 2017; Herdina and Silsbury, 1990). In a previous experiment we determined that differences between legume species concerning their competitive ability for soil N during early growth may exist in relation to seed mass, use of seed N, root exploration and start of N₂ fixation and thereby can affect weed growth during the first stages (Dayoub et al., 2017). Some species have lower N₂ fixation but higher biomass and higher soil N uptake during early growth such as chickpea, soybean, peanut and pea due to a fast lateral root expansion and a high seed mass unlike to another group of species having a low soil N uptake including alfalfa, common vetch, lentil and fenugreek. Faba bean was classed separately due to the particularity to simultaneously fix N₂ and take up soil N (Dayoub et al., 2017).

Crop-weed competition depends also on morphological features of weed species such as aboveground and belowground biomass, high density root system and response to mineral soil N availability (Blackshaw et al., 2003; Moreau et al., 2013; Swanton et al., 2015).

Black nightshade (*Solanum nigrum* L.) is a major weed problem in many crops such as potatoes, field pea, soybean, field bean (Defelice, 2003; Ogg Jr et al., 1981). In addition to its crop losses from competition, it is also a host for tomato viruses diseases contributing in the spread of this virus within Europe (Bedford et al., 1998). Black nightshade growth is favoured by high soil N availability (i.e. nitrophilic species) (Bvenura and Afolayan, 2014; Khan et al., 1995; Opiyo, 2004). The competitiveness of legume species against black nightshade has not been highlighted before.

The aim of our study is to evaluate the response of black nightshade to different legume species having contrasting traits in the early growth in relation to different strategies of nitrogen acquisition. An experiment under controlled conditions using rhizotrons was carried out testing the competitive ability of faba bean, chickpea and common vetch when grown in combination with black nightshade compared to legumes and weed grown in pure stands under two soil N availabilities during early growth.

5.2. Materials and Methods

5.2.1. Experiment Design

three legume species; faba bean, chickpea and common vetch were grown in an additive design (1:1), in mixed stand with black nightshade (Table 5.1). The experiment was conducted under natural light in a greenhouse from June 5th to July 7th, 2014 in western France (Angers; 47.5°N, 0.6°W). The mean temperature and humidity during the experiment were 23.3 °C ± 2.1 and 56.0 % ± 9.2, (mean ± standard deviation), respectively. This experiment was carried out using transparent rhizotrons (Jamont et al., 2013) made of methacrylate with inner dimensions of 50 × 20 × 3 cm, in a completely randomized design with five replications. The rhizotrons were inclined at 45° to allow the roots to grow towards the underside and covered with black polyethylene plastic sheeting to exclude light except when root measurements were being conducted. Each rhizotron was filled with 4.09 kg of a mixture of sandy fresh soil (25%) and dry sand (75%). The soil was sieved (7 mm) prior to being homogeneously mixed with dry sand. The sand was previously washed and dried at 105°C for 48 h. The final texture of the soil sand mixture was 2.9% clay, 6% loam, 90.6% sand and 0.4% organic matter, with 8.65 mg NO₃⁻ N kg⁻¹ of dry soil and the pH of the mixture was 6.9. The phosphorous (P Olsen) and potassium (K) contents were 30.31 (P₂O₅) and 78.73 (K₂O) mg kg⁻¹ of dry soil, respectively.

150 ml of N-free nutrient solution was added to each rhizotron at the following concentrations (mmol l⁻¹): 1.07 (KCl), 0.34 (KH₂PO₄), 0.24 (MgSO₄+7H₂O), 0.18 (CaCl₂) and (0.04) FeCl₃ with controlled pH of 6.8. In the half of the rhizotrons, the soil was supplemented with a solution KNO₃ to reach a concentration of 36.48 mg NO₃⁻ N kg⁻¹ dry soil. The nutrition solutions were mixed with the soil before filling the rhizotrons.

Legume and weed species in mixed and in pure stand were grown under two levels of soil nitrogen 0N (8.65 ± 0.16) and N (36.48 ± 0.70) N-NO₃ mg Kg⁻¹ of dry soil (mean ± standard error).

Table 5.1. Description of the studied legume and weeds species.

| Species (cultivar for legumes) | Common name | Code | | | Phenological | |
|---|------------------|-------|--------|------------|----------------------------------|---|
| | | Class | Family | | Stages at 33 DAG ³ | Rhizobium strain for legumes |
| <i>Vicia faba</i> L. (cv Divine) | Faba bean | Fb | Dicot | Fabaceae | 15 | <i>Rhizobium leguminosarum</i> bv <i>viciae</i> (FH34) ² |
| <i>Cicer arietinum</i> L. (cv Principé) | Chickpea | Cp | Dicot | Fabaceae | 22 | <i>Mesorhizobium ciceri</i> (UMPCa7) ¹ |
| <i>Vicia sativa</i> L. (cv Nacre) | Common vetch | Cv | Dicot | Fabaceae | 39 | <i>Rh.leguminosarum</i> bv <i>viciae</i> (P221) ² |
| <i>Solanum nigrum</i> L. ⁴ | Black nightshade | Bn | Dicot | Solanaceae | 26 | |

Source of Rhizobium strain:

¹INRA, UMR Eco&Sols (Montpellier SupAgro – CIRAD – INRA – IRD), France

²UMR 1347 - Agroecologie, Pôle MERS, Microbiologie Environnementale et Risque Sanitaire, Center INRA, Dijon, France

³According to BBCH scale of (JKI, 2010) at sampling date (35 days after seed germination)

⁴Sources of weed seeds: UMR 1347 Agroécologie (INRA Dijon), France

The legume seeds were weighed to reduce the variation in the individual seed mass to less than 3.5%. Black nightshade seeds were soaked in a solution of Gibberellic acid (GA3) (5×10^{-3} mol) in incubated temperature (28°C) under light conditions and germinated after 72 hours to break dormancy. To allow for simultaneous germination and uniform emergence of legumes and weeds species, seeds were firstly germinated on filter paper in different dates until the radicle emerged from the seeds (BBCH stage 05) (JKI, 2010). Following this, one legume seed with one weed seed were sown per rhizotron with inter plant distance of 5 cm (mixed stand). Moreover, legumes and weed species were sown also in pure stand: one seed per rhizotron. After that, each legume species was inoculated with its specific strain of *Rhizobia* (Table 5.1) at an estimated rate of 10^8 cells per plant to allow for N₂ fixation (Naudin et al., 2011). After seedling emergence, a 1-cm thick layer of sand (100 g per rhizotron) was added to the surface of the rhizotron to limit water evaporation from the soil. During the experiment, the soil moisture was kept at field capacity.

Plants of each legume species were also grown simultaneously in N-free sand to determine the ¹⁵N abundance of plants relying only on N₂ fixation (β -value) and thus estimate the N₂ fixation using the natural abundance method (Amarger et al., 1979). The pots were supplied once per week with 100 ml of N-free nutrient solution with a composition of (mmol l⁻¹) 0.54 KCl, 0.17 KH₂PO₄, 0.12 MgSO₄ 7H₂O, 0.09 CaCl₂, and 0.02 FeCl₃ with a controlled pH of 6.8 and maintained at field capacity.

5.2.2. Harvest and Measurements

The root depth and lateral root expansion of legumes and weeds were monitored using a grid of 5 mm × 5 mm squares printed on transparent plastic sheet fixed on the underside of the rhizotron. The root presence in the grid was marked to denote vertical and horizontal root distribution. Root measurements were performed twice weekly for 19 days after seed germination (DAG).

Legumes and weed species were harvested 33 days after germination (DAG) while still in early growth phase. Legumes and weeds root systems were well separated, removed from the rhizotron and washed with demineralized water. Legume seed residues were collected to quantify the remaining seed dry matter. Plants grown in N-free sand were harvested at the same time and sorted into shoot and root samples. After oven-drying (48 h at 70°C), the dry matter of the roots, shoots of legumes and weed and legume seed residues were determined. All of the samples were ground to fine powder for total N and ¹⁵N:¹⁴N ratio measurements in the original seeds, roots, shoots, and legumes seed residues using a CHN analyser (EA3000, Euro Vector, Milan, Italy) and a mass spectrometer (IsoPrime, Elementar, Hanau, Germany).

At each sowing and harvest date, soil samples were collected for all species (legumes and weed) and the soil inorganic N content was measured using a segmented flow analysis (SKALAR SA3000), which enables the determination of the nitrate content by KCl extraction according to the international standard ISO 14256-2.

5.2.3. Calculations and Statistics

The part of legume N derived from the seed was calculated using the amount of N from seeds (QN seed) consumed during the experiment by each legume. QN seed is the difference between the seed N content at sowing and that at harvest. Exogenous N is the difference between N accumulated in plant and QN seed. The relative contributions of exogenous sources of N (air and soil) were then determined.

The part of the plant exogenous N derived from the air (%Ndfa) was determined using the ^{15}N natural abundance method (Amarger et al., 1979). Black nightshade grown as sole weed was used as non-fixing reference for each N treatment (equation 1),

$$\% \text{Ndfa} = 100 \times [(\delta^{15}\text{N}_{\text{legume}} - \delta^{15}\text{N}_{\text{weed}}) / (\beta - \delta^{15}\text{N}_{\text{weed}})] \quad [1],$$

where $\delta^{15}\text{N}_{\text{legume}}$ and $\delta^{15}\text{N}_{\text{weed}}$ are the natural ^{15}N enrichment of legume and weed, respectively. The β value is the isotopic fractionation factor measured from each species of legumes grown in N-free sand. The $\delta^{15}\text{N}$ in the whole plant (for legumes and weed) was calculated using equation 2:

$$\delta^{15}\text{N whole plant} = [(\delta^{15}\text{N shoot} \times \text{shoot DW}) + (\delta^{15}\text{N root} \times \text{root DW})] / (\text{shoot DW} + \text{root DW}) \quad [2]$$

The values of $\delta^{15}\text{N}$ for legumes were corrected considering N derived from the seed as another source of N for the plant (Jensen et al., 1985), (equation 3):

$$\delta^{15}\text{N corrected} = [(\delta^{15}\text{N legume} - ((\text{QN seed} / \text{QN legume}) \times \delta^{15}\text{N seed}))] / [(1 - (\text{QN seed borne} / \text{QN legume}))] \quad [3],$$

where $\delta^{15}\text{N seed}$ expresses the natural ^{15}N enrichment of the original seeds.

The contribution of soil N was calculated as the difference between the exogenous N and the N derived from air.

The amount of N accumulated in weeds represented only the QN derived from soil while QN seed was considered negligible.

The effect of legume species on weeds growth species (root depth and lateral root expansion, total biomass and weed N) was tested by a one-way analysis of variance (type III sum of squares; $\alpha=0.05$). Normality of residues and homoscedasticity were tested using Shapiro-Wilk and Bartlett tests, respectively ($\alpha =0.05$). Means were compared using Tukey's HSD test (Honest Significant Differences; $\alpha =0.05$) to determine whether a main effect was significant. All statistical analyses were performed using R Commander package in R software, version 3.1.2 (RCore, 2012).

5.3. Results and Discussion

5.3.1. Functional traits of legume species and black nightshade grown as sole crops without N supply in pure stands

Differences were observed between chickpea, faba bean and common vetch for most investigated functional traits during early growth, including shoot and root biomass, root: shoot ratio, root depth, root lateral exploration and N acquisition (Fig. 5.1). Chickpea was able to accumulate the greatest total biomass ($1.40 \text{ g plant}^{-1}$) and had a fast lateral root expansion compared to faba bean and common vetch. However, the three legume species did not differ concerning root: shoot biomass ratio (on average 0.74) and root depth exploration (on average 363 mm). Faba bean accumulated three times more total biomass ($0.62 \text{ g plant}^{-1}$) than common vetch ($0.26 \text{ g plant}^{-1}$) but the two species showed no significant differences concerning root: shoot biomass ratio and root exploration.

The three legume species differed also for N acquisition (Fig. 5.1). Chickpea had the greatest mineral soil N uptake (20 mg plant^{-1}) and total N ($32.75 \text{ mg plant}^{-1}$). Although faba bean accumulated four times more total N than common vetch, these two species had similar and low ability for mineral soil N uptake (on average $2.93 \text{ mg plant}^{-1}$).

The contribution of the three N sources (seed, soil, atmospheric N_2) to total plant N differed between species (Fig. 5.2, a). Chickpea relied only on two N sources: seed N (40.72%) and soil N (59.28%). Chickpea did not start N_2 fixation during the studied early growth period. Faba bean relied mainly on seed N reserve (86.43%), 12.04% of N came from soil N and 1.53% from atmospheric N_2 . For common vetch, unlike chickpea and faba bean, the three N sources contributed significantly to plant N. Common vetch had the highest contribution of N derived from atmosphere (22.84%) and 36% of plant N was derived from seed and 40.40% from soil N.

Legumes displayed a low soil N uptake ability in this study as previously observed in other studies (Dayoub et al., 2017; Kurdali et al., 1996; Thorup-Kristensen, 1994) but we demonstrated that differences among species can occur during the first month. Large and N-rich legume seeds contain a substantial amount of N and are potentially able to sustain seedling growth for a significant part of the plant cycle (Herdina and Silsbury, 1990) leading to a low use of exogenous sources but differences among legume species can occur during the first month. Chickpea had a higher soil N uptake due to a lower dependency on seed N without any contribution of N_2 fixation and due to a high root lateral expansion. In contrast to the other species, common vetch relied on the three sources of N similarly and had a moderate uptake for soil N.

The traits of black nightshade were then compared to those of legume species in pure stands and the level of contrast between the weed and each legume was evaluated. The non- N fixing weed, black nightshade, in pure stand differed significantly from chickpea for most studied traits, a lower biomass

(-69%) and a reduced total plant N (-43%) (Fig. 5.1). Black nightshade allocated a reduced dry matter to roots than that chickpea. Its root: shoot ratio was lower (-36%) than that of chickpea. Black nightshade had reduced both root depth and lateral compared to chickpea (Fig. 5.1). Although black nightshade accumulated a reduced amount of total N than chickpea, the two species had a similar competitive ability of soil N uptake (19 mg plant^{-1}) (Fig. 5.1).

Black nightshade in pure stand had close traits to faba bean and common vetch concerning total biomass and root exploration. Nevertheless, nightshade was more able to take up soil N than faba bean and common vetch.

Chickpea was able to significantly reduce the initial mineral soil N level (-57%) and in the same way as black nightshade (-43%), whereas faba bean and common vetch did not reduce this level during the early growth period in the 0N treatment (Table 5.2).

Table 5.2. Quantity of Soil N-NO₃⁻ remained in the soil (mg kg^{-1}) for mixed stands of black nightshade with chickpea (Cp Bn) or faba bean (Fb Bn) or common vetch (Cv Bn) and for pure stands of black nightshade (Bn), chickpea (Cp), faba bean (Fb) and common vetch (Cv) without N supply (8.65: 0N) and with N supply (36.48: N) N-NO₃ mg Kg^{-1} of dry soil at 33 days after seed germination

| | | 0N | N |
|-----------------------------|-------|------------------------------------|---------------------------------|
| Mixed stand | Cp Bn | $3.65 \pm 0.47 \text{ b}$ | $23.93 \pm 1.69 \text{ ab}$ |
| | Fb Bn | $2.74 \pm 0.30 \text{ b}$ | $21.58 \pm 2.15 \text{ b}$ |
| | Cv Bn | $2.61 \pm 0.19 \text{ b}$ | $25.81 \pm 1.94 \text{ ab}$ |
| Pure stand | Bn | $4.90 \pm 0.99 \text{ b}$ | $24.40 \pm 2.15 \text{ ab}$ |
| | Cp | $3.69 \pm 0.33 \text{ b}$ | $23.87 \pm 1.10 \text{ ab}$ |
| | Fb | $8.34 \pm 0.65 \text{ a}$ | $26.75 \pm 2.05 \text{ ab}$ |
| | Cv | $8.07 \pm 0.33 \text{ a}$ | $31.52 \pm 1.54 \text{ a}$ |
| <i>P-value</i> ¹ | | $4.52 \times 10^{-07} \text{ ***}$ | $4.85 \times 10^{-2} \text{ *}$ |

Means within each column with different letters are significantly different (Tukey's HSD; $\alpha=0.05$)

***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$)

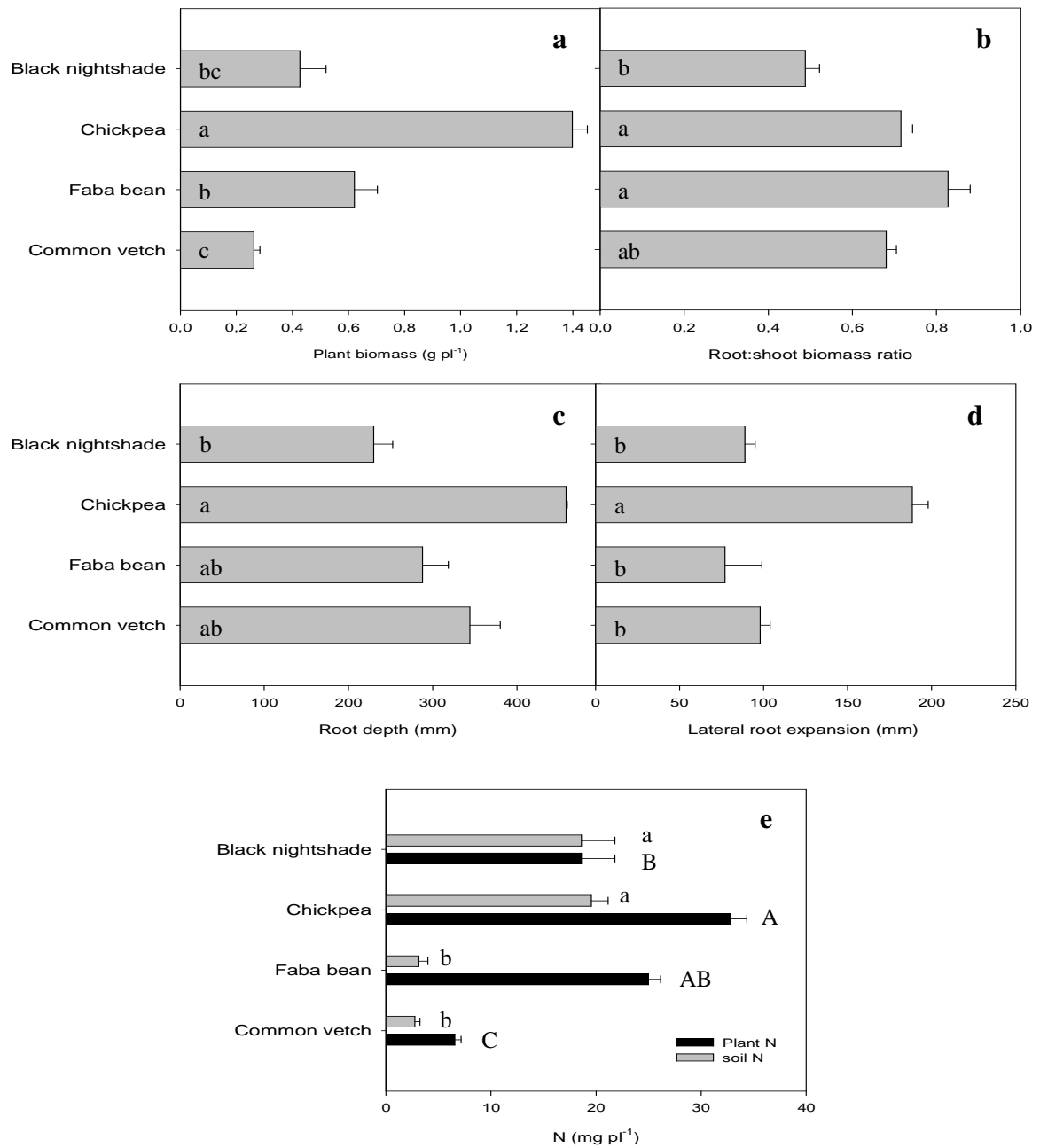


Figure 5.1. Pure stands: Plant biomass (a), root: shoot biomass ratio (b), root depth (c), lateral root expansion (d), plant N and soil N uptake (e) for black nightshade and legume species chickpea, faba bean and common vetch without N supply (0N). Values represent mean ($n=5$, except for chickpea and weed where $n=4$) \pm standard error. Values followed by the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$): capital letters and small letters for total plant N and N derived from soil, respectively.

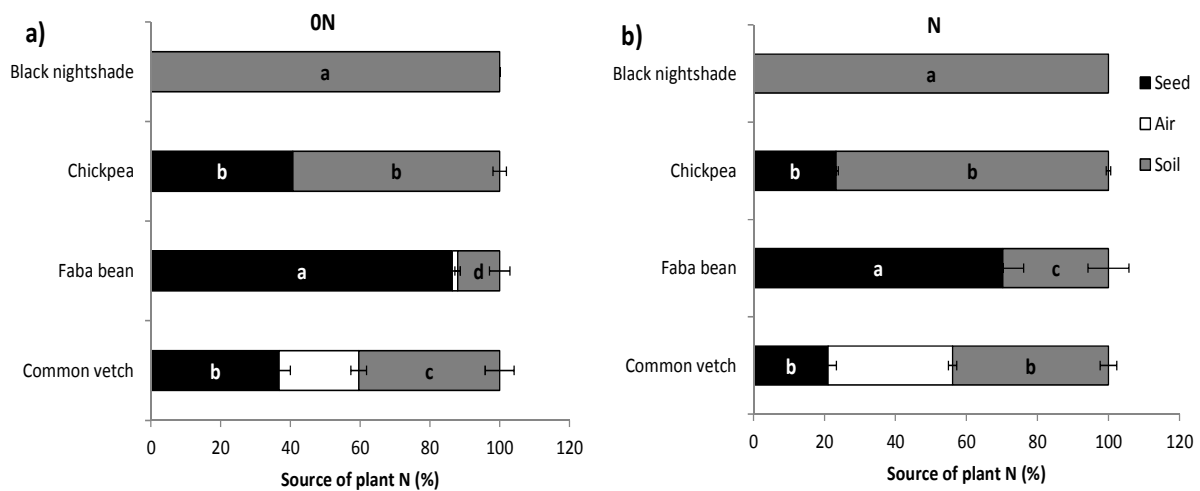


Figure 5.2. Pure stands: Contribution of the three N sources; seed N, N₂ atmospheric and soil N uptake to the total plant N for chickpea, faba bean and common vetch: a) without (0N) and b) under (N) N supply. Values represent mean ($n=5$, except for chickpea and weed where $n=4$) \pm standard error. Values followed by the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$).

5.3.2. Effects of legume species on black nightshade growth and N accumulation without N supply

The response of black nightshade (in terms of aboveground and belowground biomass, root depth, lateral root exploration and N accumulation) differed as a function of legume species during early growth (Fig. 5.3). Chickpea entailed the greatest reduction in black nightshade total biomass (-77%) and N accumulation (-85%) compared to pure stand. That may be due to the greatest total biomass of chickpea, its rapid root depth and lateral root expansion and thus a fast access to soil N early without any trend to fix N₂ from atmosphere. The high competitive ability against weeds of chickpea has been previously observed in early stages in relation to its aboveground biomass (Radicetti et al., 2012).

The root: shoot ratio of black nightshade increased in mixture with only chickpea from 0.49 (sole weed) to 0.71 (with chickpea). In general, a higher root: shoot ratio is a good indicator of plant compensation in case of a reduced resource availability (Dixon, 2007). This result was consistent with a previous study showing that the root: shoot ratio of black nightshade was reduced under high nutrition supply (Bvenura and Afolayan, 2014).

Chickpea was more competitive against black nightshade than faba bean and common vetch. Indeed, faba bean and common vetch did not bring about a decrease in total biomass and N accumulation of

black nightshade compared to sole weed. Root: shoot ratio of weed was also not modified. That may be due to the slow early growth of faba bean and common vetch, their low N demand and their high dependency on other resources of nitrogen as symbiotic fixation (common vetch) and seed N (faba bean) (Fig. 5.3).

Dynamics of root depth penetration and lateral root expansion of black nightshade were slightly modified by the addition of a legume species compared to sole black nightshade. Differences in black nightshade root depth began to appear early two weeks after germination (Fig. 5.4). The lateral root expansion of black nightshade had a similar pattern either grown in pure or in mixture with legumes. Lateral root expansion started at 5 DAG in all treatments except with chickpea where lateral root expansion started earlier at 2 DAG (Fig. 5.4). Furthermore, positive effects on black nightshade root depth were observed when mixed with both faba bean and common vetch. The two legume species increased root depth of black nightshade (on average +73%) compared to sole weed. In weed-chickpea mixture; legume roots grew fast and early occupying the soil and with a high competitive ability for mineral soil N, resulting in a reduced weed root depth. Since faba bean and common vetch roots had a lower ability to explore the soil as well as to take up mineral soil N compared to chickpea, black nightshade grew with small interferences from legumes which may improve its root depth. A previous field experiment showed that barley had exhibited a faster and deeper root depth growth when intercropped with pea in comparison with sole cropped barley, leading to a potential improvement of nutrient uptake (Hauggaard-Nielsen et al., 2001a).

This work reveals different competitive effects of three legumes species against black nightshade. Chickpea was a good competitor against weeds while faba bean and common vetch provided an environment favourable for the weed and facilitated its growth during the first growth stages.

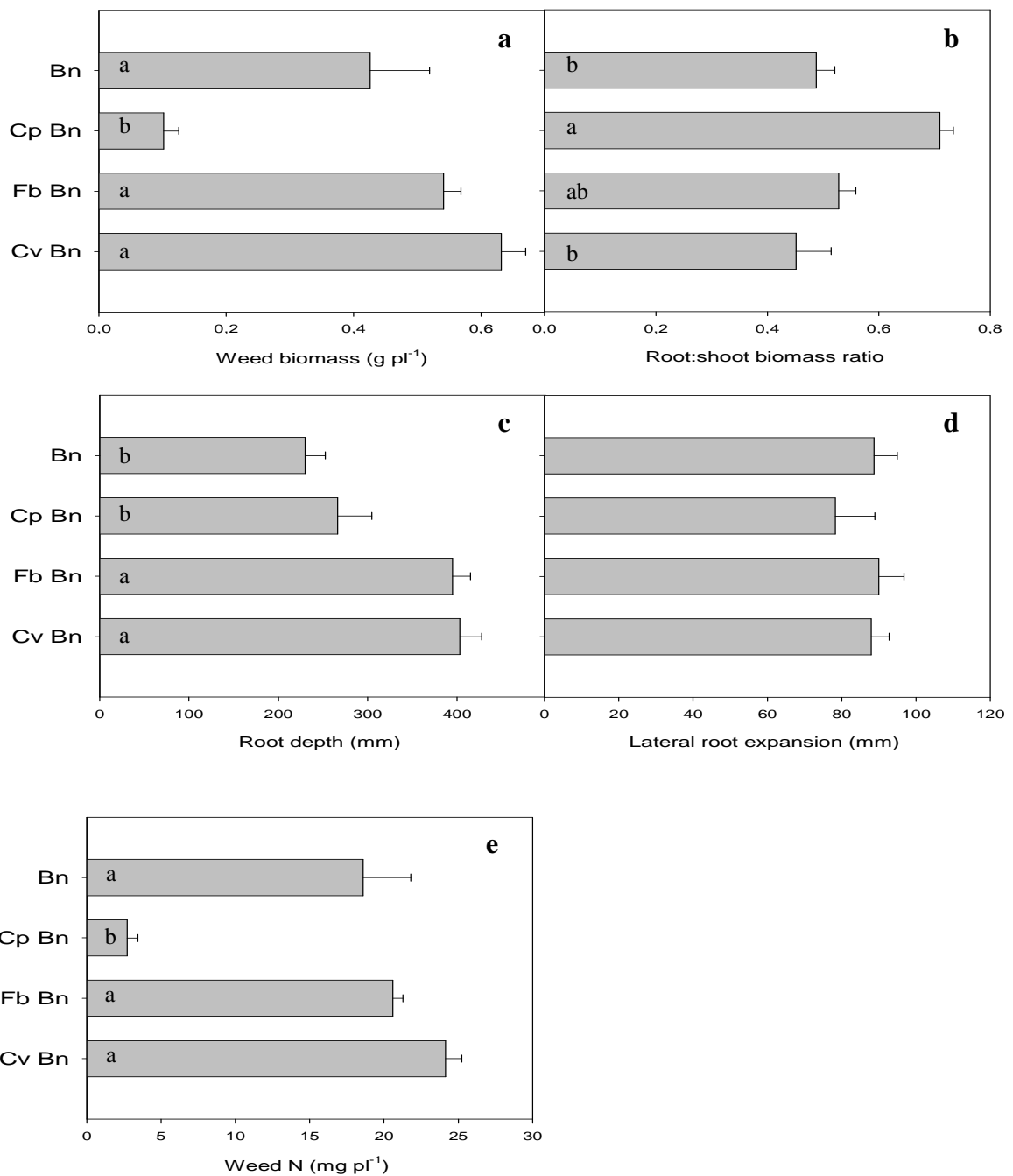


Figure 5.3. Effects of legume species on total biomass (a), root: shoot ratio (b), root depth (c), lateral root expansion (d) and total N of black nightshade when grown in pure stand (Bn) or in mixed stands with legume species; faba bean (Fb Bn), chickpea (Cp Bn), and common vetch (Cv Bn) without N supply (0N). Values represent mean ($n=5$, except for chickpea and weed where, $n=4$) \pm standard error. Treatments with the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$).

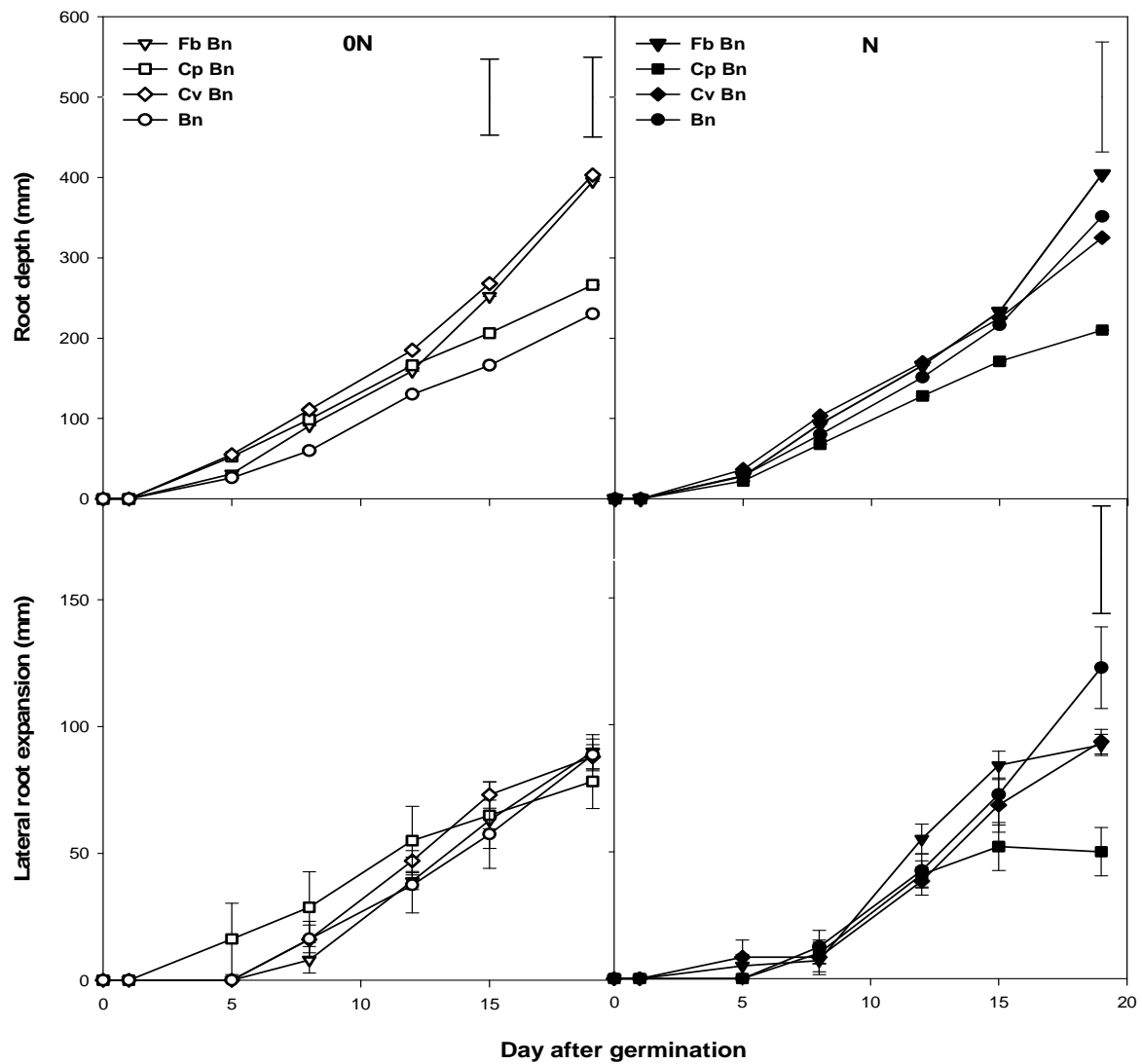


Figure 5.4. Root depth penetration and lateral root expansion of black nightshade grown in pure stand (Bn) or in mixed stands with legume species; faba bean (Fb Bn), chickpea (Cp Bn), and common vetch (Cv Bn) without (0N) and under N supply (N) at 19 days after seed germination. Values represent mean ($n=4$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare weed at each date. Vertical bars represent Tukey's HSD ($\alpha=0.05$).

5.3.3. Soil N sharing between legume species and black nightshade without N supply

When associated with faba bean or common vetch, black nightshade accumulated a very large part (on average 85%) of soil N accumulation of the mixture (Fig. 5.5). Unlike to faba bean and common vetch, when black nightshade was added to chickpea, the weed was able to take up only 11% of soil N. When black nightshade was added to faba bean and common vetch, an important decreasing in remaining soil N was observed up to 67% compared to sole crops (Table 5.2). The remaining soil N was low under chickpea in pure stand and the addition of black nightshade did not reduce more soil N. The contribution of each species to soil N depletion differed between the three mixtures but the remaining soil N was not significantly different between them (Table 5.2). Soil N sharing has been rarely investigated in weed-crop competition studies during early growth. The focus is usually on aboveground traits involved in competition for light more than belowground traits (McDonald, 2003; Uchino et al., 2011). Nevertheless these results are consistent with previous results that suggested that the variability in soil N uptake can better explain the differences in competitiveness against weeds between pea sole crop and barley sole crop than contrasting levels of leaf area and aboveground biomass (Corre-Hellou et al., 2011; Hauggaard-Nielsen et al., 2001b). Moreover (Hauggaard-Nielsen et al., 2001b) found that competition for soil N between weeds and pea began early at 46 days after emergence.

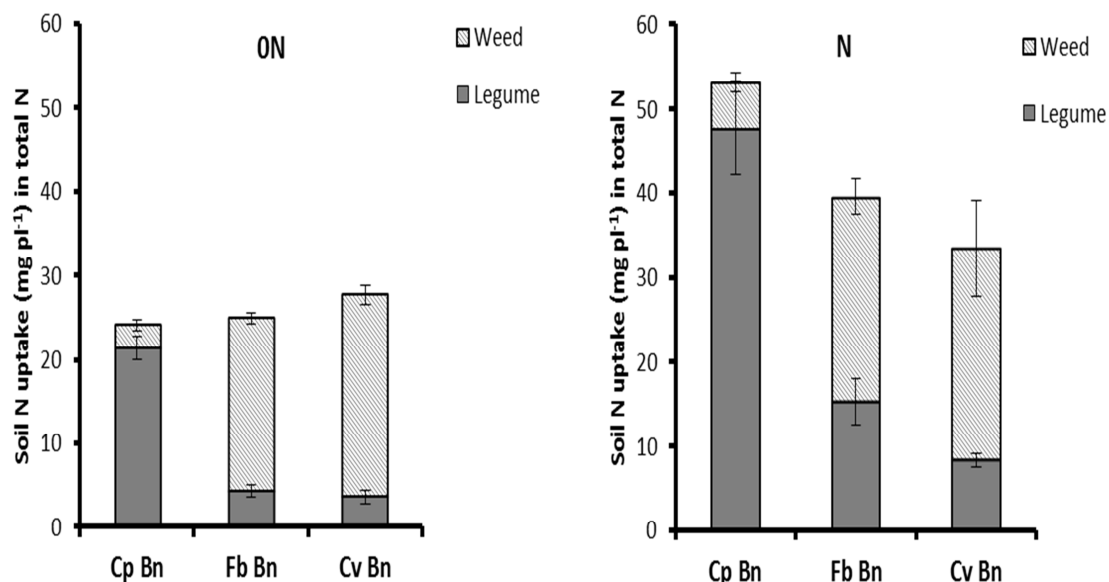


Figure 5.5. Soil N sharing between legume species and black nightshade when weed grown in mixed stands with chickpea (Cp Bn), faba bean (Fb Bn) and common vetch (Cv Bn) without (0N, a) and under (N, b) N supply. Values represent mean ($n=4$) \pm standard error.

5.3.4. Response of legume species and black nightshade to N supply

Pure stands

Chickpea in pure stand responded to increased mineral soil N by accumulating more total biomass. Total biomass of faba bean and common vetch in pure stands did not significantly modified by N supply vetch ($P=0.97$ and 0.21 for faba bean and common vetch, respectively) (Fig. 5.6 a). Root: shoot biomass ratio decreased with N supply only in common vetch ($P<0.005$) (Fig. 5.6 b). Root depth penetration and lateral root expansion were similar for the two levels of soil N availability whatever legume species (Fig. 5.6 c, d).

For faba bean and chickpea, the contribution of soil N to N accumulation increased when grown with N supply (Fig. 5.2 b, Fig.5.6, f). N_2 fixation in faba bean and chickpea was inhibited under N supply. In contrast, common vetch maintained its ability to fix N_2 even with a high soil N availability; the contribution of soil N to total N accumulation also increased but less than that in faba bean and chickpea (Fig. 5.2 b).

Whatever legume species, each legume was able to deplete mineral soil N more than without N supply; (-27% for faba bean, -35% for chickpea and -14% for common vetch) (Table 5.2). The differences between legume species for N uptake were lower than under 0N treatment.

Root depth penetration of black nightshade in pure stand increased by 53% with N supply ($P<0.01$) (Fig. 5.4, Fig. 5.6 c). Total biomass, root: shoot biomass ratio, lateral root expansion and total N tended to increase with N supply but the differences were not significant ($0.08 \leq P < 0.2$) (Fig. 5.6 a, b, d, e). A positive response on aboveground growth of black nightshade with increasing rates of nitrogen application during first stages was previously observed (Masinde et al., 2009) but the effect of N supply on root traits were not measured

Mixed stands

The differences in the response of black nightshade to legume species were maintained with N supply. Total biomass and N accumulation of black nightshade under N supply were still highly reduced by chickpea but not by faba bean and common vetch (Fig. 5.6, a, e). When mixed with chickpea under N supply, black nightshade accumulated a low part (10%) of soil N accumulation of the mixture like under 0N treatment (Fig. 5.5, b). Unlike to chickpea, when black nightshade was added to faba bean and common vetch under N supply, black nightshade took up respectively 61% and 75% of soil N (Fig. 5.5, b) compared to 82% and 87% under 0N treatment (Fig. 5, a). Faba bean and common vetch benefited from a higher N availability more than black nightshade. Soil N uptake increased by 257% and 134% for faba bean and common vetch, respectively and only by 18% and 4% for black nightshade mixed with faba bean and common vetch, respectively (Fig. 5.5, a, b).

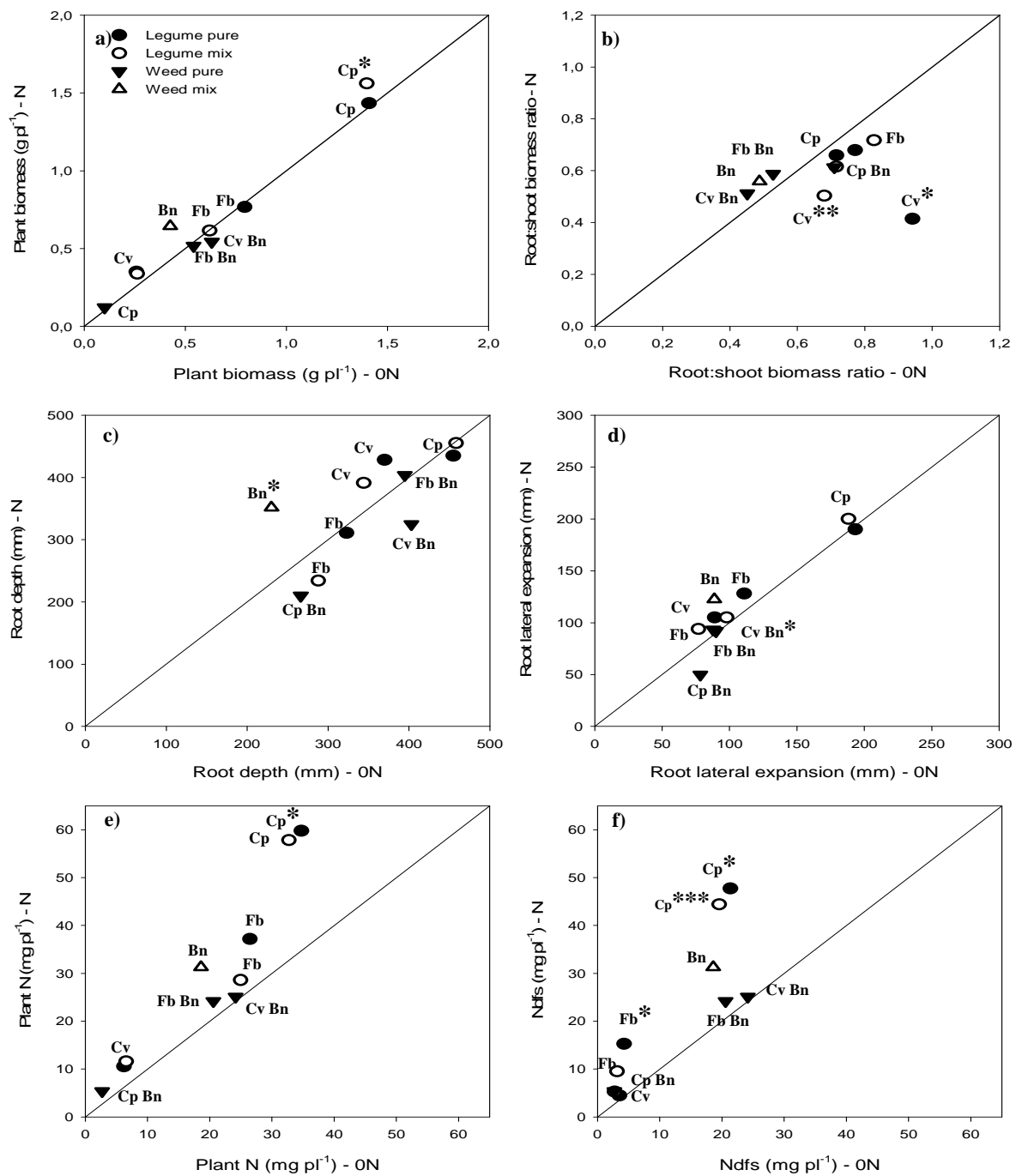


Figure 5.6. Plant biomass (a), root: shoot biomass ratio (b), root depth penetration (c), lateral root expansion (d), plant N (e) and soil N uptake (f) with (N) and without N supply (ON) for legumes and weed species: chickpea (Cp), faba bean (Fb), common vetch (Cv) and black nightshade (Bn) grown in pure stands or in mixed stands. ***, **, * indicate significant differences between the two levels of soil N availability at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively.

As a result, the response of black nightshade differed as a function of legume species in relation with their functional traits involved in soil N uptake. Indeed, chickpea showed a strong competition against black nightshade whatever N supply because of its great early growth and thus its high competitive ability for soil N. *A contrario*, faba bean and common vetch facilitated the growth of black nightshade by leaving a high amount of nitrogen available.

Our study allowed to better understand the establishment of weed-legume competition in relation to belowground traits involved in soil N uptake during the early growth. We have demonstrated that weed response to legume species began very early at the beginning of the crop cycle. This early competition between legume species and weeds was also observed previously. Croster and Masiunas (1998) reported that the interspecific competition between Eastern black nightshade (*S. ptycanthum*) and pea (*P. sativum* L.) began early, thereby the weed must be controlled during the first 2 weeks of the growing season. Several previous studies investigated the effect of weeds on legume growth (Avola et al., 2008; Bensch et al., 2003; Dusabumuremyi et al., 2014; Tepe et al., 2005) but the reverse effect, the influence of legume species on early growth of weeds has been less investigated. We highlighted that aboveground biomass and even belowground traits especially root depth penetration of a high nitrophilic weed may be influenced early when associated with a legume

In field situations with a high infestation with nitrophilic weeds such as black nightshade (*S.nigrum* L.), redroot pigweed (*Amaranthus retroflexus* L.) and Common lambsquarters (*Chenopodium album* L.) (Blackshaw et al., 2003), a legume species, such as chickpea, having a high soil N uptake might ensure efficient weed suppression during early growth. An early N supply may increase legumes biomass and soil N uptake while inhibiting or delaying N₂ fixation (Harper and Gibson, 1984; Tang et al., 1999; Turpin et al., 2002; Voisin et al., 2002), thereby improving the competitiveness against weeds during early growth. However, the effect of N supply on weed-legume competition was low in our study during early stages and such N supplies may reduce the contribution of legume N₂ fixation to N pool in cropping systems. The effect of N supply on relative growth of each component and on soil N sharing may be higher at later stages in relation to differences in N demand and root exploration as observed in legume-non legume mixtures (Corre-Hellou et al., 2007).

Other traits may also influence weed and crop growth during early growth such as the ability to secrete allelopathic substances (Adler and Chase, 2007; Ayub et al., 2013; Xuan and Tsuzuki, 2002).

In our study, we focused on individual plants in weed-legume mixtures without taking into account the effect of plant densities on weed-crop competition and on the traits involved in early growth. Crop-weed competition depends not only on traits of each component but also on environment and cultural practices (Campiglia et al., 2012; Meiss et al., 2008).

We demonstrated that weeds may be differently affected early by the choice of legume species in relation to early growth, depth and lateral root expansion and dependence on seed N. Differences between genotypes of a given species may also exist (Den Hollander et al., 2007; McDonald, 2003;

Uchino et al., 2011) . Such traits should be more taken into account in legume breeding programs including the objective of increasing weed suppression ability.

Acknowledgements

We gratefully acknowledge the technical staff of the research unit of LEVA (Légumineuses, Ecophysiologie Végétale, Agroécologie) for their excellent technical assistance. We are most grateful to the PLATIN' (Plateau d'Isotopie de Normandie) core facility for all element and isotope analyses used in this study. We thank also the National Institute for Agricultural Research (INRA, Dijon, France) for providing us the weed seeds. This work was partly funded by CASDAR n°5376 (Compte d'Affectation Spéciale pour le Développement Agricole et Rural) and the Syrian government.

Dans les chapitres 3 et 4, nous avons quantifié en plein champ l'impact de l'association d'une légumineuse, cultivée en tant que plante de service avec une culture principale, sur la croissance des adventices, la composition de la flore adventice ainsi que le rendement de la culture principale.

Le chapitre 3 étudie une association colza-légumineuse à l'échelle annuelle, répété deux ans sur deux sites expérimentaux. En dispositif substitutif, le colza a été associé à la féverole de printemps ou la vesce commune ayant des traits contrastés de capture de l'azote du sol comme nous avons déjà montré dans le chapitre 1. Nous avons quantifié l'effet de l'association sur la régulation des adventices ainsi que sur la croissance et la productivité de colza. Nous avons aussi montré une complémentarité entre espèces associées contribuant à l'atteinte de services dont la gestion des adventices et l'amélioration du rendement de colza.

**6. Chapitre 3. Improving weed suppression and
crop productivity of oilseed rape when
intercropped with legume species by increasing
trait diversity**

Ce chapitre correspond à un article soumis dans le journal Filed crops research

Improving weed suppression and crop productivity of oilseed rape when intercropped with legume species by increasing trait diversity

Elana Dayoub¹, Guillaume Piva¹, Christophe Naudin¹, Steven J. Shirtliffe², Joëlle Fustec¹ and Guénaëlle Corre-Hellou^{1*}

¹ USC LEVA, INRA, Ecole Supérieure d'Agricultures, Univ. Bretagne Loire, SFR 4207 QUASAV, 55 rue Rabelais, 49100 Angers, France

² Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada

Abstract

Developing new practices that are less dependent on herbicides is a key challenge. Increasing trait diversity in oilseed rape by introducing legume species could improve weed suppression and oilseed rape productivity. Winter oilseed rape (*Brassica napus* L.) was intercropped simultaneously with spring faba bean (*Vicia faba* L.) or common vetch (*Vicia sativa* L.) in a replacement design without herbicide applications. Intercrops and sole crops were compared over two field experimental years in Western France. The presence of faba bean with oilseed rape reduced weed aboveground biomass by 35% and weed N accumulation by 11% compared to the oilseed rape sole crop under high weed infestation conditions. In addition, the growth and the competitive ability of oilseed rape for soil N were increased when intercropped with faba bean. Both grain yield and grain number per plant increased in oilseed rape intercropped compared to sole cropped. Despite the effects of intercrops in weed suppression, no changes were found in the weed community composition in both years. The oilseed rape-faba bean intercrop was more able to suppress weeds than the oilseed rape-common vetch intercrop. Due to both the high aboveground traits (height, leaf area, biomass) of faba bean and the strong competitive ability for soil N of oilseed rape, they appeared complementary in resource utilization, and thus they left less available resources for weeds. Common vetch had similar aboveground morphological traits as oilseed rape, and thus it did not improve weed suppression compared to the oilseed rape sole crop. We observed that a level of aboveground biomass greater than 2 t ha⁻¹ and a soil N uptake at approximately 80 kg ha⁻¹ was needed to obtain good weed control, but such levels are difficult to reach systematically in sole crops under low input cropping systems. Intercropping oilseed rape and legumes is a relevant practice that promotes increased crop biomass and soil N uptake, thus ensuring weed suppression. The combination of intercropped species with contrasting traits appears to be a key factor for the success of intercropping.

Keywords: Winter oilseed rape, faba bean, common vetch, soil nitrogen uptake, weed control

6.1. Introduction

Weed management on conventional farms is primarily accomplished with herbicides. The use of herbicides as the sole weed management tool is seriously being questioned due to herbicide costs and technical problems related to resistance in weed populations to one or several herbicides (Heap, 2014). Moreover, there is an increasing concern about the environmental impacts of herbicide residues in surface and ground water. Winter oilseed rape (*Brassica napus* L.) is the dominant oilseed crop in northern Europe and is known to require large amounts of nitrogen inputs for growth (Malagoli et al., 2005) and pesticides to manage several critical biotic factors. Weeds are one of the important factors limiting oilseed rape growth and grain yield. In France, where herbicide tolerant oilseed rape is prohibited, the cost of herbicide treatments is the major component of a farmer's total variable operating costs for winter oilseed rape (Valantin-Morison et al., 2004). Because of the difficulty to find selective post-emergence herbicides against dicotyledonous weeds, weed infestation management is based mainly on pre-emergence herbicide applications as the systematic practice in France in winter oilseed rape (Hebinger, 2013; Primot et al., 2006; Valantin-Morison et al., 2004). To increase the sustainability of the cropping system, new and innovative crop practices are required in order to both optimize oilseed rape performance and suppress weeds with less dependence on herbicides. Several studies have examined crop-weed competition to identify the main functional traits involved in competitiveness against weeds. These studies have investigated plant functional traits relating mainly to competition for light such as height, leaf area, early growth, and canopy closure (Beckie et al., 2008; Pakeman et al., 2015).

In the current agricultural systems in Europe, fields consist mainly of monocultures of one crop with low genetic diversity and few plant functional traits. Intercropping, defined as the simultaneous growing of two or more crops in the same field (Willey, 1979), can be a relevant practice to increase the diversity of functional traits. The combination of intercropped species with contrasting traits could thus increase the use of the available growth resources and increase the competitive ability of the mixture against weeds. Indeed, intercropped legumes and non-legumes, sown simultaneously, are known to improve weed suppression. (Banik et al., 2006; Carr et al., 1995; Corre-Hellou et al., 2011; Gronle et al., 2014; Hauggaard-Nielsen et al., 2001b; Liebman and Dyck, 1993). However, most of these studies focused on intercropped legume species with cereals. Few studies have investigated the effects of oilseed rape intercropped with legumes on weed suppression and crop productivity (Cadoux et al., 2015; Gharineh and Telavat, 2009; Lorin et al., 2015).

Oilseed rape and legumes differ in their morphological traits and ability to acquire resources. An oilseed rape sole crop is known to have a greater capacity to take up soil mineral N compared to legumes (Andersen et al., 2005; Thorup-Kristensen, 1994) due to its rapid and deep root growth (Thorup-Kristensen, 1994; Thorup-Kristensen, 2001). Thus, less soil N could be available for weeds in oilseed rape crops. The availability of soil N in the early winter is a main factor explaining the

competitive ability of oilseed rape against weeds (Valantin-Morison and Meynard, 2008). On the other hand, legume species are known to have a reduced ability to take up soil mineral N compared to non-legumes due to their shallow rooting depth and low soil N demand and their ability to fix N_2 from the atmosphere (Dayoub et al., 2017; Schröder and Köpke, 2012; Thorup-Kristensen, 1994). This is apparent mostly at the beginning of the crop cycle (Corre-Hellou et al., 2007). Moreover, some legume species are able to produce greater biomass than brassica species (Andersen et al., 2005; Szumigalski and Van Acker, 2006), which may lead to a strong competitive ability against weeds for light. However, there is variability among legume species for both aboveground traits and their ability to uptake soil N (Hamblin and Hamblin, 1985; Herridge et al., 2008; Liu et al., 2011).

Intercropping oilseed rape with a legume may increase the diversity of functional traits, and thus, the interspecific complementary combination can exploit growth resources (nitrogen and light) differently and more efficiently compared to that in oilseed rape sole crops. Therefore, intercropping may lead to less soil N and light available for weeds. Moreover, the choice of a legume species having very contrasting traits with oilseed rape should be the determining factor to increase the success of this practice.

The complementarity between intercropping species may also increase crop growth and productivity, which is usually observed in intercrops based on legumes and non-legumes. Legumes and non-legumes have the ability to uptake soil N, but legumes have the ability to use an additional source of N: N_2 from the atmosphere. Moreover, the contribution of N_2 fixation to N accumulation is greater in intercrops compared to sole crops (Andersen et al., 2005; Hauggaard-Nielsen et al., 2009). This complementarity for the use of N sources is considered the one of the main factors explaining the increase of crop productivity in intercrops compared to sole crops (Corre-Hellou et al., 2006; Jensen, 1996). In brassica-legume intercrops, an increase of brassica biomass and N accumulation has previously been observed (Andersen et al., 2005; Banik et al., 2000; Szumigalski and Van Acker, 2005). This intercropping effect can reinforce the competitive ability of the crops against weeds while improving crop productivity.

Previous studies showed that weed growth and weed density are influenced by the level of inorganic N in the soil as a function of weed species (Blackshaw et al., 2003; Moreau et al., 2013; Poggio, 2005; Saucke and Ackermann, 2006). Moreover, the composition of a weed community is determined by environmental factors and competition between and within weeds and crops (Spitters and Van Den Bergh, 1982). Soil N availability is also an important determinant of the weed community composition (Pyšek and Lepš, 1991). Thus, intercropping legumes species with oilseed rape may change the use of available resources, which could modify the weed species community compared with sole crops. However, few studies have investigated this point in intercropping systems.

In the present study, oilseed rape is the main crop, and frost-sensitive legume species were introduced as service plants. The insertion of frost-sensitive legume species with oilseed rape was often practised

with an additive design (Cadoux et al., 2015), with intercropped oilseed rape sown at the same density as the sole crop.

In our study, we expected to maximize the interspecific complementarity between oilseed rape and legume species thanks to a replacement design, which may lead to both the suppression of weeds and the increase in or at least the avoidance of negative effects on oilseed rape yield.

The objectives of this study were (i) to determine the ability of oilseed rape intercropped with faba bean or common vetch to suppress weeds and (ii) to investigate the effects on oilseed rape productivity in relation with the complementarity between crops traits involved in competition for light and soil N uptake during the winter. To test these objectives, two experiments were carried out in Western France under field conditions for two years in order to compare intercrops and sole crops without weed control in a replacement design.

6.2. Material and Methods

6.2.1. Site characteristics

Field experiments were carried out in 2013/14 and 2015/16 at the FNAMS (Fédération Nationale des Agriculteurs Multiplicateurs de Semences) experimental field station near Angers, France (47.5°N, 0.6°W). In the ploughed layer (0–30 cm), the soil texture was a sandy clay in 2013/14 and a sandy loam clay in 2015/16. The main characteristics of each site are presented in Table 6.1. The soil contained 125 and 124 kg ha⁻¹ KCl-extractable inorganic N at the 0–90 cm depth at sowing in 2013/14 and 2015/16, respectively. The average annual temperature and precipitation are 11.2 °C and 565 mm (1993–2013), respectively. The weather conditions during the experimental years are presented in Table 6.2. Rainfall and the number of cumulated degree-days between September and July were quite similar for both years. The previous crop was winter wheat (*Triticum aestivum* L.) for 2013/14 and barley (*Hordeum vulgare* L.) for 2015/16.

Table 6.1. Characteristics of the measured topsoil (0 to 30 cm) before crop establishment in 2013/14 and 2015/16

| | 2013/14 | 2015/16 |
|--|---------|---------|
| % Clay | 14.7 | 16.2 |
| % Silt | 22 | 20.3 |
| % Sand | 61.1 | 38.5 |
| PH _(Water) | 7.3 | 8.2 |
| % Organic matter | 2.2 | 2.6 |
| % C | 1.26 | 1.51 |
| P ₂ O ₅ (mg kg ⁻¹) | 176 | 60 |
| K ₂ O (mg kg ⁻¹) | 466 | 392 |
| MgO (mg kg ⁻¹) | 316 | 250 |
| CaCO ₃ Total (%) | < 0.1 | 22.5 |

Table 6.2. Mean air temperature and precipitation during the 2013/14 and 2015/16 experimental years

| Month | 2013/14 | | 2015/16 | |
|--------------|----------------------|--------------------|----------------------|--------------------|
| | Air temperature (°C) | Precipitation (mm) | Air temperature (°C) | Precipitation (mm) |
| | Average | Total | Average | Total |
| September | 16.62 | 41.00 | 14.58 | 81.2 |
| October | 14.21 | 75.40 | 11.73 | 73.8 |
| November | 7.81 | 91.20 | 11.68 | 66.8 |
| December | 5.34 | 76.00 | 9.23 | 37.4 |
| January | 7.40 | 105.00 | 6.69 | 111 |
| February | 7.76 | 102.00 | 6.87 | 72.6 |
| March | 8.85 | 26.20 | 7.19 | 88.8 |
| April | 11.65 | 40.00 | 9.42 | 22.8 |
| May | 13.59 | 79.60 | 14.39 | 119.4 |
| June | 18.07 | 27.20 | 17.42 | 62.4 |
| July | 20.07 | 49.80 | 19.82 | 14 |
| Total | 4025 ¹ | 713 | 3962 ¹ | 750 |

¹Sum of degree-days at base temperature = 0 °C

6.2.2. Experimental design and crop management

Winter oilseed rape (OR) (*Brassica napus* L.) was grown as a sole crop (SC) and intercropped (IC) with spring faba bean (F) (*Vicia faba* L.) or common vetch (V) (*Vicia sativa* L.). Faba bean and common vetch were also grown as sole crops. Intercrops were studied using a replacement design; each species was sown at 50% of the recommended sole crop density in alternate rows. The target seeding rates for winter oilseed rape (cv. Boheme), spring faba bean (cv. Divine) and common vetch (cv. Nacre) sole crops were 60, 46, and 90 seeds m⁻², respectively. The experiments were arranged in a block design with four replicates. The size of each plot was 5 × 10 and 6.3 × 10 m in 2013/14 and 2015/16, respectively.

Soil preparation was carried out by ploughing, followed by harrowing. The crops were sown on 19 September in 2013 and on 08 September in 2015. For the intercrop treatments, the two species were sown simultaneously with a seed drill that allowed differential sowing depth for each species. The sowing depth was 2 cm for oilseed rape and common vetch and 4 cm for faba bean. Row spacing was 35 cm. The crops were irrigated one day after sowing at a rate of 15 mm to ensure homogeneous emergence.

All plots were supplied 70 kg N ha⁻¹ in the form of ammonium nitrate based fertilizer (NH₄NO₃) divided twice after winter in February and March. This nitrogen fertilization rate was calculated according to the balance sheet method (Lagarde and Champolivier, 2006) for intercrops and based on the need of oilseed rape in intercrops. No weed control was performed. Pests and diseases were controlled with appropriate pesticides. Anti-slug treatments of ferric phosphate (Sluxx) were applied each year. In addition, the insecticide deltamethrin (Decis Protech; 0.33 L ha⁻²) was applied in 2013/14, and the fungicide prothioconazole (Prosaro; 1 L ha⁻¹) was applied in 2015/16.

6.2.3. Sampling measurements and analytical methods

Crop and weed biomass were harvested twice during the oilseed rape life cycle: prior (December) to and after winter (February) (Table 6.3). Plants were harvested from a 1.2 m² area (4 row × 0.75 m) in 2013/14 and a 1.05 m² area (4 row × 0.75 m) in 2015/16 within each plot. Total aboveground biomass included legumes residues after the frost period. The green leaves were separated from the other parts of the plant for each species. The leaf area (LA) of the crops and weeds were determined for each sampling date, except at harvest, by measuring the green leaf area of 5 plants for cultivated crops and for all weeds present in the sampling area. Leaf area (LA) was measured using an LI3100 area metre (LI-COR Inc., NE, USA). In 2015/16, due to the destruction of leaves of faba bean and common vetch by frost damage and disease, leaf area was measured only before winter. Crops and weeds samples were dried at 70°C for 48 h. Samples harvested before and after winter were ground to measure N content (for weeds and crops) and the 15N:14N ratio (for legumes) in aboveground biomass using a

CHN analyser (EA3000, Euro Vector, Milan, Italy) and a mass spectrometer (IsoPrime, Elementer, Hanau, Germany).

Crop height for five plants per harvested area was measured from the soil surface to the cover height in the field. Crop density was estimated in the same harvested areas before sampling, and weed density was also measured for each species separately at each sampling date.

Yield and the yield components of oilseed rape (grain yield per hectare and per plant, weight per 1000 grain, number of grain per m², and number of grain per plant) were measured at harvest.

Table 6.3. Dates of weed and crop biomass samplings with the corresponding crop growth stages (BBCH) in 2013/14 and 2015/16

| | | | 2013/14 | | | 2015/16 | | | | |
|-----------------------|--|-------------|---------------|---------------------------|-----------|--------------|---------------|---------------------------|-----------|--------------|
| | | | Sampling date | Growth stage ¹ | | | Sampling date | Growth stage ¹ | | |
| | | | | Oilseed rape | Faba bean | Common vetch | | Oilseed rape | Faba bean | Common vetch |
| | | | 2013 | | | | 2015 | | | |
| Crop and weed biomass | | 02 December | 16 | 21 | 34 | 02 December | 16 | 21 | 34 | |
| | | | 2014 | | | | 2016 | | | |
| Crop and weed biomass | | 17 February | 32 | 55 | 51 | 23 February | 51 | – | – | |
| Oilseed rape yield | | 02 July | 81 | – | – | 28 June | 81 | – | – | |

– indicates that the legumes species were partially or totally killed by frost and disease

¹According to BBCH scale of (JKI, 2010) at sampling date

6.2.4. Calculations and statistical analysis

The nitrogen content in the crop and weed biomass was calculated as the product of aboveground biomass and %N content.

The percentage of accumulated exogenous N derived from the atmosphere (%Ndfa) was determined using the ^{15}N natural abundance method (Amarger et al., 1979). The oilseed rape sole crop was used as a reference crop to calculate N_2 fixation in the legume species by using the following equation:

$$\% \text{Ndfa} = 100 \times [(\delta^{15}\text{N}_{\text{oilseed rape}} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{oilseed rape}} - \beta)],$$

where $\delta^{15}\text{N}_{\text{legume}}$ and $\delta^{15}\text{N}_{\text{oilseed rape}}$ are the natural ^{15}N enrichment of the legume and oilseed rape sole crops, respectively.

The β value is the isotopic fractionation factor measured for each species from legumes grown in N-free conditions. $\beta = -1.70$ for faba bean shoots grown in N free medium (López-Bellido et al., 2010), and $\beta = -0.9$ for common vetch (Yoneyama et al., 1986).

The amount of N_2 fixed was calculated as the product of aboveground biomass, %N content and the proportion of plant N derived from N_2 fixation (%Ndfa). The amount of N derived from soil (Ndfs) was calculated as the difference between the aboveground N content and the amount of N_2 fixed from the air.

The land equivalent ratio (LER) is defined as the relative land area required when growing sole crops to produce the yield achieved in an intercrop. LER for an oilseed rape-legume intercrop is the sum of the partial LER values for oilseed rape (LER_{OR}) and legume (LER_{L}) (Willey, 1979). LER was calculated for crop aboveground biomass and crop soil N uptake as followed:

$$\text{LER} = \text{LER}_{\text{OR}} + \text{LER}_{\text{L}},$$

$$\text{LER}_{\text{OR}} = \text{OR}_{\text{IC}} / \text{OR}_{\text{SC}}, \text{LER}_{\text{L}} = \text{L}_{\text{IC}} / \text{L}_{\text{SC}},$$

where OR and L are the assessed variables for oilseed rape and legumes, respectively, in the intercropped (IC) and sole-cropped (SC) designs. LER values higher than 1 indicate an advantage from intercropping compared to the respective sole crops in terms of crop aboveground biomass and crop soil N uptake.

The percentage of weed biomass in the total aboveground biomass (crops + weeds) was calculated in each treatment. The percentage of soil N accumulated by weeds in the total soil N accumulated by both weeds and crops was also calculated.

The species diversity of the weed community was assessed by calculating different indices at each sampling date. Species richness (S) was the mean number of species observed per treatment (Magurran, 1988). Shannon-Wiener's indicator (H') was used to describe the diversity of the weed population using the following equation:

$$H' = -\sum(p_i * \ln p_i) \quad p_i = n_i/N$$

where n_i represents the number of observed individuals for one weed species, and N the total number of observed individuals per treatment. The value of H varied from 1 (low diversity) to 4 (high diversity).

The Simpson evenness index (D) was used as a measure of equitability of the weed population (Magurran, 1988) and was calculated using the following equation:

$$D = \sum (n_i(n_i-1) / N(N-1)),$$

D varied from 0 (a very dominant weed species) to 1 (weed species are equally distributed).

The statistical analysis was conducted using the combined experimental years, except for weeds variables and oilseed rape yield components, where the analyses were performed separately for each year. The effect of the different treatments on weeds (density, leaf area (LA), aboveground biomass and soil N uptake) was tested by a one-way analysis of variance (type III sum of squares; $\alpha = 0.05$). To test for the normality of residues and homoscedasticity, the Shapiro-Wilk and Bartlett tests were used, respectively ($\alpha = 0.05$). Means were compared using Tukey's HSD test (Honest Significant Differences; $\alpha = 0.05$). To meet the assumptions of ANOVA, weed proportion data were transformed using the arcsine square root transformation, whereas weed density and weed leaf area were squared and ln transformed, respectively, for the after-winter sampling date in 2013/14. All statistical analyses were performed using package R Commander in R software, version 3.1.2 (RCore, 2012). We compared the intercrops and sole crops each year for several variables (crop traits and weed infestation) by radar plots using scores calculated for each variable through the minimum (Min) and maximum (Max) observed values as follows: score = 10 x (value-Min)/(Max-Min).

6.3. Results

6.3.1. Comparison between oilseed rape and legumes growth and soil N uptake in sole crops

Significant differences were observed between sole crops concerning crop growth and soil N uptake before and after winter (Table 6.4). Faba bean had a higher crop height, crop growth and soil N uptake than common vetch before and after winter. Faba bean obtained a greater leaf area index (+139%), height (+236%) and aboveground biomass (+101%) than oilseed rape before winter. Despite having lesser crop biomass, oilseed rape was able to uptake a high amount of soil N. The differences between faba bean and oilseed rape in crop height were maintained after winter, whereas the differences were not significantly different for crop biomass. The high competitive ability of oilseed rape for soil N was also maintained after winter.

Unlike faba bean, common vetch had similar aboveground traits (leaf area index, crop height and biomass) as oilseed rape before and after winter. Nevertheless, common vetch had a significantly lower (-70%) soil N uptake than oilseed rape before and after winter (Table 6.4).

Table 6.4. Comparison between oilseed rape (OR), faba bean (F) and common vetch (V) growth and soil N uptake as sole crops (SC) before winter in December and after winter in February. Values are mean ($n=4$) \pm standard error for 2013/14 and 2015/16. Crop leaf area index was measured only before winter in 2015/16

| | Before winter | | | <i>P-value</i> ¹ |
|---|----------------------------|----------------------------|---------------------------|-------------------------------------|
| | OR SC | F SC | V SC | |
| Density (plt m ⁻²) | 45.48 \pm 1.57 b | 36.90 \pm 1.80 c | 70.83 \pm 4.69 a | 1.39 \times 10 ⁻⁰⁶ *** |
| Leaf area index (m ² m ⁻²) | 1.38 \pm 0.14 b | 3.30 \pm 0.26 a | 1.08 \pm 0.09 b | 9.84 \times 10 ⁻⁰⁷ *** |
| Aboveground biomass (t ha ⁻¹) | 0.99 \pm 0.10 b | 1.99 \pm 0.06 a | 0.79 \pm 0.06 b | 1.98 \times 10 ⁻⁰⁸ *** |
| Soil N uptake (kg ha ⁻¹) | 28.14 \pm 3.15 a | 18.86 \pm 1.11 a | 8.43 \pm 1.05 b | 4.09 \times 10 ⁻⁰⁶ *** |
| Height (cm) | 21.10 \pm 1.25 b | 70.90 \pm 4.41 a | 18.20 \pm 0.97 b | 3.07 \times 10 ⁻¹¹ *** |
| | After winter | | | |
| Density (plt m ⁻²) | 45.83 \pm 3.10 ab | 35.71 \pm 1.37 b | 48.57 \pm 4.27 a | 3.58 \times 10 ⁻² * |
| Leaf area index (m ² m ⁻²) | 02.16 \pm 0.51 | 2.39 \pm 0.32 | 3.25 \pm 0.18 | NS |
| Aboveground biomass (t ha ⁻¹) | 01.42 \pm 0.28 ab | 2.94 \pm 0.35 a | 1.21 \pm 0.28 b | 1.69 \times 10 ⁻² * |
| Soil N uptake (kg ha ⁻¹) | 47.76 \pm 10.7 a | 33.64 \pm 6.92 ab | 14.32 \pm 4.30 b | 4.66 \times 10 ⁻² * |
| Height (cm) | 14.13 \pm 1.61 b | 66.73 \pm 4.28 a | 21.60 \pm 2.95 b | 5.65 \times 10 ⁻⁰⁹ *** |

Means in the same row with different letters are significantly different at $P<0.05$

NS: Means were not significantly different

***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively

¹ P -values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$)

6.3.2. Effects of intercropping on crop growth and productivity

Oilseed rape growth and soil N uptake were greater when intercropped with faba bean compared with the oilseed rape sole crop (Fig. 6.1). These positive effects appeared before winter and increased after winter. The leaf area per plant of intercropped oilseed rape increased by 63% and 88% compared to the oilseed rape sole crop before and after winter, respectively. The aboveground biomass per plant of intercropped oilseed rape with faba bean increased by 32% and 56% before and after winter, respectively, compared to the oilseed rape sole crop (Fig. 6.1). The presence of faba bean resulted in an increased height of oilseed rape by 47% before winter and by 108% after winter. In addition, accumulated N per plant increased significantly by 66% and 60% before and after winter, respectively, in intercrops compared to the oilseed rape sole crop.

Unlike the oilseed rape-faba bean intercrop, in the oilseed rape-common vetch intercrop, oilseed rape growth and soil N uptake were similar as in the sole crop before winter. However, aboveground biomass, soil N uptake and height increased by 43%, 37% and 29%, respectively, in intercrops compared to sole crops after winter (Fig. 6.1). The intercrop effect was lower than that with faba bean. The leaf area and crop height of oilseed rape intercropped with faba bean were higher by 77% and 57%, respectively, compared to oilseed rape intercropped with common vetch after winter.

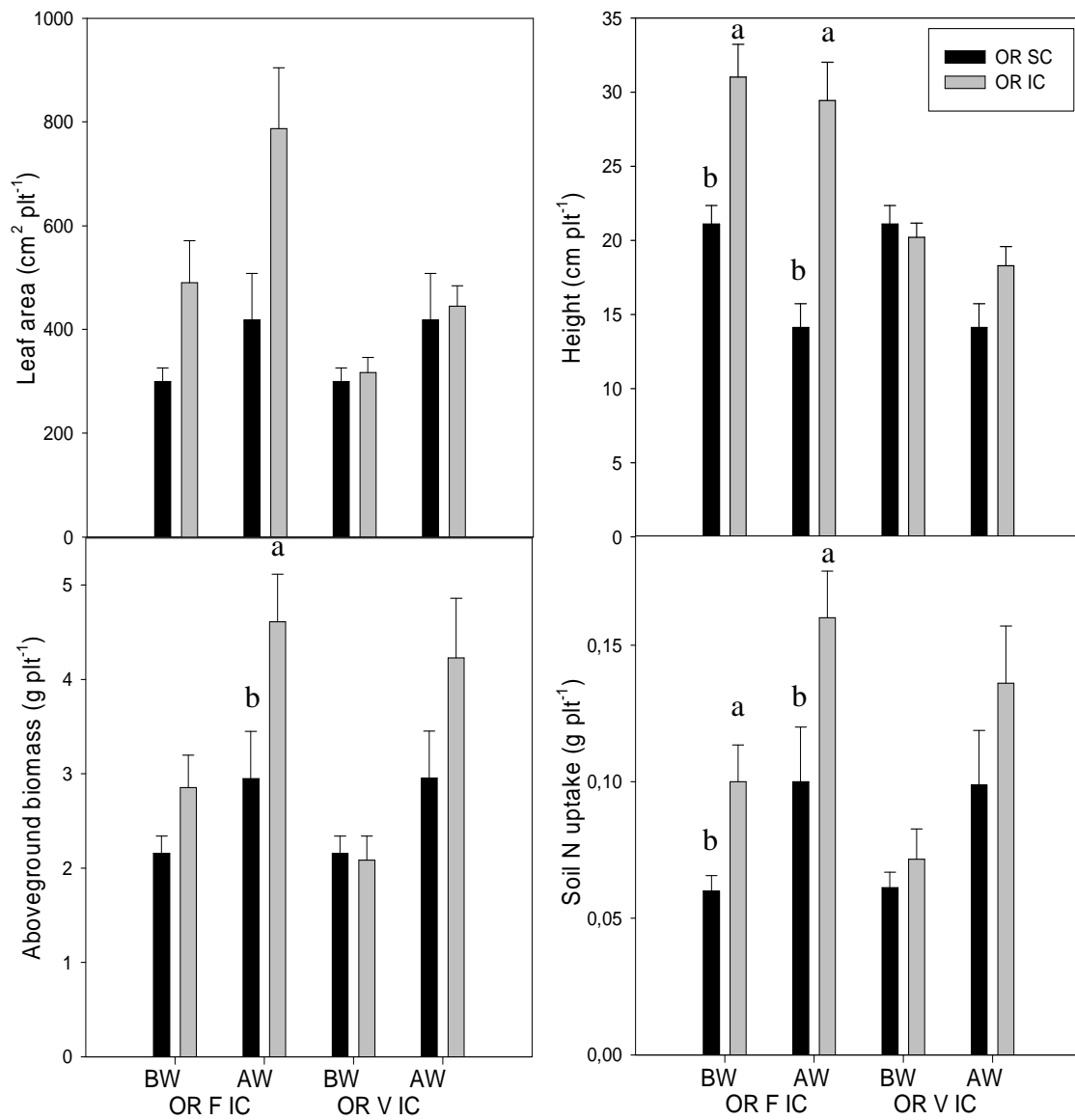


Figure 6.1. Effects of legume species on intercropped oilseed rape (OR) with faba bean (OR F IC) or common vetch (OR V IC) before winter (BW) in December and after winter (AW) in February. Values are mean ($n=4$) \pm standard error for the two experimental years (2013/14 and 2015/16), except for leaf area after winter, which was measured only in 2013/14. Columns with the different letters are significantly different using Tukey's studentized range test (Tukey's HSD; $\alpha=0.05$)

The total LER obtained by oilseed rape-faba bean for aboveground biomass and soil N uptake were greater than 1, indicating an increase of crop growth and soil N accumulation in intercrops compared to sole crops. The LER values increased between December (before winter) and February (after winter). The values ranged from 1.17 to 1.69 for aboveground biomass and from 1.35 to 1.75 for soil N uptake before and after winter, respectively (Fig. 6.2). When oilseed rape was intercropped with common vetch, the LER values were less than that with faba bean and varied from 0.94 to 1.28 for aboveground biomass and from 1.01 to 1.31 for soil N uptake before and after winter, respectively. Partial LER values were greater for oilseed rape than for legumes, indicating that oilseed rape benefited more than the legumes from intercropping (Fig. 6.2).

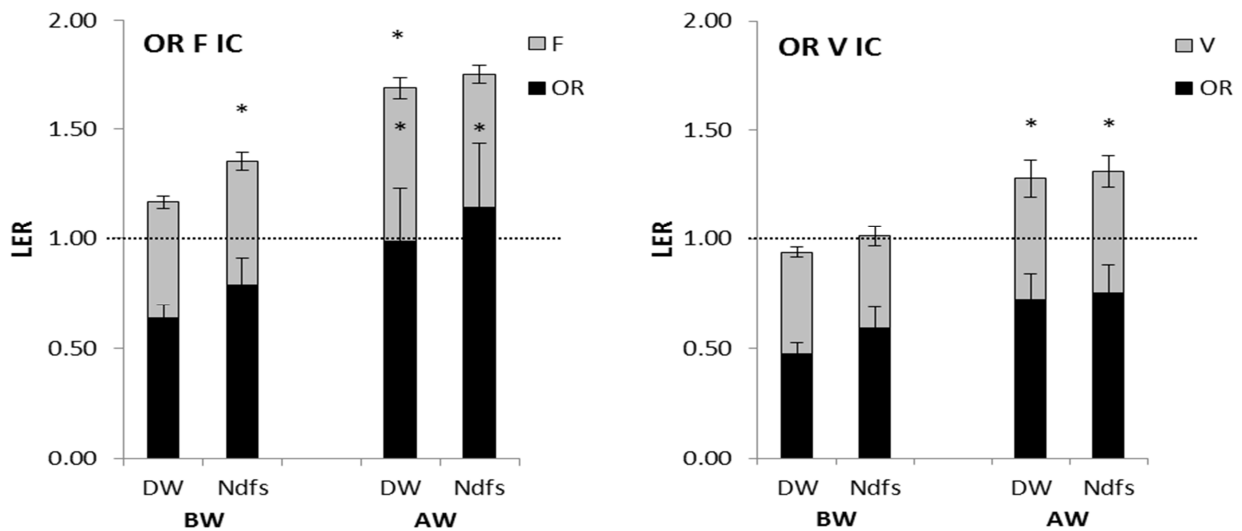


Figure 6.2. Total and partial Land Equivalent Ratio (LER) of oilseed rape (OR), faba bean (F) and common vetch (V) using aboveground biomass (DW) and soil N uptake (Ndfs) before winter (BW) in December and after winter (AW) in February when oilseed rape is intercropped using a replacement design with faba bean (OR F IC) or common vetch (OR V IC). Values are mean ($n=4$) \pm standard error for the two experimental years 2013/14 and 2015/16. “*” above the histogram indicates that a total LER > 1 was significant at $P=0.05$, whereas “*” on the histogram indicates that a partial LER > 0.5 was significant at $P=0.05$

6.3.3. Effects of intercropping on weed suppression

Weed infestation in 2015/16 was higher than that in 2013/14. Weed density and weed biomass before winter were four times greater in 2015/16 than in 2013/14 in the oilseed rape sole crop (Table 6.5). The most prevalent annual weed species in 2013/14 was *Vulpia myuros* L., whereas *Veronica persica* L. and *Stellaria media* L. dominated the weed community in the second experimental year (data not shown). Before winter, no significant differences were observed between the treatments for weed biomass, weed N uptake and weed leaf area index. Weed biomass contributed to 13% of the total biomass (weeds + crops) in 2013/14 and up to 51% in 2015/16 before winter in the oilseed rape sole crop. In 2015/16, in oilseed rape-faba bean, the percentage of weeds in the total biomass was significantly reduced compared to the oilseed rape sole crop before winter.

After winter, significant differences were observed between the treatments for weed biomass and weed N uptake but not for the weed leaf area index. In 2013/14, weed biomass and weed N uptake were significantly lower under the faba bean sole crop than the vetch sole crop. However, intercropping did not reduce weed biomass and weed N uptake compared to the oilseed rape sole crop. In 2015/16, the oilseed rape-faba bean intercrop reduced weed biomass compared to the oilseed rape sole crop but did not reduce significantly weed N uptake. The percentage of weeds in the total biomass (weeds + crops) was 25% in the oilseed rape-faba bean intercrop compared to 64% in the oilseed rape sole crop. Unlike the oilseed rape-faba bean intercrop, the oilseed rape-vetch intercrop was not able to reduce weed biomass compared to the oilseed rape sole crop.

The proportion of total soil N taken up by weeds after winter was greater in common vetch (42% in 2013/14 and up to 94% in 2015/16) compared to that in the faba bean sole crop (14% and 61% in 2013/14 and 2015/16 respectively). Similar proportions were observed for faba bean and oilseed rape. In 2015/16, the amount of nitrogen taken up by weeds in the oilseed rape-faba bean intercrop was lower (36%) than in the oilseed rape sole crop (61%) (Table 6.5). There was a negative relationship between the crop and weed aboveground biomass accumulation after winter ($R^2=0.86^{***}$) (Fig. 6.3, a). Moreover, a negative relationship was found between the crop N uptake and weed aboveground biomass in both years after winter ($R^2=0.72^{**}$) (Fig. 6.3, b). Weed density and crop aboveground biomass were negatively correlated ($R^2=0.67^*$) but in a lesser extent than weed biomass (Fig. 6.3, c).

Table 6.5. Effects of oilseed rape (OR), faba bean (F) and common vetch (V) grown as sole crops (SC) or as intercrops (IC) on weed infestation before winter (BW) and after winter (AW) in 2013/14 and 2015/16. Values are mean ($n=4$) \pm standard error for the two separate experimental years

| | Weed aboveground biomass (t ha ⁻¹) | | | | Weed biomass in total aboveground biomass (%) | | Weed N (kg ha ⁻¹) | | Weed N in total aboveground N (%) | | Weed density (m ⁻²) | | Weed leaf area index (m ²) | | | |
|------------------------------|--|---------------------------|-----------------------------|----------------------------|---|----------------------------|-------------------------------|---------------------------|-----------------------------------|---------------------|---------------------------------|-----------------|--|--|----|--|
| | BW | | AW | | BW | | AW | | BW | | AW | | BW | | AW | |
| | | | | | | | | | | | | | | | | |
| 2013/14 | | | | | | | | | | | | | | | | |
| OR SC | 0.20 \pm 0.03 | 0.48 \pm 0.08 ab | 13.80 \pm 1.85 ab | 18.13 \pm 3.03 ab | 6.28 \pm 1.24 | 15.24 \pm 2.65 ab | 14.62 \pm 1.8 b | 16.26 \pm 2.34 b | 224.29 \pm 55.28 | 153.10 \pm 43.92 | 0.31 \pm 0.07 | 0.67 \pm 0.15 | | | | |
| F SC | 0.17 \pm 0.07 | 0.25 \pm 0.08 b | 8.08 \pm 3.79 b | 6.27 \pm 2.19 c | 3.51 \pm 1.27 | 6.97 \pm 1.76 b | 16.82 \pm 5.25 b | 13.99 \pm 4.29 b | 228.57 \pm 79.13 | 138.33 \pm 34.79 | 0.40 \pm 0.17 | 0.50 \pm 0.15 | | | | |
| V SC | 0.30 \pm 0.09 | 0.67 \pm 0.11 a | 28.15 \pm 6.67 a | 24.73 \pm 3.16 a | 7.72 \pm 1.93 | 20.52 \pm 3.33 a | 49.97 \pm 9.47 a | 42.69 \pm 3.52 a | 182.38 \pm 72.73 | 183.10 \pm 43.02 | 0.47 \pm 0.11 | 1.10 \pm 0.21 | | | | |
| OR F IC | 0.23 \pm 0.05 | 0.39 \pm 0.06 ab | 12.32 \pm 3.07 ab | 9.62 \pm 1.76 bc | 6.17 \pm 1.15 | 11.58 \pm 1.68 ab | 16.72 \pm 3.97 b | 15.11 \pm 1.62 b | 168.33 \pm 31.91 | 205.95 \pm 40.28 | 0.33 \pm 0.05 | 0.76 \pm 0.13 | | | | |
| OR V IC | 0.31 \pm 0.09 | 0.48 \pm 0.06 ab | 24.94 \pm 6.26 a | 16.01 \pm 1.43 ab | 9.68 \pm 3.16 | 15.11 \pm 2.04 ab | 31.78 \pm 9.36 ab | 21.32 \pm 1.66 b | 204.76 \pm 94.03 | 165.48 \pm 33.16 | 0.71 \pm 0.25 | 0.74 \pm 0.11 | | | | |
| <i>P</i> -value ¹ | NS | 4.33 $\times 10^{-2}$ * | 8.32 $\times 10^{-3}$ ** | 1.97 $\times 10^{-3}$ ** | NS | 3.70 $\times 10^{-2}$ * | 7.55 $\times 10^{-3}$ ** | 1.61 $\times 10^{-4}$ *** | NS | NS | NS | NS | | | | |
| 2015/16 | | | | | | | | | | | | | | | | |
| OR SC | 0.77 \pm 0.10 | 1.17 \pm 0.10 ab | 51.16 \pm 4.43 a | 64.41 \pm 4.89 a | 22.05 \pm 1.42 | 26.03 \pm 2.41 ab | 51.58 \pm 3.92 | 59.33 \pm 5.47 b | 906.67 \pm 185.63 | 786.90 \pm 148.59 | 1.28 \pm 0.14 | — | | | | |
| F SC | 0.53 \pm 0.04 | 1.00 \pm 0.12 bc | 20.66 \pm 1.66 c | 33.21 \pm 4.24 b | 22.11 \pm 2.36 | 33.37 \pm 3.99 ab | 50.20 \pm 2.76 | 61.03 \pm 5.27 b | 585.00 \pm 64.45 | 561.19 \pm 177.17 | 1.34 \pm 1.80 | — | | | | |
| V SC | 0.57 \pm 0.11 | 1.37 \pm 0.10 a | 38.61 \pm 7.28 abc | 76.30 \pm 2.07 a | 20.85 \pm 3.37 | 35.52 \pm 2.00 a | 66.73 \pm 6.48 | 94.04 \pm 1.03 a | 719.76 \pm 246.65 | 715.48 \pm 235.56 | 1.19 \pm 0.26 | — | | | | |
| OR F IC | 0.62 \pm 0.12 | 0.76 \pm 0.06 c | 27.36 \pm 4.49 bc | 25.46 \pm 4.53 b | 24.77 \pm 5.23 | 23.11 \pm 0.96 b | 42.75 \pm 5.4 | 35.83 \pm 5.56 c | 695.48 \pm 198.57 | 438.10 \pm 111.11 | 1.53 \pm 0.24 | — | | | | |
| OR V IC | 0.71 \pm 0.09 | 1.29 \pm 0.08 a | 46.83 \pm 4.07 ab | 62.50 \pm 3.99 a | 23.09 \pm 3.04 | 32.02 \pm 3.04 ab | 54.83 \pm 5.76 | 62.42 \pm 3.07 b | 712.14 \pm 98.53 | 603.81 \pm 159.35 | 1.36 \pm 0.17 | — | | | | |
| <i>P</i> -value ¹ | NS | 3.49 $\times 10^{-5}$ *** | 2.49 $\times 10^{-3}$ ** | 4.53 $\times 10^{-6}$ *** | NS | 1.35 $\times 10^{-2}$ * | NS | 5.55 $\times 10^{-5}$ *** | NS | NS | NS | NS | | | | |

Means within each column and experimental year with different letters are significantly different ($P<0.05$)

NS: Means were not significantly different

***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$)

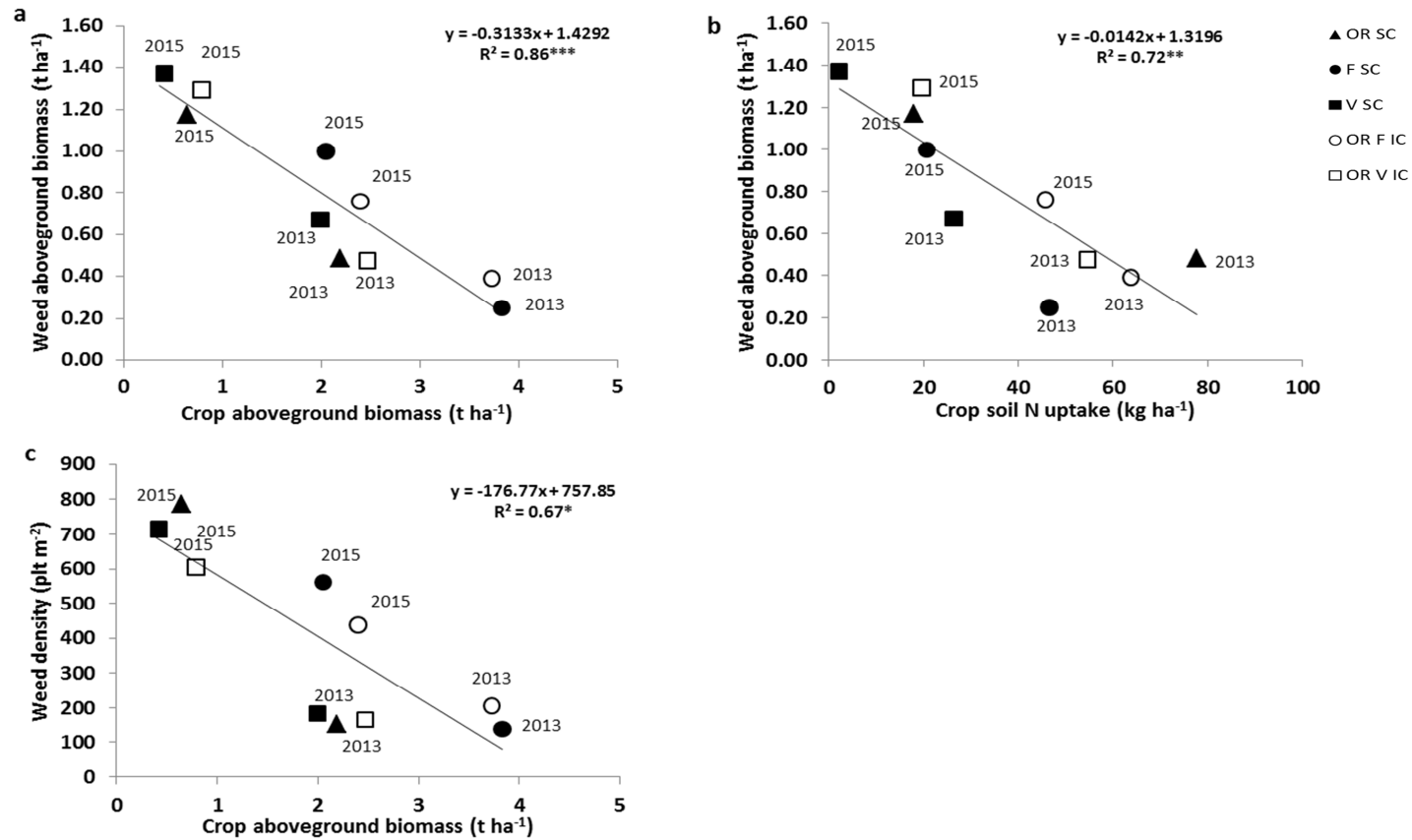


Figure 6.3. Weed aboveground biomass ($t\ ha^{-1}$) as a function of the crop aboveground biomass (a) and crop soil N uptake (b), and weed density as a function of crop aboveground biomass (c) for all treatments. Linear regression was carried out for the combined two years and included both the sole and intercropped treatments after winter in February. “*”, “**”, “***” indicate that a regression is significant at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively, according to the table proposed by Fisher and Yates (1938).

6.3.4. Competitive abilities profiles

The differences between the treatments in competitive abilities against weeds after winter were represented with radar plots (Fig. 6.4). Faba bean and oilseed rape displayed large differences concerning aboveground traits. Faba bean had a greater crop height and crop biomass than oilseed rape. Moreover, despite its lower aboveground growth, oilseed rape had a high ability to uptake soil N. The complementarity between faba bean and oilseed rape increased crop growth and soil N uptake in the intercrops. Intercropping faba bean with oilseed rape was efficient in reducing weeds compared to the oilseed rape sole crop especially in 2015/16.

Common vetch had reduced values for aboveground traits compared to faba bean each year, and these values were close to those of oilseed rape. Moreover, common vetch had low soil N uptake ability. Thus, common vetch had a lower competitive ability against weeds. Intercropping common vetch with oilseed rape was not able to reduce weeds compared to the oilseed rape sole crop.

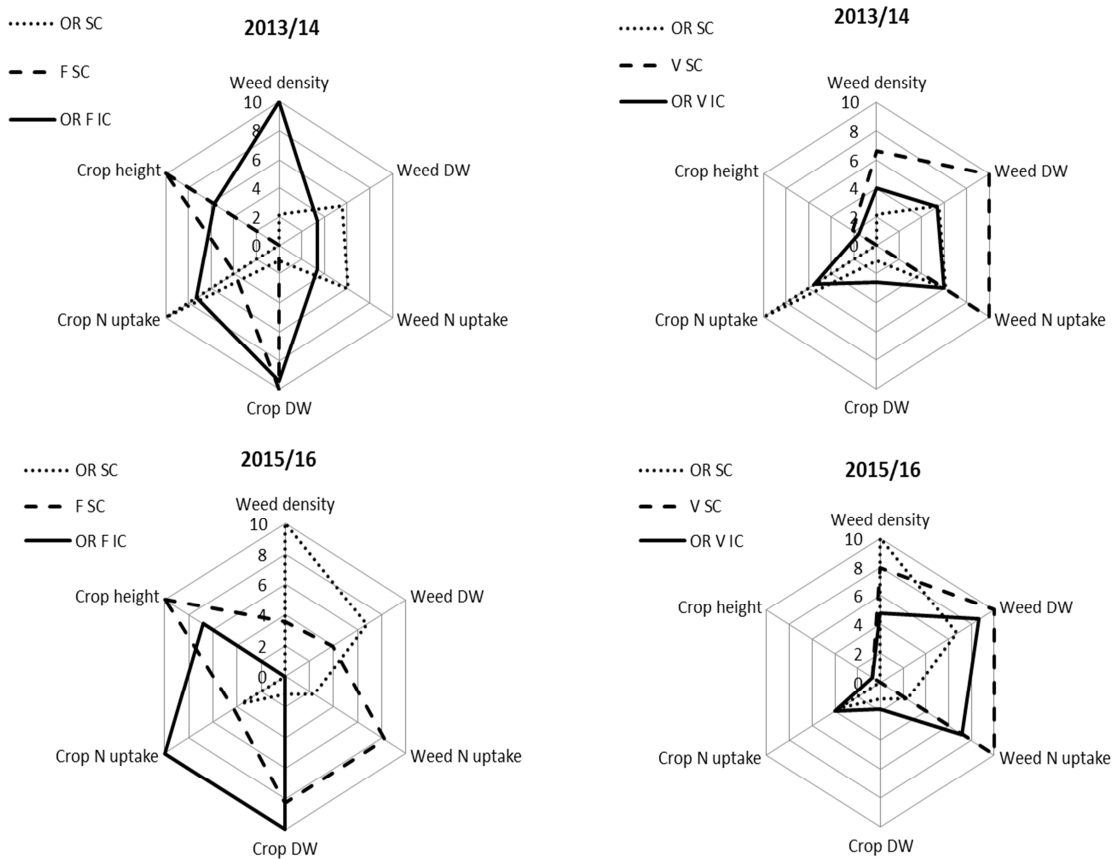


Figure 6.4. Comparison between the sole crops and intercropping simultaneously for their studied traits and their effects on weed suppression after winter in February by using radar plots for each experimental year in 2013/14 and 2015/16. Oilseed rape sole crop (OR SC), faba bean sole crop (F SC), common vetch sole crop (V SC), and oilseed rape intercropped with faba bean (OR F IC) or common vetch (OR V IC). Weed biomass (%) and weed N (%) indicate the proportion of weed aboveground biomass and weed aboveground N in the total (weeds + crops), respectively.

6.3.5. Weed diversity and species richness

Species diversity (H') of the weed communities was low and was not influenced by crop treatment regardless of the sampling date (Table 6.6). The number of weed species (S) was similar under the different treatments. Weed species evenness (D) values were close to 1 regardless of the sampling date and treatment, which indicated that weed species were equally distributed in the population during the two experimental years.

Table 6.6. The Shannon-Wiener index (H'), species richness (S) and the Simpson evenness index (D) corresponding to the treatments of oilseed rape (OR), faba bean (F) and common vetch (V) grown as sole crops (SC) or as intercrops (IC) carried out using the means ($n=4$) \pm standard error in 2013/14 and 2015/16.

| Treatment | Before winter | | | After winter | | |
|-----------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|
| | H' | S | D | H' | S | D |
| OR SC | 1.52 \pm 0.13 | 16.25 \pm 0.93 | 0.65 \pm 0.05 | 1.55 \pm 0.19 | 14.25 \pm 1.06 | 0.65 \pm 0.06 |
| F SC | 1.70 \pm 0.18 | 17.50 \pm 0.56 | 0.68 \pm 0.05 | 1.51 \pm 0.12 | 13.25 \pm 0.82 | 0.66 \pm 0.05 |
| V SC | 1.66 \pm 0.20 | 16.63 \pm 1.13 | 0.68 \pm 0.07 | 1.92 \pm 0.46 | 15.13 \pm 1.08 | 0.65 \pm 0.06 |
| OR F IC | 1.64 \pm 0.19 | 16.38 \pm 0.73 | 0.65 \pm 0.06 | 1.38 \pm 0.15 | 14.13 \pm 0.91 | 0.64 \pm 0.05 |
| OR V IC | 1.66 \pm 0.19 | 15.13 \pm 1.10 | 0.69 \pm 0.07 | 2.19 \pm 0.52 | 14.63 \pm 0.88 | 0.64 \pm 0.06 |

6.3.6. Yield components of oilseed rape

The grain yield of the oilseed rape sole crop reached 1.82 and 1.57 t ha⁻¹ in 2013/14 and 2015/16, respectively (Table 6.7). Although the planting density of oilseed rape in the intercrops was half of the planting density in the sole crop, similar grain yield were obtained in the intercrops and sole crops in both 2013/14 and 2015/16. The number of grains per square metre and weight per 1000 grains were also similar in the intercrops and sole crops. Intercropping increased the number of grains and yield per plant. In 2015/2016, the number of grains per plant and seed yield increased by 3 times in the intercrop compared to the oilseed rape sole crop.

Table 6.7. The grain yield components of oilseed rape grown as a sole crop (OR SC) or intercropped with faba bean (OR F IC) or common vetch (OR V IC) in 2013/14 and 2015/16. Values are mean ($n=3$) \pm standard error.

| Treatment | plant m ⁻² | Grain yield (t ha ⁻¹) | Grain yield (g plt ⁻¹) | Weight per 1000 seed (g) | Number of grains m ⁻² | Number of grains per plant |
|------------------------------|------------------------------------|--------------------------------------|---------------------------------------|-----------------------------|----------------------------------|----------------------------------|
| 2013/14 | | | | | | |
| OR SC | 37.14 \pm 0.45 a | 1.82 \pm 0.19 | 4.89 \pm 0.51 | 4.40 \pm 0.05 | 41186.99 \pm 3937.55 | 1109.06 \pm 105.83 |
| OR F IC | 15.24 \pm 1.62 b | 1.17 \pm 0.33 | 8.08 \pm 2.82 | 4.66 \pm 0.10 | 25371.09 \pm 7566.53 | 1771.05 \pm 654.21 |
| OR V IC | 20.63 \pm 1.44 b | 1.51 \pm 0.18 | 7.6 \pm 1.41 | 4.58 \pm 0.16 | 32747.42 \pm 2959.81 | 1632.20 \pm 245.75 |
| <i>P</i> -value ¹ | 2.76 \times 10 ⁻³ *** | NS | NS | NS | NS | NS |
| 2015/16 | | | | | | |
| OR SC | 49.52 \pm 1.56 a | 1.57 \pm 0.45 | 3.24 \pm 0.99 b | 3.41 \pm 0.06 | 45649.03 \pm 12620.40 | 939.72 \pm 275.48 b |
| OR F IC | 20.95 \pm 0.78 b | 1.97 \pm 0.16 | 9.39 \pm 0.54 a | 3.42 \pm 0.05 | 57514.60 \pm 3998.53 | 2740.56 \pm 137.66 a |
| OR V IC | 23.17 \pm 1.13 b | 1.47 \pm 0.23 | 6.28 \pm 0.81 ab | 3.34 \pm 0.05 | 43864.49 \pm 6348.88 | 1874.13 \pm 225.20 ab |
| <i>P</i> -value ¹ | 1.76 \times 10 ⁻⁴ *** | NS | 4.92 \times 10 ⁻² * | NS | NS | 3.96 \times 10 ⁻² * |

Means within each column and experimental year with different letters are significantly different ($P<0.05$)

NS: Means were not significantly different

***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$)

6.4. Discussion

Annual intercrops of cereal-legumes have been reported in previous studies to be more productive and more able to suppress weeds than comparable sole crops (Begna et al., 2011; Corre-Hellou et al., 2011; Gronle et al., 2014; Hauggaard-Nielsen et al., 2001b; Pridham and Entz, 2008; Szumigalski and Van Acker, 2005). However, there is little information regarding the intercropping of brassica and legume species and its impact on weed suppression.

This work demonstrates that the oilseed rape-legume intercrop is a relevant strategy when trying to reduce weed infestation and favour crop productivity, especially under high weed infestations. We observed that the effects on weed suppression differed greatly as a function of legume species and interspecific complementarity that resulted from contrasting traits between oilseed rape and legumes.

Intercropping effects on weed suppression in relation to crop functional traits

Oilseed rape-faba bean intercrops tended to suppress weeds greater than oilseed rape-common vetch intercrops. The greater competitiveness of intercropping against weeds notably appeared after winter for each experimental year. Oilseed rape-faba bean intercropping reduced weed aboveground biomass by 35% compared to the oilseed rape sole crop under high weed infestations (2015/16). Moreover, the proportion of N accumulated by weeds in the total aboveground N in weeds and crops was reduced by 60% in the oilseed rape-faba bean intercrops compared to the oilseed rape sole crop. The main factor explaining these effects on weed infestation seems to be the interspecific complementarity between oilseed rape and faba bean. Indeed, faba bean showed morphological traits that contributed to a high competitive ability for light, which included greater aboveground biomass and plant height. Moreover, oilseed rape produced less aboveground biomass and shorter plants than faba bean, but it showed a high competitive ability for soil N uptake. This variability between oilseed rape and faba bean traits contributed to achieving the complementary use of growth resources when intercropped. Thus, reduced amounts of light and soil N may have been available for associated weeds.

Unlike the oilseed rape-faba bean intercrop, the oilseed rape-common vetch intercrop was less efficient at reducing weed parameters for both experimental years. Common vetch displayed a very low aboveground biomass and soil N uptake compared to faba bean. Moreover, common vetch had similar aboveground traits as oilseed rape, but oilseed rape was more competitive for soil N. As a result, more light and soil N were probably available for weeds, and the level of complementarity for sharing resources between oilseed rape and common vetch was not enough to reduce weeds.

Previous studies have shown that the effects of oilseed rape-legume intercrops on weed suppression differ as a function of legume species and plant densities. Lorin et al. (2015) found differences between legume species intercropped with oilseed rape in relation with differences in aboveground biomass. A larger amount of total biomass (oilseed rape + legume) was associated with a larger

decrease in weed abundance (Lorin et al., 2015). Gharineh and Telavat (2009) observed high weed suppression with an oilseed rape-faba bean intercrop, particularly with high densities of faba bean. In these studies, competition for light was assumed to be the main driver of competitive ability for such intercrops against weeds and the traits of both components (oilseed rape and legumes) explaining the competitiveness of intercrops against weeds were not investigated. Our work reveals that competition and complementarity for soil N may be a key point in the interactions among legumes, non-legume plants and weeds. Since aboveground traits are usually more investigated than belowground, we suggest that the identification of the main root traits involved in soil N acquisition should be also investigated in future studies.

Factors influencing the competitiveness of the intercrops against weeds compared to the sole crops

The weed infestation level was greater in 2015/16 compared to 2013/14. In 2013/14, after winter, the oilseed rape sole crop accumulated a greater biomass (2.19 t ha^{-1}) and was able to take up more soil N (77.65 kg ha^{-1}) than all the other treatments (Fig. 6.3). Therefore, the oilseed rape sole crop was a greater competitor against weeds, and the presence of legumes intercropped with oilseed rape did not improve weed control. Oilseed rape developed slowly in 2015/16 and had less aboveground biomass (0.65 t ha^{-1}) and a weak ability to uptake soil N (17.87 kg ha^{-1}) after winter, and thus, it provided low weed suppression. In this year, the presence of faba bean with oilseed rape increased aboveground biomass to 2.40 t ha^{-1} and soil N uptake to 45.96 kg ha^{-1} , thus contributing to a reduction in weed biomass by 35% compared to the oilseed rape sole crop. In our experimental conditions, oilseed rape was able to suppress weeds alone once it reaches a threshold of 2 t ha^{-1} of aboveground biomass and 80 kg ha^{-1} of soil N uptake. Our relationships between aboveground biomass and weed biomass are consistent with previous studies. A previous work showed effective competition against weeds when aboveground biomass of both intercropped plants reached a threshold of 2 t ha^{-1} in November (Cadoux et al., 2015). Additionally, Valantin-Morison and Meynard (2008) showed that when an oilseed rape sole crop was able to accumulate 100 kg ha^{-1} of soil N in the early winter, it was highly competitive against weeds. In low input systems, as in our study, it is difficult to reach these thresholds in sole crops. Oilseed rape-legume intercropping may be a relevant practice to attain the sufficient amount of aboveground biomass and N uptake in order to ensure weed suppression in low input systems under high weed pressure.

Effects on weed density and weed species diversity

We did not observe a reduction of weed density in the intercrops compared to the sole crops, as previously observed by Szumigalski and Van Acker (2005) in an oilseed rape-pea intercrop. Weed biomass was more influenced than weed density as a function of crop treatment (Fig. 6.3).

Nevertheless, other studies also found negative effects of intercropping on weed density (Lorin et al., 2015; Teasdale, 1996).

Weed species diversity (H') was low due to the dominance of one species over other species and was not affected by the presence of legumes because the same species number was observed in all treatments. Similarly, Saucke and Ackermann (2006) observed no changes in weed species diversity between treatments; species numbers were identical in the sole crops and the false flax-pea intercropping treatments. In another study in Argentina, it was found that barley-pea intercrops displayed a lower weed species diversity compared to a pea sole crop but a similar diversity as barley sole crops, and weed species diversity was found more stable across the experiments in intercrops compared to sole crops (Poggio, 2005).

Effects of intercropping on crop growth and productivity

Intercropping improved and facilitated the growth of oilseed rape by increasing its height, aboveground biomass and leaf area and its ability to uptake soil N compared to an oilseed rape sole crop. This may be related to the interspecific complementarity for resource use that resulted from the differences between crops traits. The positive effects of intercropping on oilseed rape performance had already been shown in some previous studies. Intercropped oilseed rape accumulated a 20% greater amount of N per plant than sole crops, but the net N transfer from faba bean to oilseed rape in autumn was found to be negligible (Jamont et al., 2013). Pea increased the ability of oilseed rape to uptake soil N in an intercropping replacement design (Andersen et al., 2005). Andersen et al. (2005) was also found that soil N availability for oilseed rape was greater in intercropping.

All studied intercrops displayed land equivalent ratio values close to or exceeding 1 for both aboveground biomass and soil N uptake, indicating a complementary use of growth resources before and after winter. However, the evaluation of intercropping performance according to the LER values showed a greater benefit of intercropping over sole cropping under oilseed rape-faba bean than under oilseed rape-common vetch intercrops. Andersen et al. (2005) observed that the LER values of aboveground biomass in intercropped pea-oilseed rape varied from 1.32 to 1.16 at low and high fertilization levels, respectively, in a replacement design, indicating the abilities of legume and non-legume plants to exploit different N pools leading to yield advantages over sole crops especially in low nitrogen input systems. When mustard was intercropped with pea, lentil or chickpea in a replacement design, all intercrops reached LER values more than 1, except in mustard-chickpea intercrops (Banik et al., 2000). The LER values were affected negatively by increasing the N fertilization rate in oilseed rape-pea intercrops (Andersen et al., 2005) and mustard-pea intercrops (Waterer et al., 1994). A high amount of soil N at sowing (approximately 100 kg ha^{-1} , as in our study) contributed to increasing the competitive ability of oilseed rape against weeds (Valantin-Morison and Meynard, 2008). However, in intercropping, this level of soil N availability can decrease or delay complementary effects.

In our study, oilseed rape produced similar grain yield per hectare in both sole crops and intercrops for both experimental years. Moreover, in 2015/16, grain yield (g per plant) and the number of seeds produced per plant were significantly greater in intercrops with faba bean than in sole crops. Oilseed rape can demonstrate high plasticity for yield build-up in replacement intercrops when sown at half density. Banik et al. (2000) observed that mustard-pea intercrops performed better than (mustard-lentil) and (mustard-chickpea) intercrops, where mustard (*B. campestris*) had the highest yield (1 t ha⁻¹) compared to mustard sole crops (0.70 t ha⁻¹) in a replacement design. In our work, because of the absence of weed control, the grain yield of oilseed rape (1.59 t ha⁻¹) was low compared to the average yield of oilseed rape in France (4 t ha⁻¹). However, Valantin-Morison and Meynard (2008) obtained similar grain yields (1.19 t ha⁻¹ on average) in organic oilseed rape sole crops where similar weed pressures were observed.

Effects of intercropping design and crop management

The effects of intercropping on weed suppression and productivity depend also on crop management such as sowing date. In such intercrops, the legumes were sown at a different time than their recommended sowing times in sole crops with potential negative impacts on their development and sensitivity to pests and disease. In our study, the early sowing of common vetch (one month before the recommended sowing time in a sole crop) increased its sensitivity to fungal diseases (rust), especially in 2015/16 (data not shown). Our study focused on species traits, but it would also be interesting to study different cultivars within a species with a similar approach but also included specific criteria in relation to potential limiting factors in such systems (e.g. sensitivity to disease, sensitivity to frost). Other studies also displayed interest in using legume mixtures intercropped with oilseed rape (Cadoux et al., 2015; Lorin et al., 2015). The choice of contrasted legumes concerning competition for light and soil N uptake could further improve weed suppression ability. Our results highlighted the advantages of a replacement design regarding weed suppression and favouring the interspecific complementarity between intercropped species associated with positive effects on oilseed rape performance. It would be interesting to compare in a same study replacement and additive designs and to compare intercropping effects with a control treatment with herbicides (non-selective to legumes) in various weed pressure conditions. In our study, in intercrops, only the oilseed rape was harvested, whereas legumes were considered as frost-sensitive legumes even if the temperature was not low enough to completely kill the legumes in both years. However, other studies indicated the possibility of harvesting the two species and having an additional benefit of intercropping (Andersen et al., 2005).

6.5. Conclusions

Our study demonstrates that oilseed rape-legume intercrops is a relevant strategy when trying to reduce weed infestation and favour oilseed rape productivity, especially under high weed infestations. We observed that the effects on weed suppression differed greatly as a function of legume species and interspecific complementarity that resulted from contrasted traits between oilseed rape and legumes. Our results also highlighted the advantages of a replacement design compared to a sole crop regarding weed suppression and favouring the interspecific complementarity between intercropped species associated with the positive effects on oilseed rape performance under a low input cropping system. The combination of intercropped species with contrasted traits appears to be a key factor for the success of intercropping in order to improve both weed suppression and crop productivity.

Acknowledgements

We gratefully acknowledge the technical staff of the research unit of LEVA (Légumineuses, Ecophysiologie Végétale, Agroécologie) for their excellent technical assistance. We are most grateful to the PLATIN' (Plateau d'Isotopie de Normandie) core facility for all element and isotope analyses used in this study. This work was funded by CASDAR n°5376 (Compte d'Affectation Spéciale pour le Développement Agricole et Rural) and the Syrian government.

Dans le chapitre 4, nous avons exploré un autre type d'association à l'échelle pluriannuelle au champ en production de maïs semence afin de montrer à nouveau l'effet sur des adventices et la composition de la flore adventice. Cette expérimentation teste différents couverts de légumineuses et de graminées. En plus de l'effet sur les adventices et le rendement de maïs, un résultat marquant concerne l'impact sur la flore adventice en fonction de la disponibilité en azote minéral dans le sol qui varie selon les couverts.

**7. Chapitre 4. Weed suppression and changes in
species composition of weed communities
occurring in no-tillage seed maize with living or
dead mulches**

Ce chapitre correspond à un article en préparation pour le journal *Crop Protection*

Weed suppression and changes in species composition of weed communities occurring in no-tillage seed maize with living or dead mulches

Abstract

Introducing legume and grass cover crops as living or dead mulch before and throughout maize growing season could be a strategy for weed management while maintaining maize productivity. A 2-year field experiment was carried out in Western France to assess the effect of cover crops managed as living or dead mulch on weed control (density and biomass), on weed species composition and yield of a seed maize crop. Two legume cover crops, alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.), two grasses, oat (*Avena sativa* L.) and English ryegrass (*Lolium perenne* L.), and a mixture of English ryegrass-white clover were sown in autumn 2012 and mowed or killed by glyphosate in May in both 2013 and 2014. A no cover treatment with maize was conducted with similar cultural practices. The effect of cover crops on weed density, weed biomass and weed composition began early even before maize sowing, grasses were more competitive against weeds than legumes cover crops. Although all living cover crops were able to reduce weed density and biomass (on average by 89%) compared to no cover treatment, differences were found among them. Living alfalfa had the least weed suppression ability and the highest species diversity of weed community, species richness and species evenness. Conversely, white clover, ryegrass and their mixture showed a strong competitive ability against weeds. Legume living mulches were more favorable for high nitrophilic weed species than for low ones whereas ryegrass was able to better control them. A high and negative correlation was observed between the percentage of low nitrophilic weed species in weed community and soil N availability. Dead mulches were able to keep on a low weed biomass under a threshold of 2 t ha⁻¹ at maize flowering. Dead mulch cover crops did not affect weed composition with a preference for high nitrophilic weed species. Maize grain yield under dead mulch was higher than that obtained under living mulch; the highest yield reached 6.20 t ha⁻¹ with dead alfalfa, twice more than that obtained with living alfalfa, and similar to that obtained in conventional maize (cultivated with herbicides). The approach of using cover crops in seed maize could be a relevant strategy in order to control weeds providing a good management of cover crops in order to mitigate the interference between maize and cover.

Keywords: Living mulch, dead mulch, seed maize, weed suppression, weed diversity

7.1. Introduction

The loss potential of maize yield due to weeds can reach 40 % worldwide (Oerke, 2006). France is the largest exporter country of seed maize worldwide with a cultivated surface reached at 69800 hectares in 2014/2015 (Source: GNIS - Eurostat - FNPSMS - 2014). However, farmers rely intensively on herbicide applications for weed control in such systems based mainly on seed maize monoculture. The use of herbicides has long protected crop yield satisfactorily. However, shifts to herbicides-resistant weed species are predicted to occur in continuous cropping systems worldwide. In Europe and USA, maize have selected 58 resistant weed species due to the high herbicide use over the large area during long period in simplified cropping systems (Heap, 2014). For example, the occurrence of glyphosate-resistant weeds in maize systems are becoming important particularly in *Amaranthus* spp. and *Ambrosia* spp. (Heap, 2014; Shaner, 2000).

Generally, the beginning of the critical period for weed control varied from 3 to 14-leaf stage of maize development (Hall et al., 1992). Thus, it is necessary to focus on weed suppression during this period before weeds affect maize yield.

As a result to this shift in herbicides-resistant weeds, more integrated approaches are required to manage weed species including high biomass cover crops in order to reduce weed establishment for a long-term and thus to minimize herbicide applications (Blackshaw et al., 2007; Webster et al., 2013).

One of the options recommended might be the insertion of different living cover crops in maize in order to increase traits diversity and to provide several benefits for both maize productivity and weed suppression. Indeed, living cover crops suppress weeds by disturbing weed emergence and competing for resources. Moreover, their decaying residues may also inhibit weeds through physical, biotic and allelopathic interactions (Hartwig and Ammon, 2002; Weston, 1996).

However, a successful living mulch must simultaneously provide a maximal weed control and a minimal competition with maize for water, light, and nutrients with a constant and sufficient of nitrogen supply in the case of legumes living mulch (Enache and Ilnicki, 1990).

Although legume species are less competitive than grasses against weeds, they are very recommended as cover crops thanks to their advantages including economizing of nitrogen fertilization, minimizing soil erosion, maintaining soil organic matter, improving soil structure and suppressing weeds (Fisk et al., 2001; Sarrantonio and Gallandt, 2003; Teasdale et al., 1991), thereby enhancing crop productivity.

The effects on weed density and biomass could differ as a function of cover crop species in relation with morphological and physiological traits among species and even among cultivars. White clover was more competitive with weeds than subterranean clover when introducing as living mulch in organic winter wheat (Hiltbrunner et al., 2007a). In addition, hairy vetch was more effective in weed suppression than different clover species in organic farms (Uchino et al., 2011). Abdin et al. (2000) observed that a living mulch of alfalfa and a mixture of white clover and English ryegrass were able to

reduce weed biomass by 75% and 62%, respectively compared to unweeded control at maize flowering stage (Abdin et al., 2000). A previous study showed that white clover living mulch was able to reduce weed biomass by 50% in wheat in the second year when it was well established (Thorsted et al., 2006).

In addition, cultural practices to manage the cover crops could also influence weed suppression. Indeed, the mowing practices could contribute to reduce annual weed species when realized before the dispersion of seeds and to deplete root reserves in perennial weed species (Schneider and Huyghe, 2015). A previous study observed that when maize was sown with alfalfa, the latter was able to reduce weed fresh weight by 44% when it was clipped only once at early flowering stage (Ghosheh et al., 2005).

Cover crops in the form of dead mulch residues also can influence weed populations in no-tillage cropping systems because of the proximity of the residues to the site of seed germination on the soil surface (Teasdale et al., 1991). In no-tillage maize, killed hairy vetch with paraquat before maize sowing reduced weed biomass and density by 44% and 46%, respectively compared to no vetch treatment (Reddy and Koger, 2004). Also, the reduction in weed density by hairy vetch killed by paraquat was 75% at 400 g m⁻² of residue weight and this reduction differed according to weed species compared to no cover treatments (Teasdale et al., 1991).

Moreover, some legume species residues have been shown to release allelopathic compounds that suppress weed species germination and growth (Chang et al., 1969; Dyck and Liebman, 1994; Ohno and Doolan, 2001; White et al., 1989). Some grass cover crops as oat (*Avena sativa* L.) contain allelopathic compounds that inhibit weed germination and weed emergence (Belz, 2007; Weston, 1996).

However, living cover crops have greater weed suppression capacity than dead ones but can be quite competitive with crops (Sarrantonio and Gallandt, 2003).

Cover crops can reduce weed biomass and density in different cropping systems. However, little information is known about the influence of cover crops on weed species composition. Cover crops species had contrasting morphological or physiological traits. For example, legume species produce lower aboveground biomass associated with a low soil N demand than grass cover crops (Thorup-Kristensen, 1994). Many weed species are high consumers of nitrogen (Qasem, 1992), and the growth of many of them is enhanced by higher soil nitrogen levels (Supasilapa et al., 1992). However, weed species differ in their response to soil N availability (Blackshaw et al., 2003; Ellenberg, 1974; Moreau et al., 2013). Thus, weed species composition could be modified as a function of the ability of cover crop species to deplete soil N. However, the influence of living or dead mulches, obtained from various cover crop species in maize, on weed composition in relation to weeds and cover crops traits has not yet investigated.

Our experiment tests different species of cover crops (legumes and grasses) in the form of living mulch and dead mulch in order to improve weed control in seed maize.

The aim of our study is to investigate i) the competitive ability of cover crops (English ryegrass, white clover, mixture of ryegrass/clover, alfalfa and oat) managed as living mulch or dead mulch against weeds ii) the modification in weed species composition by different cover crops and iii) the effect of cover crops on maize growth and yield compared to a control without cover crops. To test these objectives, one experiment was carried out in Western France during two years in no-tillage seed maize.

7.2. Materials and Methods

7.2.1. Field site description, experimental design and treatments

A field experiment was established in the autumn of 2012 and carried out from October 2012 to October 2014 at Saint-Mathurin-sur-Loire (47.5°N, 0.6°W) in western France. The average annual temperature and precipitation are 11.2 °C and 565 mm (1993–2013), respectively. The weather conditions during the experiment are presented in Table 7.1. The soil characteristics from 0 to 90 cm of soil depth are presented in Table 7.2. The initial nitrogen content was 72 kg h⁻¹ KCl-extractable inorganic N at 0-90 cm soil depth before cover crops sowing in November 2012. Previous crops were a seed maize in 2008, 2009, 2011 and 2012, shallots in 2010 and orchards in 2007. The field was ploughed and harrowed in October 2012 before cover crops sowing.

Table 7.1. Mean air temperature and amount of precipitation during the experimental years from November 2012 to November 2014

| Month | 2012 | | 2013 | | 2014 | |
|--------------|----------------------|--------------------|----------------------|--------------------|----------------------|--------------------|
| | Air temperature (°C) | Precipitation (mm) | Air temperature (°C) | Precipitation (mm) | Air temperature (°C) | Precipitation (mm) |
| | Average | Total | Average | Total | Average | Total |
| January | | | 4.9 | 66.8 | 7.4 | 105.0 |
| February | | | 3.8 | 70.8 | 7.8 | 102.0 |
| March | | | 6.2 | 54.0 | 8.9 | 26.2 |
| April | | | 9.9 | 66.0 | 11.6 | 40.0 |
| May | | | 11.9 | 85.9 | 13.6 | 79.6 |
| June | | | 16.6 | 34.6 | 18.1 | 27.2 |
| July | | | 21.8 | 43.6 | 20.1 | 49.8 |
| August | | | 19.2 | 11.8 | 17.6 | 96.4 |
| September | | | 16.6 | 41.0 | 17.9 | 8.6 |
| October | | | 14.2 | 75.4 | 14.0 | 47.0 |
| November | 7.6 | 34.2 | 7.8 | 91.2 | 10.4 | 60.2 |
| December | 7.3 | 18.8 | 5.3 | 76.0 | | |
| Total | 483 ¹ | 53 | 4082 ¹ | 717 | 4517 ¹ | 642 |

¹Sum of degree-days at base temperature = 0°C

Table 7.2. Characteristics of the soil (0 to 90 cm) at cover crop sowing in November 2012

| Soil depth | Soil type | Organic matter (%) | PH (KCL) |
|------------|-----------------|--------------------|----------|
| 0-30 cm | sandy clay loam | 1.8 | 6.3 |
| 30-60 cm | sandy clay loam | 1.5 | 6.3 |
| 60-90 cm | silty clay | <1.4 | 6.8 |

Prior to cover crops sowing, the soil was ploughed and harrowed in October 2012. Four cover crops were sown in November 2012. Cultivars and seeding rates were shown in Table 7.3. Due to the low establishment of alfalfa, it was over seeded (10 kg ha^{-1}) in March/2013. White clover (*Trifolium repense* L.), alfalfa (*Medicago sativa* L.), English ryegrass, oat (*Avena sativa* L.) and a mixture of ryegrass and white clover were compared. The annual oat (*A.sativa* L.) cover crop was sown again in January 2014 with the same density than in 2012.

In addition to conventional seed maize, a control without cover crops was conducted and managed in the same way as cover crop treatments but the soil of the control was harrowed also in April 2013.

Table 7.3. Characteristics of the cover crops sown in November 2012

| Common name | Latin name | Cultivar | Seeding rate (kg ha^{-1}) | Life cycle |
|--------------------------|----------------------------|----------------|---|------------|
| English ryegrass | <i>Lolium perenne</i> L. | Aberavon | 20 | Perennial |
| White clover | <i>Trifolium repens</i> L. | Alice | 20 | Perennial |
| Alfalfa | <i>Medicago sativa</i> L. | Cannelle | 12 | Perennial |
| Oat | <i>Avena sativa</i> L. | Une de mai | 80 | Annual |
| Ryegrass/ clover mixture | | Aberavon/Alice | 10/10 | Perennial |

7.2.2. Cultural practices

After the establishment during winter, cover crops were managed differently at maize sowing date in order to create two different systems in both experimental years 2013 and 2014. The first system was the living mulch system where the five cover crops were maintained but mowed twice two weeks after maize sowing in order to mitigate the interference with maize seedlings. The second system received an application of glyphosate (Roundup Max plus :450 g/l of acid glyphosate) at maize sowing date, 2 l ha^{-1} and 5.4 l ha^{-1} in 2013 and 2014, respectively, in order to kill the cover crops and establish a dead mulch system. The plot size was $8 \text{ m} \times 138 \text{ m}$ and $114 \text{ m} \times 8 \text{ m}$ for living and dead treatments, respectively. The experiment design was a strip design with three replications.

Maize was sown without tillage directly into living or killed cover crops in 2013. The particularity of seed maize is the sowing of two different cultivars (female and male) at different sowing dates. In 2013, the hybrid maize (cv. 970) was used at a planting density of 9600 and 7500 plant ha^{-1} for female (cv.DL26) and male (cv.LIMRFQ939), respectively.

In 2014, the rows were prepared by achieving a strip-till on 25 cm in depth and rototilled (15 cm depth, 40 cm width) prior to maize planting. The rototilled strip-till was achieved twice on 18 December 2013 and the second time on 07 May 2014. After that, maize was sown (cv.891) at a planting density of 10200 and 5400 plant ha^{-1} for female (cv. 224E772) and male (cv.151X716),

respectively. The row spacing was 75 cm. Hoeing practices were achieved twice for living mulch system and once for dead mulch system in the first week of June. In addition, a dose of 1 l ha⁻¹ of Auxo (tembotrione and bromoxynil) was applied precisely on the rows in living mulch system and on the whole field in dead mulch system at the beginning of July in 2014.

There was no fertilization during winter cover crops while the summer maize was fertilized. Maize female were undersown on mid-May with a fertilization starter (15kg of micro granules) applied on the rows only in 2014. The nitrogen fertilisation was conducted in 2013 by applying 100 kg ha⁻¹ at maize sowing and 133 kg ha⁻¹ the end of June for all treatments regardless of the system.

In 2014, a dose of 100 kg ha⁻¹ was added for the systems at maize sowing. After that, a dose of 80 and 60 kg ha⁻¹ nitrogen in the form of ammonitrate was added and incorporated in the soil for living and dead mulch systems, respectively, at the end of June. In addition to 40 kg ha⁻¹ was added for ryegrass in the living mulch system.

The conventional maize was ploughed and harrowed before maize sowing associated with an application of 2 l ha⁻¹ of Camix (Benoxacor, Mesotrione, S-metolachlore) and 1 l ha⁻¹ of Dual Gold (S-metolachlore) in both 2013 and 2014. In addition, three herbicides applications of Gécko (0.6 l ha⁻¹, Sulcotrione), Auxo (1 l ha⁻¹) and Banvel 4S (Dicamba 0.3 l ha⁻¹) were achieved at different dates during maize growth.

The conventional maize received 100 and 233 kg ha⁻¹ at maize sowing and at the end of June in 2013. In 2014, maize conventional received 233 and 313 kg ha⁻¹.

7.2.3. Sampling and measurements

In 2013, a first sampling (0.5 m × 0.75 m) was realized before maize sowing in order to study the cover crops and weed establishment. Cover crops height, cover crops and weed biomass and leaf area were measured. Leaf area was measured using a LI3100 area metre (LI-COR Inc., NE, USA). During maize growth in 2013, four samples were realized when maize had unfolded 3, 6, 10 leaves at the flowering stage. Only two samples were realized in 2014 at 3 unfolded leaves and at flowering stage. Maize stages were determined according to BBCH (JKI, 2010). One quadrat of 0.50 m x 0.95 m was established parallel to maize rows in each subplot. Cover crops, maize and weed species were cut at the level of soil surface, and weed species were identified and counted separately. Cover crop, maize and weed samples were dried at 70°C for 48 h in order to measure their dry weight. In addition, soil samples were collected for each sampling date and the soil inorganic N content was measured using a segmented flow analysis (SKALAR SA3000), which enables the determination of the nitrate content by KCl extraction according to the international standard ISO 14256-2.

Maize was harvested at maturity during the second week of October in 2014. Ears were harvested by hand from the two rows on area of 0.5 m × 0.75 m. Maize grain was then weighted to determine wet grain yield after which it was dried at 70°C to constant weight to determine the moisture content.

7.2.4. Weed community composition and diversity

Dicotyledons weed species were classed into four different categories as a function of weed nitrophily index (high nitrophilic ≥ 4 and low nitrophilic < 4 (Ellenberg, 1974) and stem morphology (upright or creeping stem). The categories named as follow: **A**: high nitrophilic and upright stem, **B**: low nitrophilic and upright stem, **C**: high nitrophilic and creeping stem, **D**: low nitrophilic and creeping stem, In addition, weed monocotyledons species were classed by their nitrophily into two categories **E**: high nitrophilic and monocotyledons, **F**: low nitrophilic and monocotyledons. Weed species and the categories were presented in **Appendix**.

Each category corresponds to the proportion of weed species to the total weed species density.

The species diversity of the weed community was assessed by calculating different indexes at each sampling date. Species richness (S) was the mean number of species observed per treatment (Magurran, 1988). Shannon-Wiener's indicator (H') was used to describe the diversity of the weed population using the following equation:

$$H' = -\sum(p_i * \ln p_i) \quad p_i = n_i/N$$

where n_i represents the number of observed individuals for one weed species, and N the total number of observed individuals per treatment. The value of H varied from 1 (low diversity) to 4 (high diversity).

The Simpson evenness index (D) was used as a measure of equitability of the weed population (Magurran, 1988) and was calculated using the following equation:

$$D = \sum (n_i(n_i-1) / N(N-1)),$$

D varied from 0 (a very dominant weed species) to 1 (weed species are equally distributed).

7.2.5. Statistical analysis

The statistical analysis was performed separately for each experimental year. The effect of the different cover crop treatments on weeds (density, aboveground biomass and weed species composition) was tested by a one-way analysis of variance (type III sum of squares; $\alpha = 0.05$). To test for the normality of residues and homoscedasticity, the Shapiro-Wilk and Bartlett tests were used, respectively ($\alpha = 0.05$). Means were compared using Tukey's HSD test (Honest Significant Differences; $\alpha = 0.05$). To meet the assumptions of ANOVA, data of maize biomass at flowering in 2013 was transformed using the ln transformation, whereas data of mineral N remaining in the soil at 3-leaf maize in 2014 was transformed by using $(1/x)$. All statistical analyses were performed using package R Commander in R software, version 3.1.2 (RCore, 2012).

7.3. Results

7.3.1. Cover crops and weed species establishment before maize sowing in 2013

After the establishment of cover crops during winter, differences were observed among cover crops at maize sowing for their morphological characteristics including crop height, aboveground biomass, and leaf area associated with a large variability concerning their ability to deplete soil N (Table 7.4). Indeed, oat was taller (30.6 cm) and accumulated a greater aboveground biomass (3.23 t ha⁻¹) than other cover crops. Also, it was able to deplete soil N efficiently and left only 7.44 kg ha⁻¹ of soil N. The other grass species (ryegrass) was less competitive than oat for soil N depletion. Ryegrass accumulated similar biomass as white clover but five times more aboveground biomass than alfalfa. In addition, oat, ryegrass and the mixture ryegrass-white clover had a higher ability to deplete soil N than legume cover crops. Although alfalfa and white clover had similar height and aboveground biomass, white clover showed a greater ability to deplete soil N than alfalfa. In addition, alfalfa had the lowest leaf area.

The effect of cover crops on weed density and biomass began even before maize sowing (Table 7.4). White clover had the greatest weed density (62.22 plt m⁻²) whereas oat and ryegrass showed less weed density, 6 and 14 plt m⁻², respectively. The greatest weed aboveground biomass was observed in legume cover crops, alfalfa and white clover (0.1 t ha⁻¹) whereas grass cover crops, oat and ryegrass showed a reduced weed biomass of 0.003 and 0.01 t ha⁻¹, respectively.

Differences were also observed among treatments concerning species diversity of weed communities (H'), weed species richness (S) and weed species evenness (D) from cover crops. Before maize sowing in 2013, alfalfa had the highest species diversity of weed community (H= 1.42) and the highest number of weed species (S= 6) whereas oat had the lowest ones. Moreover, weed species evenness value (D) was close to 1 in alfalfa indicating that weed species were equally distributed compared to other cover crops treatments. White clover, ryegrass and their mixture had intermediate values of the three studied parameters (Table 7.4).

At cover crops establishment, differences were observed in weed species composition as a function of cover crop species. Alfalfa showed a high weed species diversity compared to other cover crops with the presence of 4 categories (A, C, D, and E). Creeping weeds, especially high nitrophilic ones (C), were dominant with most cover crops treatments. In addition, alfalfa showed weed species in high nitrophilic categories including upright weeds (A=26%), creeping ones (C=41%) and monocotyledons (E=16%). The percentage of high nitrophilic weeds represented by A, C and E varied as a function of cover crops species and this proportion was higher in legume cover crops, alfalfa and white clover (83%) than in other treatments (on average 68%) (Table 7.5). White clover was favourable for creeping weeds (91%) more than alfalfa (58%).

Table 7.4. The characteristics of the cover crops and their effects on weed density, biomass, species diversity of weed communities (H'), weed species richness(S) and weed species evenness (D) before maize sowing in 2013

| | Oat | English ryegrass | Ryegrass-clover IC | White clover | Alfalfa | <i>P-value</i> ¹ |
|---|-----------------|------------------|--------------------|-----------------|-----------------|-----------------------------|
| Cover crop height (cm) | 30.6 ± 0.82 a | 18.7 ± 1.16 b | 15.82 ± 1.13 bc | 9.9 ± 0.9 d | 11.8 ± 1.04 cd | 1.03×10 ⁻⁰⁷ *** |
| Cover crops biomass (t ha ⁻¹) | 3.23 ± 0.3 a | 1.69 ± 0.24 b | 2.02 ± 0.22 b | 1.36 ± 0.16 bc | 0.3 ± 0.06 c | 2.01×10 ⁻⁰⁶ *** |
| Cover crops leaf area index | 2.05 ± 0.21 a | 1.25 ± 0.25 ab | 1.89 ± 0.28 a | 2.02 ± 0.29 a | 0.32 ± 0.06 b | 1.11 ×10 ⁻³ ** |
| Mineral N left in soil (kg ha ⁻¹) | 7.44 ± 0.6 d | 23.12 ± 1.12 c | 24.15 ± 1.94 c | 36.1 ± 2.96 b | 47.87 ± 4.48 a | 1.12×10 ⁻⁰⁸ *** |
| Weed density (plt m ⁻²) | 6.22 ± 1.5 b | 14.22 ± 6.85 b | 39.11 ± 11.57 ab | 62.22 ± 19.86 a | 37.78 ± 7.73 ab | 4.96×10 ⁻³ ** |
| Weed biomass (t ha ⁻¹) | 0.003 ± 0.001 b | 0.01 ± 0.004 b | 0.06 ± 0.02 ab | 0.09 ± 0.03 a | 0.1 ± 0.04 a | 4.97×10 ⁻² * |
| H' | 0.20 ± 0.12 c | 0.49 ± 0.11 bc | 0.83 ± 0.12 b | 0.69 ± 0.18 bc | 1.42 ± 0.14 a | 5.47×10 ⁻⁰⁵ *** |
| S | 1.33 ± 0.19 b | 1.50 ± 0.31 b | 2.83 ± 0.15 b | 3.00 ± 0.58 b | 5.50 ± 0.87 a | 9.28×10 ⁻⁰⁵ *** |
| D | 0.14 ± 0.08 c | 0.34 ± 0.08 bc | 0.49 ± 0.07 b | 0.39 ± 0.10 b | 0.71 ± 0.04 a | 6.34×10 ⁻⁴ *** |

Values are mean (n=6) ± standard error

Means in the same row with different letters are significantly different at $P < 0.05$

***, **, *, indicate significant differences among species at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively

¹ P -values (analysis of variance ANOVA Type III, sum of squares, $\alpha = 0.05$)

Table 7.5. The percentage of weed species categories (%) relatively to the total weed species density before maize sowing in 2013. Values are mean (n=6)

| Weed species categories | Oat | English ryegrass | Ryegrass-clover IC | White clover | Alfalfa |
|-------------------------------------|-----|------------------|--------------------|--------------|---------|
| A= high nitrophilic, upright | 0 | 7 | 0 | 4 | 26 |
| B= low nitrophilic, upright | 0 | 0 | 0 | 0 | 0 |
| C= high nitrophilic, creeping | 45 | 56 | 55 | 75 | 41 |
| D= low nitrophilic, creeping | 33 | 24 | 33 | 16 | 17 |
| E= high nitrophilic, monocotyledons | 22 | 13 | 13 | 4 | 16 |
| F= low nitrophilic, monocotyledons | 0 | 0 | 0 | 0 | 0 |

7.3.3. Living mulch growth, weed biomass and weed density during maize growth

Living mulch

After cover crops mowing in 2013, the aboveground biomass was only reduced for oat. Aboveground biomass of cover crops increased during maize growth season between sowing and flowering except for oat (Fig. 7.1, a). Differences were observed at each sampling date among cover crop biomass. At maize flowering stage, white clover and the mixture ryegrass-clover showed the greatest aboveground biomass (approximately at 6 t ha⁻¹) and twice more than that in alfalfa. At the same date, oat showed the least aboveground biomass (1.45 t ha⁻¹). White clover and the mixture ryegrass-clover kept on their great biomass (on average 4.38 t ha⁻¹), followed by ryegrass (2.03 t ha⁻¹) at maize sowing in 2014. The least aboveground biomass was observed in alfalfa and oat (on average 0.5 t ha⁻¹).

At maize flowering stage, due to particular cultural practices in 2014, all cover crops had low and stable biomass (below 2 t ha⁻¹). Owing to bad establishment of annual oat in 2014, its growth was null at this stage (Fig. 7.1, a).

English ryegrass, oat and the mixture were able to reduce soil N efficiently more than the no cover treatment at 3-leaf maize stage. English ryegrass was the sole cover crop which kept on their high ability to reduce mineral soil N whereas white clover had the lowest one at maize flowering stage. The quantity of mineral N remaining in the soil was found to be greater in 2014 than that in 2013 whatever treatment. However, ryegrass maintained its great competitive ability to deplete soil N more than all other treatments at 3-leaf maize stage. White clover had the greatest quantity of soil N (234 kg ha⁻¹) at maize flowering stage (Table 7.6).

Weeds density and aboveground biomass:

Regardless of sampling date in 2013, alfalfa allowed the greatest weed density (on average 143 plt m⁻²) and behaved as same as the no cover treatment (200 plt m⁻² on average) during maize growth (Fig. 7.1, b). However, alfalfa was more able to reduce weed biomass than no cover treatment, notably at 10-leaf stage (Fig. 7.1, c). Both white clover and the mixture ryegrass-clover had the greatest ability to reduce weed density and biomass throughout maize growth. White clover was more competitive and reduced weed biomass by 98% compared to alfalfa at flowering maize stage.

At flowering maize stage, all cover crops were able to reduce weed biomass (below 2 t ha⁻¹) compared to no cover treatment where weed biomass increased at 10 t ha⁻¹.

In 2014, no differences were observed among treatments concerning weed suppression at maize sowing date. Weed density and biomass were very low in all treatments. Weed density increased in no cover treatment and reached 1033 plt m⁻² at 3-leaf maize stage while weed density was below 200 plt m⁻² with cover crops. However, all cover crops reduced both weed biomass and density compared to no cover treatment at maize flowering stage. The effect of cover crops on weed suppression differed as

a function of cover crop species. Indeed, oat had the least competitive ability to reduce weed density (215 plt m⁻²) and weed biomass (2.73 t ha⁻¹). Moreover, white clover was more competitive for reducing weed density (5.6 plt m⁻²) than alfalfa (72.28 plt m⁻²) but the two species showed similar ability to reduce weed biomass at maize flowering stage (Fig. 7.1 b, c).

Weed biomass was highly correlated with the combined biomass of cover crops and maize. A threshold of 3 t ha⁻¹ of cover crops was adequate to reduce greatly weed biomass. In 2013, cover crop biomass was higher than in 2014. Moreover, cover crop biomass was very high in 2013 and reached 6 t ha⁻¹ leading to a high weed suppression. In 2014, cover crops biomass was low but was compensated by a high maize biomass and the combined biomass (maize + cover) reached 5 t ha⁻¹ (Fig. 7.2).

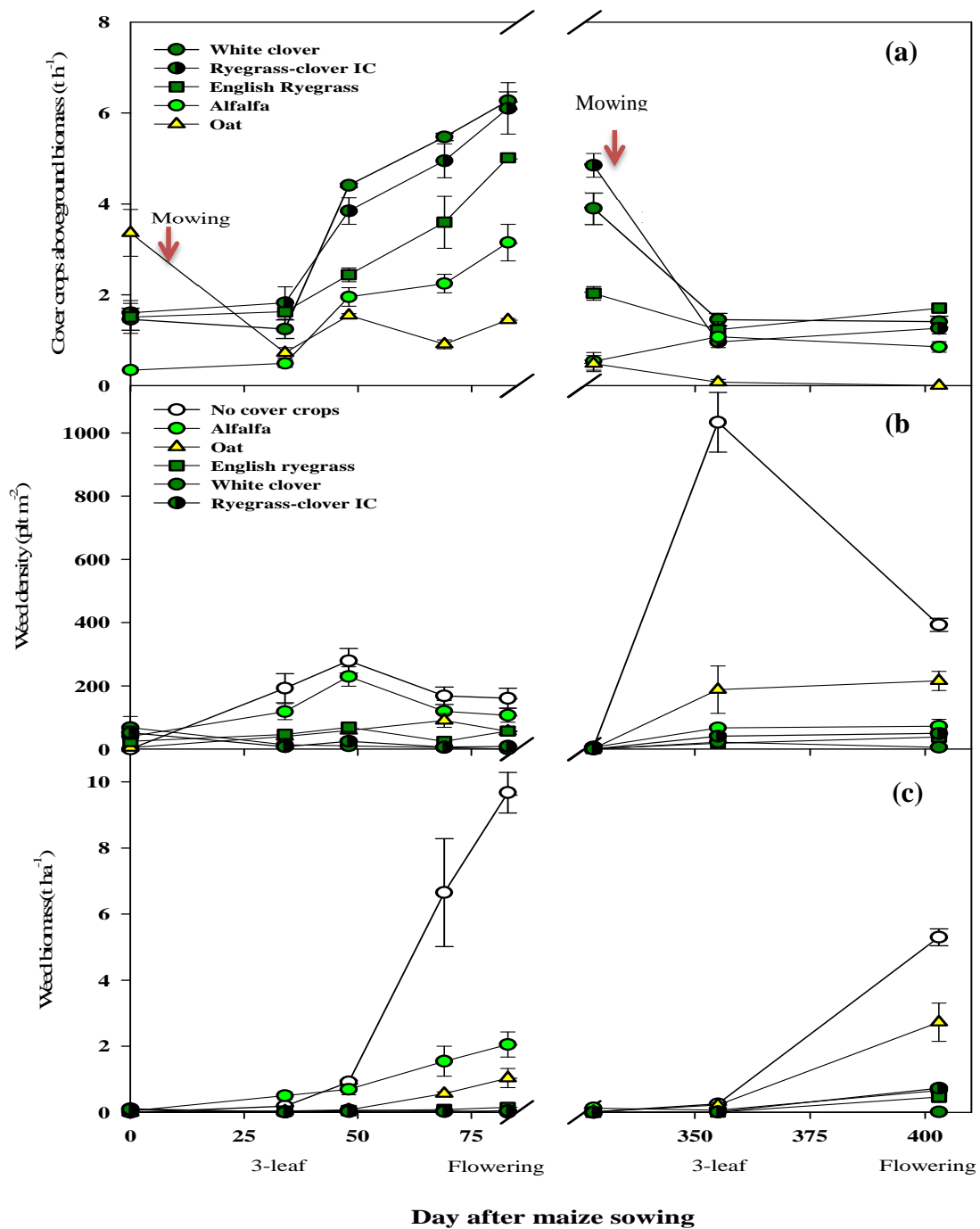


Figure 7.1. Evolution of living mulch aboveground biomass (a), weed density (b) and weed aboveground biomass (c) for five cover crop treatments compared with no cover treatment from maize sowing to maize flowering stage in both 2013 and 2014. Values are mean ($n=3$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date.

Table 7.6. The quantity of mineral nitrogen (N) remaining in the soil (kg ha^{-1}) at both sampling date for 3-leaf and flowering maize stage under living mulch in both experimental years 2013 and 2014

| | 2013 | | 2014 | |
|-----------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
| | 3-leaf maize | Maize flowering | 3-leaf maize | Maize flowering |
| Oat | 10.25 ± 1.93 b | 32.55 ± 8.63 ab | 104.3 ± 25.39 a | 92.56 ± 16.87 b |
| English ryegrass | 9.70 ± 1.97 b | 6.26 ± 0.09 b | 37.82 ± 8.52 b | 147.9 ± 33.44 ab |
| Ryegrass-clover IC | 15.07 ± 2.11 b | 34.98 ± 9.97 ab | 90.61 ± 5.35 a | 123.01 ± 22.63 b |
| White clover | 36.45 ± 6.65 ab | 57.70 ± 7.99 a | 199.58 ± 4.75 a | 234.42 ± 7.51 a |
| Alfalfa | 55.04 ± 16.14 ab | 50.37 ± 10.78 ab | 105.83 ± 9.96 a | 129.98 ± 15.97 ab |
| No cover | 73.81 ± 6.85 a | 20.33 ± 6.12 ab | 95.91 ± 2.09 a | 56.47 ± 15.9 b |
| <i>P-value</i> ¹ | 3.71×10^{-3} ** | 2.43×10^{-2} * | 8.28×10^{-3} ** | 4.07×10^{-3} ** |

Means within each column with different letters are significantly different at $P < 0.05$

***, **, *, indicate significant differences among treatments at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha = 0.05$)

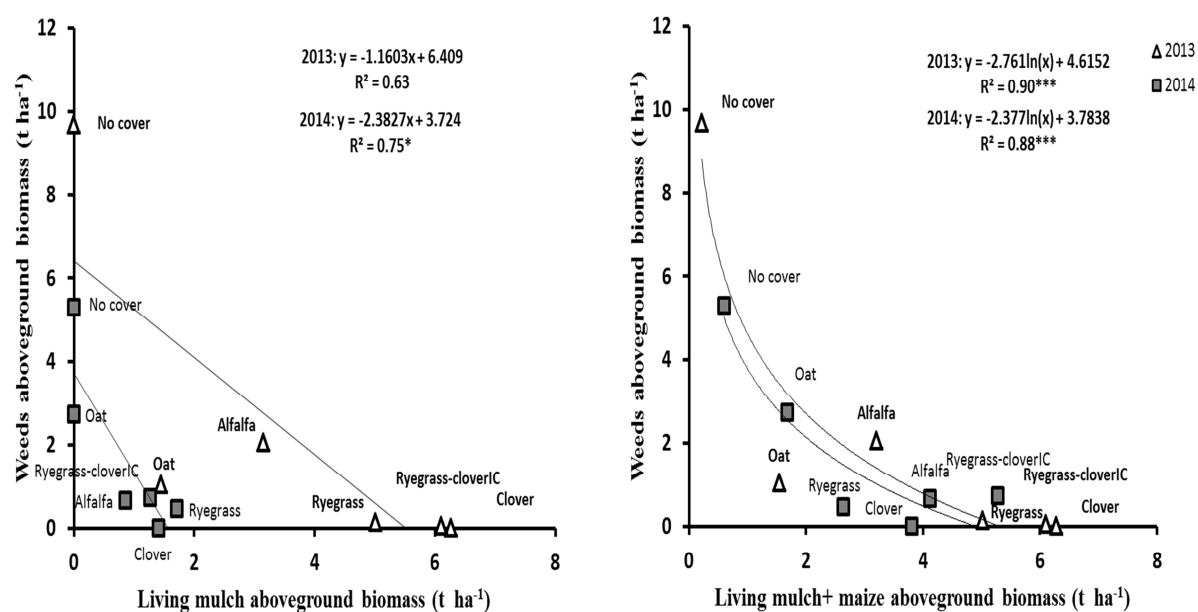


Figure 7.2. Weed aboveground biomass (t ha^{-1}) as a function of the aboveground biomass of cover crops (a) and of cover crops and maize (b). Linear and logarithmic regressions were carried out for the two years separately under living mulch at maize flowering stage. “*”, “**”, “***” indicate that a regression is significant at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively, according to the table proposed by Fisher and Yates (1938)

Effect of living mulch on species composition of weed community:

Diversity index

During maize growth, the composition of weed communities differed among treatments whatever sampling dates. At maize flowering stage in 2013, alfalfa and oat had the highest weed species diversity (on average $H'=1.51$) and specific richness (on average $S=7$ species) as that with no cover treatment whereas the mixture ryegrass-clover had the lowest ones ($H'=0.46$, $S=1.33$).

Conversely, in 2014, no differences were observed among treatments for the studied parameters concerning weed diversity before maize sowing. However, these differences appeared after maize sowing, notably at maize flowering stage. The mixture ryegrass-clover, oat and alfalfa had higher weed species diversity (on average $H'=1.58$) than white clover ($H'=0.44$). Moreover, these three cover crops showed a higher number of weed species (on average $S=7$) than that in clover ($S=2$) and close to that in no cover treatment (Fig. 7.3).

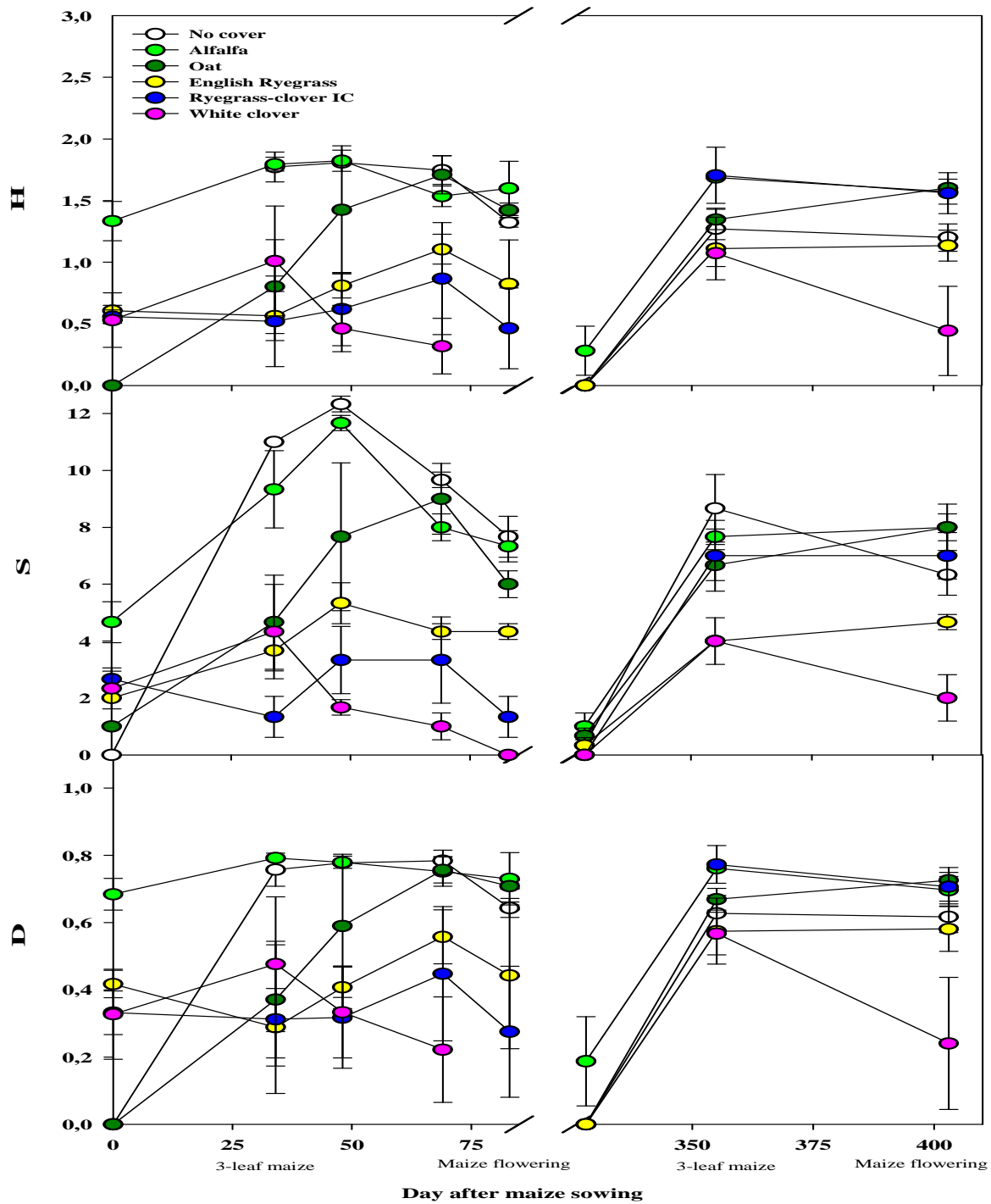


Figure 7.3. The Shannon-Wiener index (H'), species richness (S) and the Simpson evenness index (D) corresponding to the treatments of cover crops compared to no cover treatment under the living mulch system using the means ($n=3$) \pm standard error in both 2013 and 2014. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date.

Weed species categories

Under no cover treatment, the composition of weed species was stable and had a high diversity represented by the presence of four main categories (A, C, D and E), whatever sampling dates during the two experimental years. High nitrophilic weeds were mainly present in no cover treatment (above 90%) including a high percentage of upright weeds (61% in 2013 and 59% in 2014 on average) for two sampling dates.

During maize growth in both 2013 and 2014, weed species composition was different as a function of cover crops species (Fig. 7.4).

When legume cover crops, alfalfa and white clover were introduced, high nitrophilic weed species were still dominant compared to no cover during the two experimental years. Indeed, the percentage of high nitrophilic weeds in alfalfa (on average 71%) and white clover (on average 78%) was twice greater than in ryegrass (36%).

With ryegrass cover crops, the percentage of low nitrophilic weed species was high and reached on average 64% during the two years whereas with the no cover treatment, this percentage reached only 5%.

With oat, although the high nitrophilic weed species were low (28%) at the beginning of maize cycle, the composition of weed species was close to that in no cover crops with a high percentage of high nitrophilic weed species (69%) because of the low biomass of oat in the following stages.

With grass cover crops, ryegrass had a very low percentage of upright weeds (3%) compared to oat in 2013 (22%). Within legume cover crops, no upright weeds were observed with white clover whereas they are represented up to 31% of weed composition with alfalfa.

In the mixture of ryegrass-clover, the composition of weed species was unstable as that in sole cover crops. In 2014, the high nitrophilic weed species were dominant in the mixture (65%) but more than that in ryegrass (48%) and less than that in white clover (93%).

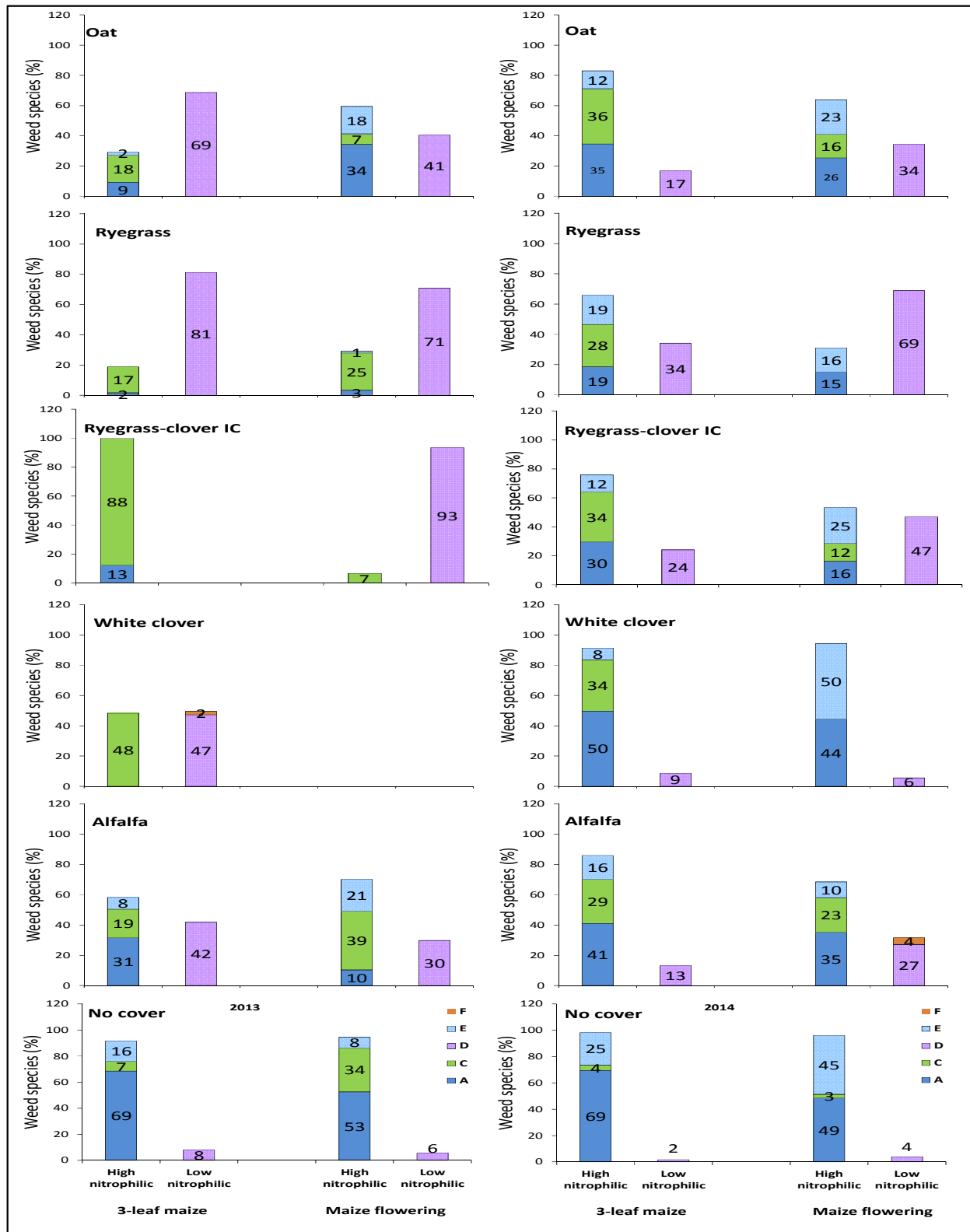


Figure 7.4. The percentage of weed species categories (%) relatively to the total weed species density under living mulch and no cover treatment. The four categories are: A= high nitrophilic and upright weeds, C= high nitrophilic and creeping weeds, D= low nitrophilic and creeping weeds, E= high nitrophilic and monocotyledons and F= low nitrophilic and monocotyledons weeds for two sampling dates at 3-leaf and at flowering maize stages in both 2013 and 2014.

A high correlation was observed between the percentage of low nitrophilic weed species and soil N availability at 3-leaf maize stage ($R^2= 0.94$) (Fig. 7.5). Cover crop species entailing a high soil N availability, such as alfalfa and white clover, had a low percentage of low nitrophilic weed species such as *Kickxia spuria* (L.) Dumort. and a high percentage of high nitrophilic weed species such as *Amaranthus retroflexus* L., *Chenopodium album* L., *Mercurialis annua* L. and *Polygonum* spp.

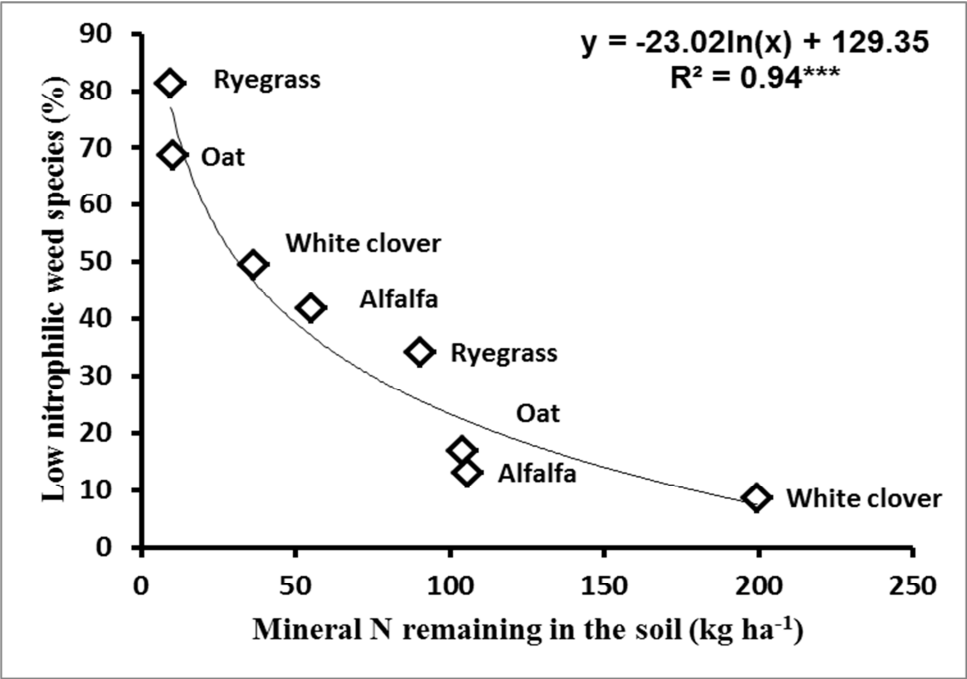


Figure 7.5. Effect of the mineral soil N availability on the percentage of low nitrophilic weed species (%) for each cover crop at 3-leaf maize stage for combined 2013 and 2014. The percentage of low nitrophilic weed species (%) was calculated relatively to the total weed species density.

7.3.4. Dead mulch growth, weed biomass and weed density during maize growth

Dead mulch

In 2013, the application of glyphosate at maize sowing date was not enough to completely kill the cover crops. Differences were observed among treatments whatever sampling date. Cover crop biomass (living mulch + residues) varied from 2.88 to 0.34 t ha⁻¹ for oat and alfalfa, respectively after glyphosate application at 3-leaf maize stage (Fig. 7.5, a).

At maize flowering stage, the mixture ryegrass-clover and sole white clover sole had a greater biomass (on average 4 t ha⁻¹) than other cover crops (on average 1.60 t ha⁻¹).

In 2014, all cover crops were killed completely by a glyphosate application except sole white clover and the ryegrass-clover mixture which showed a low biomass (below 2 t ha⁻¹) during the maize growth.

In 2013, no differences were found among cover crops treatments concerning their ability to reduce soil N whatever sampling dates.

In 2014, under grass treatments, the quantity of mineral soil N remaining in the soil was higher than under legume treatments at 3-leaf maize stage.

Although the quantity of soil N remaining in the soil in 2014 was much greater than in 2013, no differences were found among treatments concerning their ability to reduce soil N at maize flowering stage in both 2013 and 2014 (Table 7.7).

Weed density and biomass

In 2013, differences were observed between treatments in weed density and biomass for each sampling date (Fig. 7.5, b c). Alfalfa showed a low competitive ability against weeds whereas the ryegrass-white clover mixture showed a high competitive ability than other cover crops during maize growth. All cover crops were able to reduce weed density and biomass by 66% and 83% (on average), respectively compared to no cover treatment at flowering maize stage.

Unlike 2013, no differences were observed among treatments (including no cover treatment) for weed density and biomass whatever sampling dates in 2014.

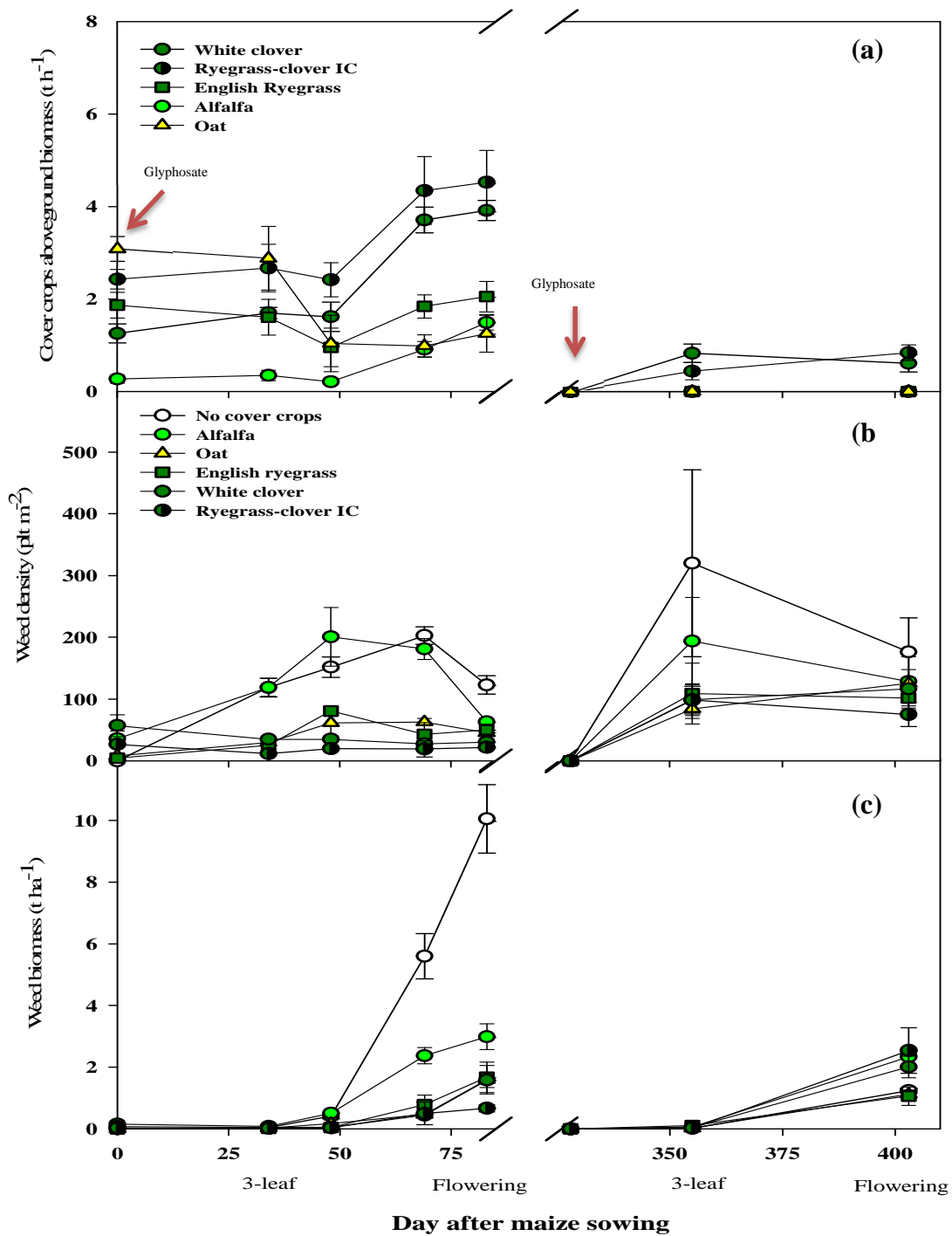


Figure 7.6. Evolution of cover crop aboveground biomass (a), weed density (b) and weed aboveground biomass (c) for five cover crops treatments compared with no cover treatment under the dead mulch system from maize sowing to maize flowering stage in both 2013 and 2014. Values are mean ($n=3$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date.

Table 7.7. The quantity of mineral nitrogen (N) remaining in the soil (kg ha^{-1}) at 3-leaf and flowering maize stages under the dead mulch system in both experimental years 2013 and 2014

| | 2013 | | 2014 | |
|-----------------------------|---------------------------|-----------------|-----------------------------|-----------------|
| | 3-leaf maize | Maize flowering | 3-leaf maize | Maize flowering |
| Oat | 38.96 ± 1.79 b | 17.30 ± 5.49 | 97.89 ± 12.6 c | 137.78 ± 44.9 |
| English ryegrass | 53.38 ± 12.26 ab | 18.86 ± 1.7 | 79.48 ± 9.27 c | 82.52 ± 22.74 |
| Ryegrass-clover IC | 61.25 ± 6.8 ab | 22.68 ± 1.37 | 158.74 ± 10.45 ab | 160.76 ± 31.95 |
| White clover | 60.42 ± 2.51 ab | 22.70 ± 2.15 | 195.55 ± 12.24 a | 151.87 ± 29.87 |
| Alfalfa | 83.79 ± 11.92 ab | 20.84 ± 4.99 | 158.35 ± 3.06 ab | 155.98 ± 29.18 |
| No cover | 98.53 ± 9.17 a | 17.07 ± 2.33 | 128.77 ± 7.35 bc | 80.54 ± 16.11 |
| <i>P-value</i> ¹ | 3.82 × 10 ⁻² * | NS | 2.40 × 10 ⁻⁴ *** | NS |

Means within each column with different letters are significantly different at $P < 0.05$

NS: Means were not significantly different

***, **, *, indicate significant differences among treatments at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha = 0.05$)

Effect of the dead mulch on species composition of weed community:

Differences were observed among treatments only in 2013 concerning species diversity of weed communities (H'), weed species richness(S) and weed species evenness (D) from cover crops during maize growth (Table 7.8). However, these differences disappeared in 2014 whatever sampling date and treatment and the weed species communities were stable. At 3-leaf maize stage in 2013, the mixture ryegrass-clover had lower diversity than under other cover crops and no cover treatment. Moreover, alfalfa had the highest number of weed species (on average S= 9.33) whereas the mixture ryegrass-clover had the lowest one (S= 3.33). Weed species evenness (D) was low under the mixture ryegrass-clover. At maize flowering stage, alfalfa had the highest species diversity and number of weed species than that in the mixture of ryegrass-clover. No differences were observed among treatments for weed species evenness (D). Under dead cover crops system, no differences were observed among treatments concerning weed species categories. The high nitrophilic weeds species were dominant (on average 80%) whatever treatment and sampling date (Data not shown).

Table 7.8. The Shannon-Wiener index (H'), species richness (S) and the Simpson evenness index (D) corresponding to the treatments of cover crops compared to no cover treatment under dead mulch system carried out using the means ($n=3$) \pm standard error in both 2013 and 2014. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at 3-leaf and flowering of maize stage

| 2013 | | | | | | |
|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-----------------|
| | 3-leaf maize | | | Maize flowering | | |
| | H' | S | D | H' | S | D |
| Oat | 1.45 \pm 0.07 ab | 6.33 \pm 0.27 cd | 0.69 \pm 0.03 ab | 1.52 \pm 0.26 ab | 7.00 \pm 1.25 ab | 0.71 \pm 0.07 |
| English ryegrass | 1.11 \pm 0.17 ab | 4.33 \pm 0.72 de | 0.58 \pm 0.07 bc | 1.41 \pm 0.22 ab | 6.33 \pm 0.98 ab | 0.67 \pm 0.09 |
| Ryegrass- clover IC | 0.90 \pm 0.21 b | 3.33 \pm 0.72 e | 0.49 \pm 0.09 c | 1.21 \pm 0.18 b | 4.33 \pm 0.54 b | 0.64 \pm 0.07 |
| White clover | 1.53 \pm 0.27 ab | 7.00 \pm 0.94 bc | 0.75 \pm 0.06 ab | 1.58 \pm 0.15 ab | 6.00 \pm 0.47 ab | 0.75 \pm 0.05 |
| Alfalfa | 1.73 \pm 0.10 ab | 9.33 \pm 0.54 ab | 0.77 \pm 0.03 ab | 1.73 \pm 0.19 a | 8.33 \pm 1.66 a | 0.76 \pm 0.04 |
| No cover | 1.80 \pm 0.07 a | 10.0 \pm 0.47 a | 0.78 \pm 0.01 a | 1.61 \pm 0.09 ab | 8.33 \pm 0.54 a | 0.74 \pm 0.03 |
| <i>P-value</i> | 2.91 $\times 10^{-2*}$ | 1.20 $\times 10^{-05***}$ | 4.27 $\times 10^{-2*}$ | 4.85 $\times 10^{-2*}$ | 1.70 $\times 10^{-2*}$ | NS |

| 2014 | | | | | | |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 3-leaf maize | | | Maize flowering | | |
| | H' | S | D | H' | S | D |
| Oat | 1.19 \pm 0.26 | 5.67 \pm 1.52 | 0.60 \pm 0.09 | 0.99 \pm 0.26 | 4.67 \pm 1.19 | 0.5 \pm 0.11 |
| English ryegrass | 1.17 \pm 0.18 | 5.67 \pm 0.27 | 0.57 \pm 0.10 | 1.43 \pm 0.11 | 6.67 \pm 0.54 | 0.69 \pm 0.04 |
| Ryegrass- clover IC | 1.21 \pm 0.06 | 6.00 \pm 0.00 | 0.60 \pm 0.04 | 1.12 \pm 0.16 | 5.33 \pm 0.72 | 0.57 \pm 0.08 |
| White clover | 1.44 \pm 0.05 | 6.67 \pm 0.27 | 0.70 \pm 0.02 | 1.25 \pm 0.16 | 6.67 \pm 0.72 | 0.59 \pm 0.07 |
| Alfalfa | 1.21 \pm 0.21 | 7.33 \pm 0.72 | 0.59 \pm 0.10 | 1.41 \pm 0.03 | 6.33 \pm 0.27 | 0.7 \pm 0.01 |
| No cover | 1.23 \pm 0.15 | 6.67 \pm 0.72 | 0.60 \pm 0.06 | 1.48 \pm 0.14 | 8.33 \pm 0.72 | 0.67 \pm 0.06 |
| <i>P-value</i> ¹ | NS | NS | NS | NS | NS | NS |

Means within each column with different letters are significantly different at $P<0.05$

NS: Means were not significantly different

***, **, *, indicate significant differences among treatments at $p<0.001$, $p<0.005$, $p<0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$)

7.3.5. Effect of living and dead mulch on maize growth and yield

Living mulch system

At flowering stage, maize biomass was considerably higher in 2014 than in 2013 and maize grain was harvested only in 2014. In both experimental years, differences were observed in maize biomass and grain yield (Table 7.9). In 2013, due to a high competitive ability between living mulch cover crops and maize, maize biomass was very low (on average 0.04 t ha^{-1}) in all cover crops and six times lesser than that in no cover treatment whereas the greatest biomass was observed in conventional maize (2 t ha^{-1}). Unlike 2013, the competition between living mulches and maize was lower in 2014. Maize biomass in alfalfa and the mixture ryegrass-clover was six times greater than that in the no cover treatment and similar to that in conventional maize (4 t ha^{-1}). Maize was harvested in 2014 and differences in grain yield were found among treatments. The grain yield was the greatest with alfalfa (3.38 t ha^{-1}) and close to grain yield obtained in conventional maize. However, the grain yield was the lowest with oat (0.5 t ha^{-1}). Maize grain yield was similar under ryegrass, white clover and the mixture (on average 2.07 t ha^{-1}).

Dead mulch system

In 2013, maize biomass was low for all treatments except conventional maize which showed the greatest maize biomass (2 t ha^{-1}). Moreover, maize biomass was three times greater in 2014 than that in 2013 whatever treatment but no differences were observed among treatments.

In 2014, maize grain yield obtained with alfalfa (6.2 t ha^{-1}) and oat (5.02 t ha^{-1}) were greater than that obtained with ryegrass, white clover, the mixture ryegrass-clover and the no cover treatment and similar to that obtained with conventional maize.

Table 7.9. Maize biomass at flowering stage and maize grain yield under living and dead mulches in both 2013 and 2014.

| | Living mulch system | | | | Dead mulch system | | | | | |
|-----------------------------|----------------------------------|--------------------------------------|----------------------------------|--------------------------------------|----------------------------------|--------------------------------------|----------------------------------|--------------------------------------|----|----------------------------|
| | 2013 | | 2014 | | 2013 | | 2014 | | | |
| | Biomass (t ha ⁻¹) | Grain yield (t ha ⁻¹) | Biomass (t ha ⁻¹) | Grain yield (t ha ⁻¹) | Biomass (t ha ⁻¹) | Grain yield (t ha ⁻¹) | Biomass (t ha ⁻¹) | Grain yield (t ha ⁻¹) | | |
| Oat | 0.10 ± 0.04 bc | | 1.68 ± 0.54 bcd | 0.50 ± 0.16 c | 0.52 ± 0.21 b | | 3.56 ± 0.96 | 5.02 ± 0.8 abc | | |
| English ryegrass | 0.01 ± 0.00 cd | | 0.93 ± 0.08 cd | 1.77 ± 0.36 bc | 0.29 ± 0.12 b | | 3.76 ± 0.94 | 1.76 ± 0.99 c | | |
| Ryegrass-clover IC | 0.003 ± 0.002 d | | 4.01 ± 0.29 ab | 1.89 ± 0.36 bc | 0.10 ± 0.05 b | | 1.87 ± 0.32 | 1.85 ± 0.53 c | | |
| White clover | 0.01 ± 0.01 cd | – | 2.40 ± 0.05 abcd | 2.56 ± 0.69 bc | 0.36 ± 0.11 b | – | 4.29 ± 1.13 | 2.20 ± 0.50 c | | |
| Alfalfa | 0.05 ± 0.01 bcd | | 3.27 ± 0.76 abc | 3.38 ± 0.67 ab | 0.90 ± 0.28 b | | 3.81 ± 0.25 | 6.20 ± 0.94 a | | |
| No cover | 0.22 ± 0.14 b | | 0.60 ± 0.14 d | 1.48 ± 0.56 bc | 0.58 ± 0.02 b | | 4.19 ± 0.37 | 2.47 ± 0.55 bc | | |
| Maize conventional | 2.01 ± 0.13 a | | 4.25 ± 0.56 a | 5.73 ± 0.09 a | 2.01 ± 0.13 a | | 4.25 ± 0.56 | 5.73 ± 0.09 ab | | |
| <i>P-value</i> ¹ | 9.90 × 10 ⁻⁰⁶ *** | | 1.05 × 10 ⁻³ ** | | 4.70 × 10 ⁻⁴ *** | | 9.64 × 10 ⁻⁰⁵ *** | | NS | 1.58 × 10 ⁻³ ** |

– No maize harvest in 2013

Means within each column with different letters are significantly different at $P < 0.05$

NS: Means were not significantly different

***, **, *, indicate significant differences among treatments at $p < 0.001$, $p < 0.005$, $p < 0.01$, respectively

¹*P*-values (analysis of variance ANOVA Type III, sum of squares, $\alpha = 0.05$)

7.4. Discussion

The approach of using cover crops established in winter and combined with maize growth as living or dead mulches, proved to be efficient for weed suppression. This practice required a good management of cover crops in order to reduce the interference between maize and cover crops. Weed suppression differed as a function of cover crops species.

Cover crop competitiveness during winter

Our results demonstrated that the differences in the competitive ability of cover crops against weeds can be observed even before maize sowing during their establishment in the winter. This competitiveness against weeds differed as a function of the characteristics of cover crops such as leaf area, crop height, aboveground biomass and their ability to deplete soil N. Our results showed that grass cover crops (oat and ryegrass) were more competitive against weeds than legume cover crops (alfalfa and white clover) while the mixture of ryegrass-white clover had an intermediate competitive ability during cover crop establishment.

Oat produced more aboveground biomass associated with a great ability for soil N depletion leading to a greater weed suppression suppress more efficiently than white clover and alfalfa. Even if ryegrass produced less aboveground biomass than oat, the two crops had a similar ability to reduce weed biomass and density which may be due mainly to a high soil N depletion for both species. The greatest weed biomass was obtained in white clover and alfalfa. Previous studies showed that legume species are known less competitive than grasses against weeds related to a low growth and a low soil N uptake (Blackshaw et al., 2007; Corre-Hellou et al., 2011; Kruidhof et al., 2008).

The effect of cover crops on the composition of weed species has not been investigated before in relation to crop and weed species traits. In our study, weed species composition was modified early and differed also as a function of cover crops. A higher percentage of high nitrophilic weed species was observed under legume cover crops in relation to the low soil N depletion ability.

Living mulch

Although mowing practices were achieved in 2013 in order to reduce cover crop growth and the interference with maize, their aboveground biomass continued to increase except for oat during maize growth entailing a strong competitive ability against both weeds and maize in 2013. Weed density and biomass were reduced by 89% under living cover crops compared to no cover treatment at maize flowering stage. Other previous studies have shown a strong reduction in weed biomass from 52 to 81% (Abdin et al., 2000; Fisk et al., 2001). Moreover, a previous study by Enache and Ilnicki (1990) has shown that a living mulch of subterranean clover (*T. subterraneum* L.) was able to reduce fall panicum biomass by 85%.

However, the competitiveness against weeds differed according to cover crop species and cultural practices. White clover, ryegrass and the mixture ryegrass/clover produced the greatest aboveground biomass that reached up to maximally 6 t ha⁻¹ in 2013, thereby they contributed greatly in weed suppression but it was associated with a large decrease in maize biomass.

Cover crops were better controlled in 2014 than in 2013 by cultural practices such as strip-till and mowing. All cover crops had a aboveground biomass below 2 t ha⁻¹ during maize growth. This threshold provided a sufficient and stable weed suppression during maize growth while allowing the maize harvest. Moreover, we observed a strong negative correlation between aboveground biomass of living mulches and weeds. Such negative correlations have been previously observed with living mulches associated with mustard (Ross et al., 2001), soybean (Sheaffer et al., 2002) or pea (Akemo et al., 2000).

Despite of the low biomass of oat in 2013 (below 2 t ha⁻¹) and its poor establishment in 2014, it was able to reduce weed biomass more than no cover treatment which may due to their allelopathic effects against weeds as mentioned previously by Weston (1996). Moreover, oat maintained a stable and a high weed species diversity with the occurrence of both high and low nitrophilic weed species in both experimental years.

Legume living mulches are usually used to increase soil N availability in cropping systems because of their low soil N uptake ability, their ability to fix N₂ from atmosphere and the supply of nitrogen fixed through rhizodeposition and mineralisation of their residues (Fustec et al., 2011; Høgh-Jensen and Schjoerring, 2001). We observed effectively a higher soil N nitrogen availability under legume than under grass cover crops. One main original result of our work is to show the influence of legumes on weed species composition in relation with soil N availability. Perennial forage legumes, alfalfa and white clover, were more favourable for high nitrophilic weed species than for low nitrophilic ones in both 2013 and 2014. On the contrary, the high nitrophilic weed species were well controlled by ryegrass more than by legume cover crops. A strong negative correlation was observed between soil N availability and the percentage of low nitrophilic weed species. The response of weed community to living mulch may depend also on other traits than nitrophilic traits. For example, when white clover growth was not controlled in 2013, it was more favourable for creeping weeds than upright ones. Because white clover possesses a particular architecture as creeping cover, having stolons, thus we assumed that it provided an environment unfavourable for upright stem weeds. Conversely, alfalfa had an upright stem allowing the appearance of all weed species.

A high competitive ability against weeds was observed with white clover, ryegrass and the mixture, was associated with the lowest species diversity, specific richness and species evenness of weed community. Alfalfa had the lowest weed suppression and the highest species diversity and richness of weed community. In addition, although alfalfa and oat were more efficient than no cover treatment to suppress weeds, the three treatments showed a similar and a high species diversity and a high species richness of weeds. A previous study in Switzerland showed also that a living mulch of white clover

was able to reduce the number of weed species by 68% compared to a no cover control (Hiltbrunner et al., 2007a). Campiglia et al. (2012) have also observed a negative correlation between weed suppression and weed species diversity.

Dead mulch

The dose of glyphosate applied in 2013 was not efficient to kill cover crops and cover crops behaved as living cover crops. Unlike to 2013, all cover crops were killed by glyphosate in 2014 except white clover and the mixture ryegrass-clover. However, cover crops maintained a sufficient level of biomass (living crop+ residues) approximately 2 t ha^{-1} allowing an efficient and stable weed suppression during maize growth with no differences among cover crops species.

The composition of weed species was not affected under dead cover crops system by the cover crops species. The high nitrophilic weed species were dominant whatever years and treatments. A higher soil N availability was observed with dead mulch compared to living mulch

As a result, we assumed that the application of glyphosate on the weed species composition was more important than the effect of cover crops species. However, the glyphosate application was not able to kill the high nitrophilic weed species. A previous study in Italy showed that oat mulch had the highest weed suppression ability which corresponded also to the lowest species richness, Shannon's H index and Shannon evenness. Other study had shown some effects on weed species diversity. Hairy vetch mulch showed high values both for community richness and Shannon's index, while the hairy vetch/oat mixture had intermediate values (Campiglia et al., 2012).

Maize yield in 2014:

The maize grain yield was not harvested in 2013. In fact, the particularity of seed maize that the two maize cultivars (males and females) should reach the flowering stage simultaneously. However, the high competition and the heterogeneity in cover crop-maize competition resulted in an important lag between the two cultivars stages. In 2014, cultural practices (strip-till) and glyphosate application were more efficient than in 2013, allowing harvest. In general, regardless of treatments, maize grain yield under dead mulch was higher than under the living mulch. The highest maize grain yield was obtained in alfalfa dead cover crops which produced (6.20 t ha^{-1}), twice more than that obtained in alfalfa living mulch. A previous study has observed also that maize grain yield was higher under living mulch of subterranean clover than that obtained in conventional maize (Enache and Ilnicki, 1990). Abdin et al. (2000) have shown that cover crops reduced maize grain yield compared to weeded control but this yield was higher than that obtained in the no cover weedy treatment.

As a result from our study, we showed that alfalfa is an interesting cover crop in maize cropping systems. The least grain yield was obtained in white clover and ryegrass and its mixture (on average 2 t ha^{-1}). However, these three cover crops; could be also used in maize cropping system if they are well managed in order to reduce their interference with maize.

As successful innovative system ensuring both weed suppression and a high crop production required a good choice of cover crops species in addition to a better management of these species since their establishment. Several ways could be integrated in maize system in order to build a system less dependent on herbicide applications. The sowing date of cover crops (Abdin et al., 2000), a low dose of selective herbicides application on the rows and cultural practices such as strip-till are key practices to be studied. When perennial cover crops are sown after maize sowing, they are established between maize rows, with a reduction of weed density and growth without a strong interference with maize. After the harvest of maize, the cover crop can continue its growth for the next year with taking into account the integration of several practices when necessary to regulate its growth.

Appendix

Weed species observed in living and dead cover crops over replications across the years of the study in maize. Latin names and symbols are from EPPO codes database and nitrophily index extracted from the ecological database of Ellenberg (1974)

| Code Bayer | Botanic name | Plant taxonomy | Family | Life cycle | Ellenberg N score | Category |
|------------|---|----------------|------------------|-----------------|-------------------|----------|
| AMARE | <i>Amaranthus retroflexus</i> L. | Dicot | Amaranthaceae | Annual | 7 | A |
| ANGAR | <i>Anagallis arvensis</i> L. | Dicot | Primulaceae | Annual | 6 | C |
| AVEFA | <i>Avena fatua</i> L. | Monocot | Poaceae | Annual | – | E |
| CAP | <i>Capsella bursa-pastoris</i> (L.) Medik. | Dicot | Brassicaceae | Annual | 6 | A |
| CHEAL | <i>Chenopodium album</i> L. | Dicot | Chenopodiaceae | Annual | 7 | A |
| CIRAR | <i>Cirsium arvense</i> (L.) Scop | Dicot | Asteraceae | Perennial | 7 | A |
| CONAR | <i>Convolvulus arvensis</i> L. | Dicot | Convolvulaceae | Perennial | – | D |
| DATST | <i>Datura stramonium</i> L. | Dicot | Solanaceae | Annual | 8 | A |
| DIGSA | <i>Digitaria sanguinalis</i> (L.) Scop. | Monocot | Poaceae | Annual | 5 | E |
| ECHspp | <i>Echinochloa</i> spp | Monocot | Poaceae | Perennial | 8 | E |
| EPIAD | <i>Epilobium tetragonum</i> subsp. <i>tetragonu</i> | Dicot | Onagraceae | Annual | 5 | A |
| EPHPE | <i>Euphorbia peplus</i> L. | Dicot | Euphorbiaceae | Annual | 7 | A |
| GERspp | <i>Geranium dissectum</i> L. | Dicot | Geraniaceae | Annual | 5 | C |
| JUNBU | <i>Juncus bufonius</i> L. | Monocot | Juncaceae | Annual | 4 | F |
| KICSP | <i>Kickxia spuria</i> (L.) Dumort. | Dicot | Scrophulariaceae | Annual | 3 | D |
| LAMPU | <i>Lamium purpureum</i> L. | Dicot | Lamiaceae | Annual | 7 | A |
| LOLPE | <i>Lolium perenne</i> L. | Monocot | Poaceae | Perennial | 4 | F |
| LYCAR | <i>Lycopsis arvensis</i> L. | Dicot | Boraginaceae | Annual | 7 | A |
| MATRE | <i>Matricaria recutita</i> L. | Dicot | Asteraceae | Annual/biennial | 5 | A |
| MERAN | <i>Mercurialis annua</i> L. | Dicot | Euphorbiaceae | Annual | 8 | A |
| MYGPE | <i>Myagrurn perfoliatum</i> L. | Dicot | Brassicaceae | Annual | 4 | B |
| MYSMI | <i>Myosurus minimus</i> L. | Dicot | Ranunculaceae | Annual | 5 | C |
| PICPE | <i>Picris hieracioides</i> L. | Dicot | Asteraceae | Biennial | 4 | B |
| POATR | <i>Poa trivialis</i> L. | Monocot | Poaceae | Perennial | 7 | E |
| POLspp | <i>Polygonum</i> spp. | Dicot | Polygonaceae | Annual | 8 | C |
| RUBFR | <i>Rubus fruticosus</i> L. | Dicot | Rosaceae | Perennial | 4 | D |
| RUMOB | <i>Rumex obtusifolius</i> L. | Dicot | Polygonaceae | Perennial | 9 | A |
| SENVU | <i>Senecio vulgaris</i> L. | Dicot | Asteraceae | Annual | 8 | A |
| SETVI | <i>Setaria viridis</i> (L.) P.Beauv. | Monocot | Poaceae | Annual | 7 | E |
| SINAR | <i>Sinapis arvensis</i> L. | Dicot | Brassicaceae | Annual | 6 | A |
| SOLNI | <i>Solanum nigrum</i> L. | Dicot | Solanaceae | Annual | 8 | A |
| SONAR | <i>Sonchus arvensis</i> L. | Dicot | Asteraceae | Perennial | – | A |
| STEME | <i>Stellaria media</i> L. | Dicot | Caryophyllaceae | Annual | 8 | C |
| TAROF | <i>Taraxacum officinale</i> F.H.Wigg. | Dicot | Asteraceae | Perennial | 8 | A |
| TRIRE | <i>Trifolium repense</i> L. | Dicot | Fabaceae | Perennial | 6 | C |
| VERPE | <i>Veronica persica</i> Poir. | Dicot | Scrophulariaceae | Annual | 7 | C |

8. Discussion générale et perspectives de la thèse

Les résultats obtenus à la suite de ce travail nous ont permis de mettre en évidence une variabilité de la capacité à capter l'azote minéral du sol pour une gamme d'espèces de légumineuse en identifiant les traits majeurs impliqués dans cette capacité au phase de croissance précoce. Ensuite, ce travail nous a permis d'évaluer la réponse d'une adventice nitrophile aux différentes espèces de légumineuse ayant des traits contrastés. Enfin, les résultats obtenus en plein champ nous ont permis de quantifier l'impact de l'association d'une légumineuse, cultivé en tant que plante de service avec une culture principale, sur la croissance des adventices, la composition de la flore adventice ainsi que le rendement de la culture principale.

8.1. Compétitivité de différentes espèces des légumineuses vis-à-vis des adventices en lien avec leurs traits fonctionnels pendant la phase de croissance précoce

8.1.1. Différences de capture précoce d'azote minéral du sol chez différentes espèces de légumineuse

Les légumineuses sont connues pour avoir une faible capacité à prélever l'azote du sol (Thorup-Kristensen, 1994) mais la caractérisation de cette faible capacité ainsi que la mise en évidence de la variabilité entre espèces avaient été peu explorés notamment au cours la croissance précoce.

Ce travail a permis d'identifier les traits fonctionnels de différentes espèces de légumineuse impliqués dans leur capacité à capter l'azote minéral du sol pendant la croissance précoce.

Nous avons testé une gamme d'espèces légumineuses cultivées en rhizotrons en conditions contrôlées pendant le premier mois de leur croissance. Toutes les espèces étudiées ont montré une faible capacité à capter l'azote minéral du sol par rapport aux espèces non-légumineuses telles que le colza ou le blé. Par contre, nous avons montré qu'il existe bien une variabilité au sein des légumineuses concernant leur compétitivité pour capter l'azote minéral du sol très tôt au début du cycle.

Ce travail a caractérisé des traits impliqués dans la capacité à capter l'azote du sol, que sont la masse de la semence, la biomasse aérienne et racinaire, l'expansion racinaire latérale, le démarrage de la fixation symbiotique de N_2 et le niveau de vidange des réserves de la semence. Ces traits ont permis de classer les espèces selon leur capacité de capture de l'azote au début de cycle. La masse de la semence et l'expansion racinaire latérale semblent particulièrement déterminants.

Certaines espèces comme le fenugrec, la lentille, la luzerne et la vesce commune apparaissent les moins compétitives pour l'azote du sol en raison de leur faible biomasse (aérienne et racinaire) et leur faible enracinement latéral en lien avec une faible masse de semence. En revanche, d'autres espèces de légumineuse comme l'arachide, le pois, le pois chiche et le soja sont caractérisées par une plus forte biomasse aérienne, une plus forte expansion racinaire latérale et l'absence de démarrage de la fixation de N_2 ; ces espèces ont présenté une plus forte capacité de capture d'azote du sol. La féverole a la particularité de démarrer tôt à la fois la capture de l'azote du sol et la fixation symbiotique de N_2 . Cette espèce s'est distinguée des deux autres groupes par une plus forte biomasse de nodules et une plus

faible utilisation des réserves de la semence. Nous mettons en évidence que durant la croissance précoce les légumineuses reposent beaucoup sur l'azote de la semence comme montré précédemment par (Herdina and Silsbury, 1990). Un grand nombre de légumineuses ont des grosses semences riches en azote, ce qui peut retarder l'utilisation d'azote exogène par rapport d'autres espèces. En revanche, nous montrons que toutes les légumineuses n'ont pas la même stratégie d'utilisation des trois sources d'azote (azote de la semence, azote de l'air et azote du sol). Comme d'autres travaux (Dürr and Boiffin, 1995; Fayaud et al., 2014; Tamet et al., 1996), nous montrons pour une gamme d'espèces que la biomasse précoce est corrélée avec la masse de semence. Néanmoins, les légumineuses ayant une masse de semence supérieure à 0.17g, telles que le soja (0.18g), le pois (0.27g), le pois chiche (0.36g), l'arachide (0.41g) et la féverole (0.54g), ont une biomasse aérienne similaire, mais présentent des traits contrastés au niveau racinaire. Les légumineuses sont connues pour avoir une faible vitesse d'enracinement en profondeur en comparaison avec des non-légumineuses (Bellostas et al., 2003; Corre-Hellou et al., 2007; Thorup-Kristensen, 2001). Dans cette étude, nous montrons qu'il existe très tôt des différences de vitesse de progression des racines à la fois en profondeur et en latéral. De plus, nous avons mis en évidence que la vitesse d'enracinement en latéral a plus d'impact sur la capture de l'azote du sol que la vitesse d'enracinement en profondeur pendant la phase précoce. A ce stade précoce, les racines latérales contribuent davantage que le pivot à l'exploration rapide du volume de sol disponible.

Plusieurs auteurs ont montré l'existence de variabilité intraspécifique sur certains traits racinaires dont le nombre des nodules, la biomasse racinaire, la longueur racinaires et le ratio root: shoot, par exemple, chez le pois (Bourion et al., 2010; McPhee, 2005) et le lupin (Chen et al., 2012; Tang et al., 1999). En revanche, peu de travaux comparent plusieurs espèces de légumineuses et ils n'étudient pas spécifiquement ces traits pendant des stades très précoces et en relation avec la fonction de capture d'N minéral. Notre travail a comparé des espèces et n'a pas exploré la variabilité entre génotypes au sein d'une espèce ; les écarts entre espèces mis en évidence dans ce travail peuvent dépendre des génotypes choisis. Toutefois le travail a permis de mettre en évidence des traits clés permettant de mieux caractériser la variabilité qu'elle soit intra et inter spécifique.

8.1.2. Réponse d'une espèce adventice nitrophile aux différentes légumineuses en liens avec leur capacité contrastée à capter l'azote du sol

Nous avons étudié la réponse d'une adventice nitrophile (la morelle noire) en présence de trois espèces des légumineuses (la féverole, le pois chiche et la vesce commune) ayant des traits contrastés pour capter l'azote du sol. Ces espèces ont été choisies dans la gamme des espèces étudiées dans le chapitre 1. Chaque espèce appartient à une de trois classes identifiées dans le chapitre 1. Ce travail a été réalisé avec un dispositif additif en rhizotrons en comparant les combinaisons légumineuse-adventice et les

espèces pures correspondantes. La morelle noire a été influencée tôt et différemment en fonction de la légumineuse associée. Dans cette étude, nous avons mis en évidence que la compétitivité des légumineuses vis-à-vis de la morelle noire commence tôt mais diffère en fonction de la capacité de la légumineuse à capter l'azote du sol. Le pois chiche a montré une forte compétition vis-à-vis de la morelle noire en raison d'une forte biomasse précoce et d'une forte capacité de capture de l'azote minéral du sol. De plus, le pois chiche a présenté le plus fort enracinement en profondeur et en latéral. Quand la morelle noire est associée au pois chiche, nous avons observé une forte réduction de la biomasse totale (-77%) ainsi que de l'azote accumulé dans la morelle noire (-85%) en comparaison avec la morelle noire pure. Au contraire, la féverole et la vesce commune n'impactent pas la biomasse de la morelle par rapport à une morelle pure. De plus, ces deux espèces améliorent la croissance racinaire de l'adventice en profondeur de 73% par rapport à la morelle noire pure. La féverole et la vesce commune fournissent un environnement favorable pour la morelle noire et facilitent sa croissance à ce stade précoce. Les principaux résultats des chapitres 1 et 2 sont présentés dans la Fig. 8.1. Les interactions (compétition, facilitation) entre la morelle noire et la légumineuse peuvent être aussi liées aux exsudats racinaires et aux effets potentiels allélopathiques de la morelle noire ou des légumineuses. En effet, une étude précédente a montré que la féverole peut avoir un effet stimulant via des exsudats sur la croissance racinaire de maïs associé ainsi que sur la disponibilité du phosphore pour le maïs (Zhang et al., 2016). De plus, certaines études ont détecté un effet allélopathique de la morelle noire sur certaines espèces des légumineuses dont le pois, la vesce et le soja (Marinov-Serafimov, 2010; Verma and Rao, 2006).

D'autres auteurs ont mis en évidence, au champ, que la compétition légumineuse-adventice démarre précocement dès les trois premières semaines (Croster and Masiunas, 1998).

Beaucoup d'études explorent l'effet des adventices sur la croissance et le rendement des légumineuses (Avola et al., 2008; Bensch et al., 2003; Dusabumuremyi et al., 2014; Tepe et al., 2005) mais la relation inverse, à savoir l'effet des légumineuses sur les adventices est moins documenté, et en particulier sur la croissance et l'exploration racinaire. Nous mettons en avant que la biomasse aérienne mais aussi les traits racinaires (profondeur d'enracinement notamment) d'une adventice nitrophile peuvent être impactés très tôt par la légumineuse associée.

Notre travail a permis de mettre en lumière l'importance de la capture d'azote en début de cycle pour comprendre la compétitivité des légumineuses vis-à-vis des adventices. Les travaux portant sur la compétitivité des cultures vis-à-vis des adventices ne prennent pas habituellement en compte les traits racinaires. Ces travaux en rhizotrons ont été conduits à deux niveaux d'azote minéral du sol. Toutefois, nous montrons que la disponibilité en azote du sol n'affecte pas la compétition légumineuse-adventice pendant la phase précoce. Une étude récente a montré aussi que la disponibilité en azote minéral du sol n'impactait pas les traits racinaires dont l'élongation et la ramification racinaire chez certaines espèces (adventices et cultures) au début de cycle (Moreau et al., 2017).

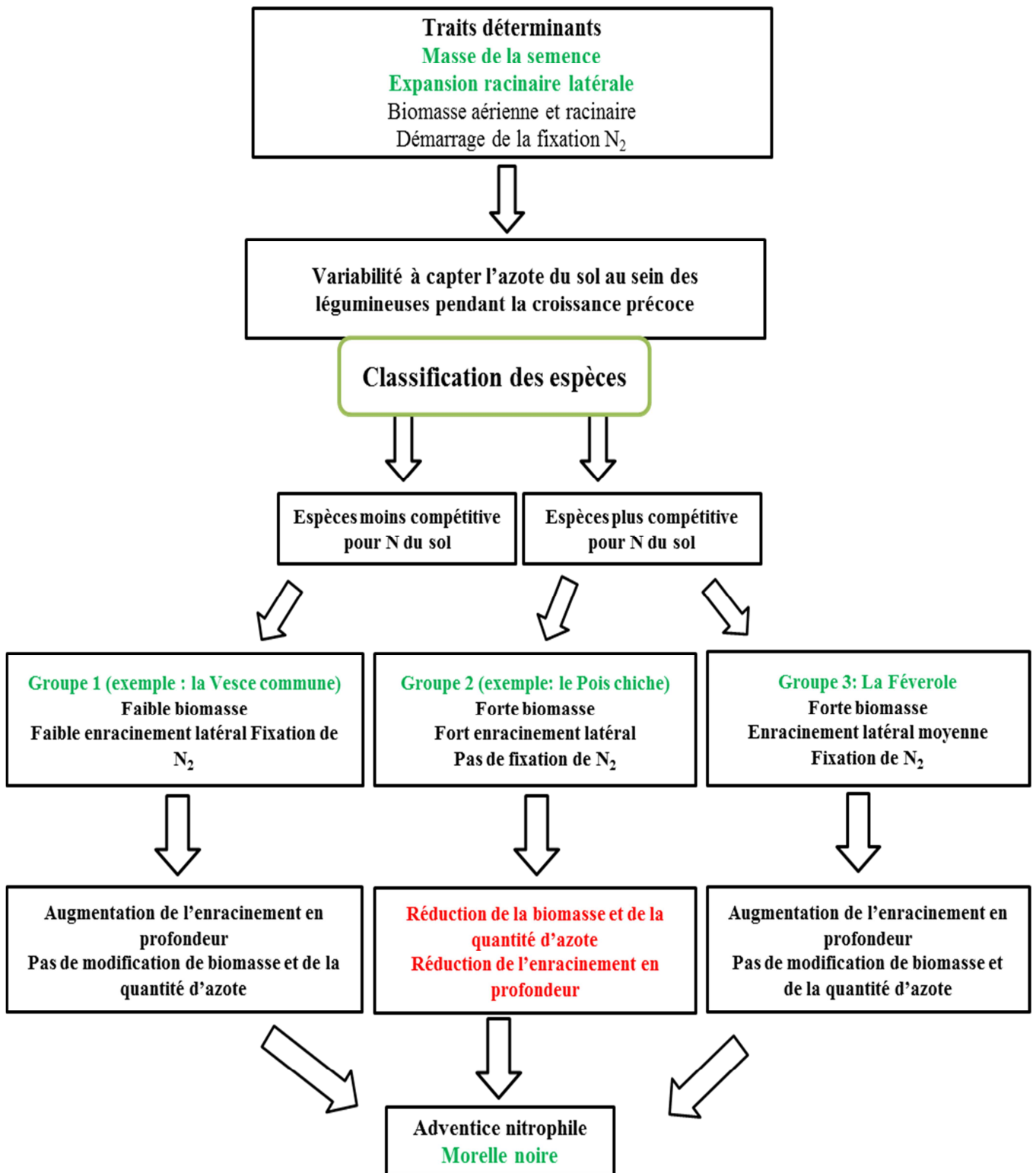


Figure 8.1. Schéma résumant les résultats obtenus sur la compétitivité des légumineuses vis-à-vis des adventices pendant la croissance précoce, en fonction des traits contrastés pour la capture de l'azote minéral du sol

8.2. L'intérêt de cette approche et ses applications à l'échelle de la parcelle

Dans notre travail nous avons mis en évidence l'existence d'une variabilité au sein des espèces de légumineuses concernant la capture précoce de l'azote du sol qui peut influencer la croissance des adventices dès les premiers stades de développement de la culture. Ces résultats obtenus peuvent nous servir à mieux comprendre la compétition légumineuse-adventice au champ et aider aux choix des espèces en fonction de leur compétitivité.

Ainsi, les espèces de légumineuse ayant des traits favorisant une croissance précoce rapide et une forte capacité à capter l'azote du sol peuvent conduire à une forte compétitivité avec d'autres espèces associées (cultures ou adventices) dès la phase précoce.

En revanche, d'autres légumineuses ayant une faible croissance précoce et une faible capacité à capter l'azote du sol, seront des espèces moins compétitives vis-à-vis des autres espèces voisines au début du cycle.

Nos résultats peuvent guider la sélection des génotypes pour leur compétitivité vis-à-vis des adventices en lien avec des traits aériens mais aussi racinaires. Des précédents travaux ont montré l'importance de certains traits impliqués dans la compétitivité vis-à-vis des adventices chez les espèces de légumineuses (Den Hollander et al., 2007; McDonald, 2003; Uchino et al., 2011). Cependant, ces travaux ont mis l'accent principalement sur les traits aériens liés à la compétition pour la lumière tels que la vitesse de la croissance aérienne, la hauteur des plantes, la couverture de sol et la surface foliaire en négligeant l'importance des traits racinaires. Notre travail met en valeur l'intérêt de s'intéresser aussi aux traits racinaires et les caractéristiques des semences (masse et richesse en azote) influençant la stratégie d'acquisition d'azote chez les légumineuses et donc la compétition légumineuse-adventice pendant la croissance précoce.

Quel que soit le mode d'insertion de la légumineuse dans les systèmes de culture, la variabilité des traits pour le choix des espèces est un levier à considérer dans la conception de systèmes de culture incluant davantage de légumineuses. En cultures pures, la compétitivité des légumineuses vis-à-vis des adventices peut varier en fonction de l'espèce et les traits présentés précédemment. Une légumineuse ayant une faible capacité à capter l'azote du sol, va laisser davantage d'azote disponible pour les adventices. La légumineuse peut être aussi cultivée en association avec une non-légumineuse. Dans ces associations où les deux espèces sont semées et récoltées en même temps, la non-légumineuse peut pallier à la faible capacité de capture d'azote de la légumineuse. La complémentarité des deux espèces en terme de stratégie d'acquisition d'azote peut contribuer à accroître la compétitivité de la culture vis-à-vis des adventices pendant la phase précoce

Des associations de deux espèces (ou plus) de légumineuse aux traits contrastés peuvent être aussi envisagées. Enfin, quand la légumineuse est un couvert dans une culture principale, le choix d'un couvert qui démarre tôt et rapidement avec une forte capacité à capter l'azote du sol peut être un atout pour accroître la compétitivité vis-à-vis des adventices au début de cycle.

8.3. Impact de l'insertion d'un couvert de légumineuses comme couvert dans une culture principale sur la régulation des adventices et la composition de la flore adventices

Dans les chapitres 3 et 4, le travail s'est situé à des échelles temporelles et spatiales très différentes de celles des expérimentations conduites dans les deux premiers chapitres. Toutefois dans les expérimentations au champ, nous nous sommes aussi intéressés à la question de la compétitivité des légumineuses vis-à-vis des adventices en relation avec les caractéristiques des légumineuses et notamment leur capacité à capter l'azote minéral du sol. Toutefois dans ces essais au champ, les légumineuses sont aussi étudiées en interaction avec le système de culture dans lequel elles sont insérées. Par ailleurs on ne s'intéresse non seulement aux traits des espèces de légumineuse mais aussi aux traits des autres espèces cultivées auxquelles elles sont associées ainsi qu'aux traits de réponse des adventices. Deux types d'expérimentations ont été conduites au champ afin d'étudier l'effet de l'insertion d'un couvert de légumineuse dans une culture principale sur la gestion des adventices et le rendement. La première expérimentation consistait en l'insertion de légumineuses annuelles dans une culture de colza à l'échelle du cycle de culture de ce dernier. La deuxième expérimentation a permis de tester différents couverts de légumineuses pérennes en comparaison de graminées dans une culture de maïs semence à l'échelle pluriannuelle (étude conduite sur deux ans, deux cycles culturaux de maïs successifs).

8.3.1. Impact de l'insertion de différentes espèces de légumineuse dans une culture principale sur la densité et la croissance des adventices

Nos travaux confirment que la diversification des systèmes de culture par l'insertion d'une légumineuse semée soit avant, soit en même temps qu'une culture principale contribue au contrôle des adventices en améliorant la compétitivité du couvert (culture principale + légumineuse). D'ailleurs, beaucoup de précédents travaux ont montré que les associations d'espèces (légumineuse/non-légumineuse) sont une stratégie efficace pour maîtriser les adventices (Abdin et al., 2000; Corre-Hellou et al., 2011; Gronle et al., 2014; Hauggaard-Nielsen et al., 2001b; Poggio, 2005). Nous observons cependant que la compétitivité des associations vis-à-vis des adventices diffère en fonction des traits des espèces associées, du mode d'insertion de la légumineuse dans le système de culture et des pratiques culturales ainsi qu'en fonction du niveau initial d'infestation des adventices. Nous avons montré que l'association colza-féverole a une plus forte capacité à maîtriser les adventices que l'association colza-vesce commune. L'association colza-féverole a permis de réduire la biomasse des adventices de 35% et la quantité d'azote accumulé dans les adventices de 11% en comparaison avec un colza pur, en 2015/16 (année présentant la plus forte infestation d'aventices). Cette réduction dans la croissance des adventices semble permise par la complémentarité observée entre le colza et la féverole, espèces présentant des traits contrastés. En effet, la féverole possède une plus forte biomasse aérienne et une hauteur plus élevée que le colza durant la phase hivernale. Le colza malgré une faible

croissance aérienne se distingue par une forte capacité à capter l'azote. La féverole, en complément de ses traits aériens intéressants, a présenté une capacité de capture d'N du sol également élevée. Par conséquent, cette combinaison de traits semble avoir permis une meilleure utilisation des ressources en particulier la lumière et l'azote de sol, laissant moins de ressources disponibles pour les adventices. La vesce, en revanche, ne permet pas comme la féverole une augmentation de la diversité des traits et ne permet pas d'accroître la compétitivité par rapport à un colza pur. Dans ce travail, nous retrouvons au champ des différences observées en conditions contrôlées en début de cycle entre la vesce et la féverole en terme de capture précoce de l'N du sol. La vesce est bien moins compétitive que la féverole.

Nous montrons que la compétitivité vis-à-vis des adventices du colza est liée à sa forte capacité de capture de l'azote. D'autres travaux ont montré que la compétitivité du colza vis-à-vis des adventices dépendait de la possibilité de bénéficier d'une grande quantité d'N disponible en début de cycle (Valantin-Morison and Meynard, 2008). Nos travaux suggèrent que l'association d'une autre espèce avec le colza permet de sécuriser cette compétitivité en garantissant une forte biomasse et une forte capture d'azote qui est plus difficile à atteindre systématiquement en culture seule notamment dans des systèmes moins dépendants des intrants (azote et herbicides). Nous avons observé que l'effet de l'association d'un colza avec une légumineuse sur la gestion des adventices était plus efficace sous une forte infestation initiale des adventices.

D'autres travaux récents ont aussi montré l'intérêt d'associer un colza avec une légumineuses ou avec un mélange d'espèces en comparaison avec un colza pur pour la gestion des adventices (Gharineh and Telavat, 2009; Lorin et al., 2015). Ces travaux ont comparé différentes espèces de couvert et mettent aussi en évidence une relation forte entre la biomasse du couvert et la biomasse ou la densité des adventices. L'accent a été jusque-là davantage mis sur les différences entre les caractéristiques des couverts plutôt que sur la complémentarité qu'ils apportent avec la culture principale. Par ailleurs l'accent est surtout mis, dans ces travaux, sur les traits relatifs à la compétitivité pour la lumière négligeant les traits impliqués dans la compétitivité pour capter des ressources du sol. Nos travaux sur ces associations confirment l'intérêt de s'intéresser aussi aux traits influençant les capacités de capture de l'N minéral du sol comme expliqué dans les chapitres 1 et 2.

L'insertion de différents couverts vivant ou détruit dans une culture du maïs semence a contribué à réduire la densité et la biomasse des adventices par rapport à un traitement sans couvert avant et pendant la croissance du maïs lors de deux cycles de maïs successifs. Cependant, le couvert a influencé la croissance des adventices différemment en fonction des traits des espèces et la gestion de système. Pendant la période d'installation des couverts avant le semis du premier maïs, les couverts ayant accumulé le plus de biomasse aérienne et ayant réduit fortement l'azote disponible dans le sol, se sont montrés les plus efficaces à maîtriser des adventices. Les légumineuses étaient les couverts les moins compétitifs pendant cette phase d'installation. Nous retrouvons au champ la faible croissance et capture précoce d'N des légumineuses comme cela avait été identifié en conditions contrôlées dans les

deux premiers chapitres. Ensuite, pendant la croissance du maïs, la compétitivité vis-à-vis des adventices dépend de la dynamique de croissance de chaque couvert. Après une installation très rapide et très efficace, l'avoine a ensuite une plus faible croissance ensuite en lien avec son cycle annuel. Les couverts de trèfle blanc, ray-grass anglais et leur mélange sont les plus compétitifs vis-à-vis des adventices. La luzerne, faiblement compétitive dès l'installation, reste un couvert peu compétitif vis-à-vis des adventices pendant la croissance du maïs. Abdin et al. (2000) observait aussi que tous les couverts et les mélanges insérés dans une culture de maïs étaient capables de maîtriser les adventices par rapport à un témoin sans couvert mais de manière différente en fonction des espèces.

8.3.2. Modification de la composition de la flore adventice selon les espèces et le mode d'insertion

Dans les associations colza-légumineuse, les critères relatifs à la diversité des adventices ont montré une stabilité dans la composition de la flore adventice dans les associations et les cultures pures indépendamment de l'année et de la modalité. L'association du colza avec une légumineuse peut contribuer à réduire la densité ainsi que la biomasse des adventices en maintenant en revanche la composition de la flore adventice et la richesse des espèces. Une étude précédente en Argentine a montré qu'une association pois-orge produit des effets similaires sur les communautés des adventices par rapport à l'orge pure ; en revanche, le pois en culture pure était moins compétitif et la flore adventice était plus diversifiée par rapport à l'association et l'orge pure (Poggio, 2005).

D'autre part, quand un couvert pérenne est inséré et maintenu dans une culture du maïs semence, la composition de la flore adventice est modifiée et de façon différente selon les couverts. Nous mettons en avant que les légumineuses pérennes, la luzerne et le trèfle blanc, ont favorisé la préférence d'aventices ayant une forte nitrophilie (morelle noire, chénopode blanc, amarante réfléchie) par rapport à d'autres couverts en raison de leur faible capacité à réduire l'azote minéral du sol. Par contre, les adventices fortement nitrophiles ont été maîtrisées par le couvert de ray-grass ayant lui une forte capacité à réduire l'azote du sol. Nous avons dégagé une relation étroite entre la disponibilité en azote et la proportion d'espèces nitrophiles présentes dans ce système. Les légumineuses laissent beaucoup d'azote disponible et ont considérablement enrichi le sol contrairement à des couverts de graminées, entraînant une réponse de la flore adventice différente.

En revanche, quand le couvert a été détruit avec du glyphosate, aucune modification dans la composition de la flore adventice n'a été observée; les adventices fortement nitrophiles dominent dans toutes les modalités. Dans le système où le couvert est détruit, la disponibilité en N est encore plus forte que dans le système où les couverts sont maintenus vivants.

Par ailleurs, nous pouvons noter que le couvert le moins compétitif (la luzerne) vis-à-vis des adventices est aussi le couvert le plus diversifié avec une richesse spécifique élevée quel que soit le système. Une étude précédente comparant différents couverts a montré aussi que le couvert le plus

compétitif (l'avoine) était en même temps le moins diversifié (Campiglia et al., 2012). Même si nous n'avons pas quantifié ce phénomène, nous avons observé que l'avoine est capable de mieux maîtriser les adventices que d'autres couverts alors que sa biomasse est inférieure.

D'autres phénomènes, non étudiés dans ce travail, peuvent influencer la composition de la flore adventice dans les systèmes étudiés. Certaines espèces comme l'avoine peuvent être intéressantes comme couvert grâce à leur effet allélopathique vis-à-vis des adventices (Belz, 2007; Weston, 1996).

Le couvert peut aussi attirer des prédateurs des semences de certaines adventices et impacter le stock semencier (Pullaro et al., 2006). Des auteurs ont montré que l'insertion de légumineuse pouvait favoriser la pourriture de semences d'adventices via des pathogènes du sol dont *Fusarium* et *Trichoderma* (Davis et al., 2006; Gómez et al., 2014).

8.3.3. Amélioration du rendement de la culture principale par l'insertion d'un couvert

Plusieurs travaux soulignent la difficulté de réussir *via* l'insertion d'un couvert à maîtriser les adventices sans pénaliser le rendement de la culture principale. Dans nos travaux, nous montrons qu'il peut être possible, dans certaines situations, de combiner le contrôle des adventices et le maintien voire l'amélioration du rendement de la culture principale. Nous avons ainsi observé des effets positifs de l'association colza-féverole sur la croissance aérienne, la hauteur, la surface foliaire et l'azote accumulé et le rendement du colza par plante. Ces effets semblent issus de la complémentarité pour l'utilisation des ressources résultant des traits contrastés des espèces associées. La capacité d'un colza associée à capter l'azote de sol de façon plus efficace qu'un colza pur a été aussi observée dans quelques études précédentes (Andersen et al., 2005; Jamont et al., 2013).

Nous montrons que les LER (*Land Equivalent Ratio*) concernant la biomasse aérienne et l'azote absorbé du sol peuvent être supérieurs à 1 indiquant une plus forte croissance et accumulation d'azote dans l'association colza-féverole par rapport aux cultures pures. Banik et al. (2000) ont observé aussi des valeurs de LER > 1 dans les associations brassicacée-légumineuse, moutarde-lentille ou moutarde-pois dans des dispositifs substitutifs comme dans nos travaux. Dans nos essais, malgré que le colza associé était semé à 50% de sa densité en pur, le rendement en grains du colza dans l'association colza-féverole était similaire au colza pur. Cette complémentarité entre les deux espèces a permis à la fois d'accroître la compétitivité vis-à-vis des adventices et d'atteindre des niveaux de rendement sans désherbage satisfaisants par rapport à la culture pure. Cette complémentarité a été probablement favorisée par le choix d'un dispositif substitutif. Ces associations colza-légumineuse et de façon plus générale les associations ayant une culture principale et un couvert non récolté sont le plus souvent étudiées avec un dispositif additif. La densité importante de plantes dans un système additif peut rendre difficile l'expression d'une complémentarité interspécifique. Le choix d'un dispositif substitutif peut être conseillé dans le cas d'une plante ayant une forte plasticité telle que le colza.

Le couvert végétal pérenne inséré en culture de maïs semence a influencé négativement la biomasse et l'élaboration du rendement du maïs pendant la première année. En effet, certains couverts ont produit une forte biomasse lors de l'installation (favorisée par un automne et un hiver doux). La maîtrise du couvert n'a pas été suffisante avant le semis du maïs. Les variétés en maïs semences sont moins vigoureuses que les variétés de maïs utilisées pour la production de maïs (grain ou ensilage). La croissance du maïs dans les premiers stades a été particulièrement délicate surtout que, là encore, les conditions n'étaient pas optimales : températures froides et forte pluviométrie. Par ailleurs, la réussite de la production de maïs semence nécessite des conditions particulières dont une floraison simultanée pour les mâles et femelles. La forte compétitivité de certains couverts vis-à-vis du maïs ont provoqué un grand décalage dans le stade de floraison et ont empêché la récolte du maïs la première année. La gestion technique du couvert a été améliorée en 2ème année en intégrant le *strip-till* et la compétition entre le couvert et le maïs a été beaucoup réduite. Les rendements ont été satisfaisants avec certains couverts. L'insertion de luzerne a permis l'obtention d'un rendement de maïs de 3.38 avec un couvert vivant et 5.06 t ha⁻¹ avec un couvert mort, rendement équivalent à celui obtenu en maïs conventionnel (sans couvert, conduite classique).

D'autres travaux ont montré aussi l'effet négatif de l'insertion de couverts sur le rendement du maïs et ils ont aussi insisté sur la nécessité de gérer correctement le couvert, par l'utilisation d'herbicides ou le travail du sol entre les rangs de maïs (Abdin et al., 2000; Carruthers et al., 1998). Les principaux résultats des chapitres 3 et 4 sont présentés dans les Fig. 8.2, 8.3, et 8.4.

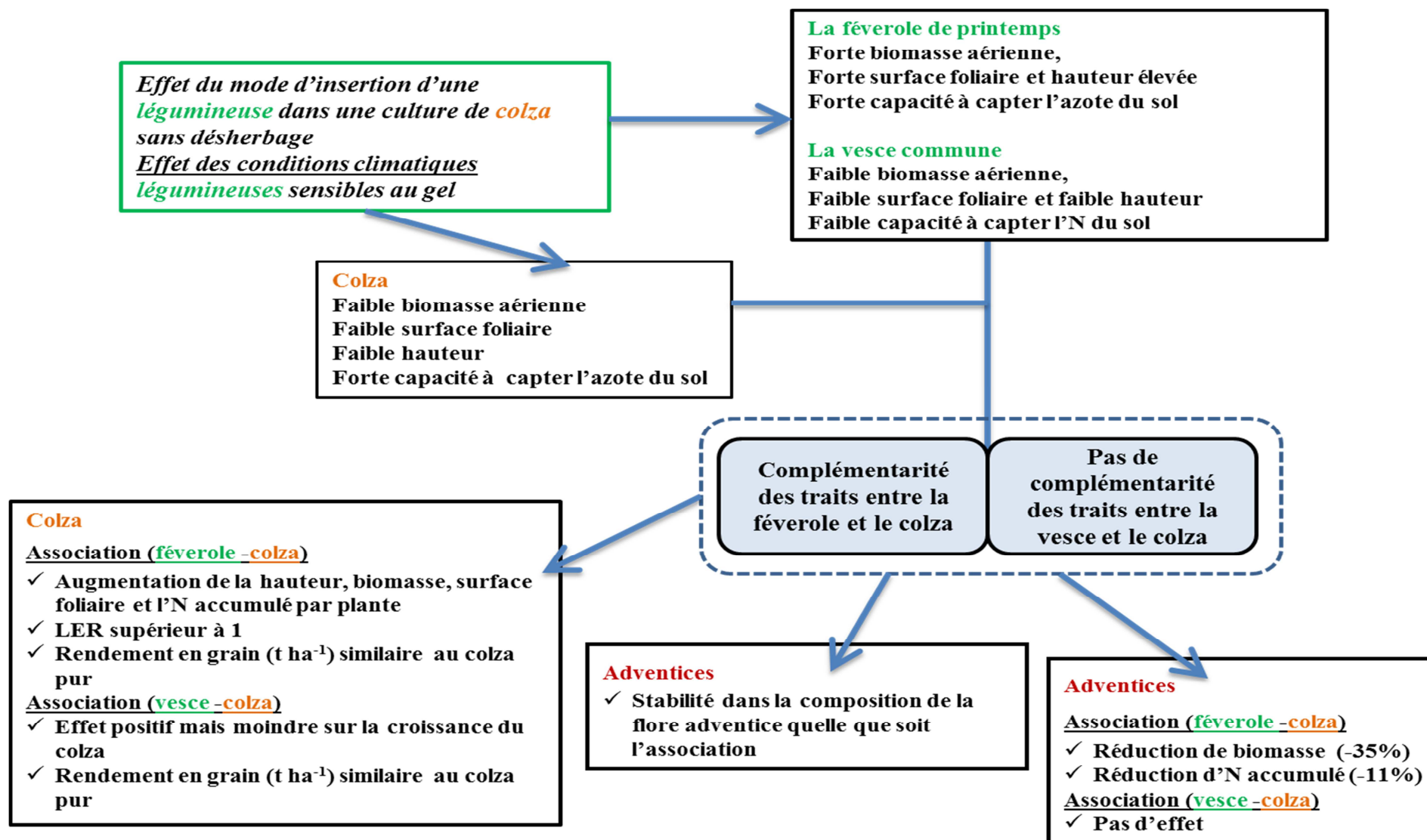


Figure 8.2. Schéma résumant les résultats obtenus dans les associations légumineuses-cola

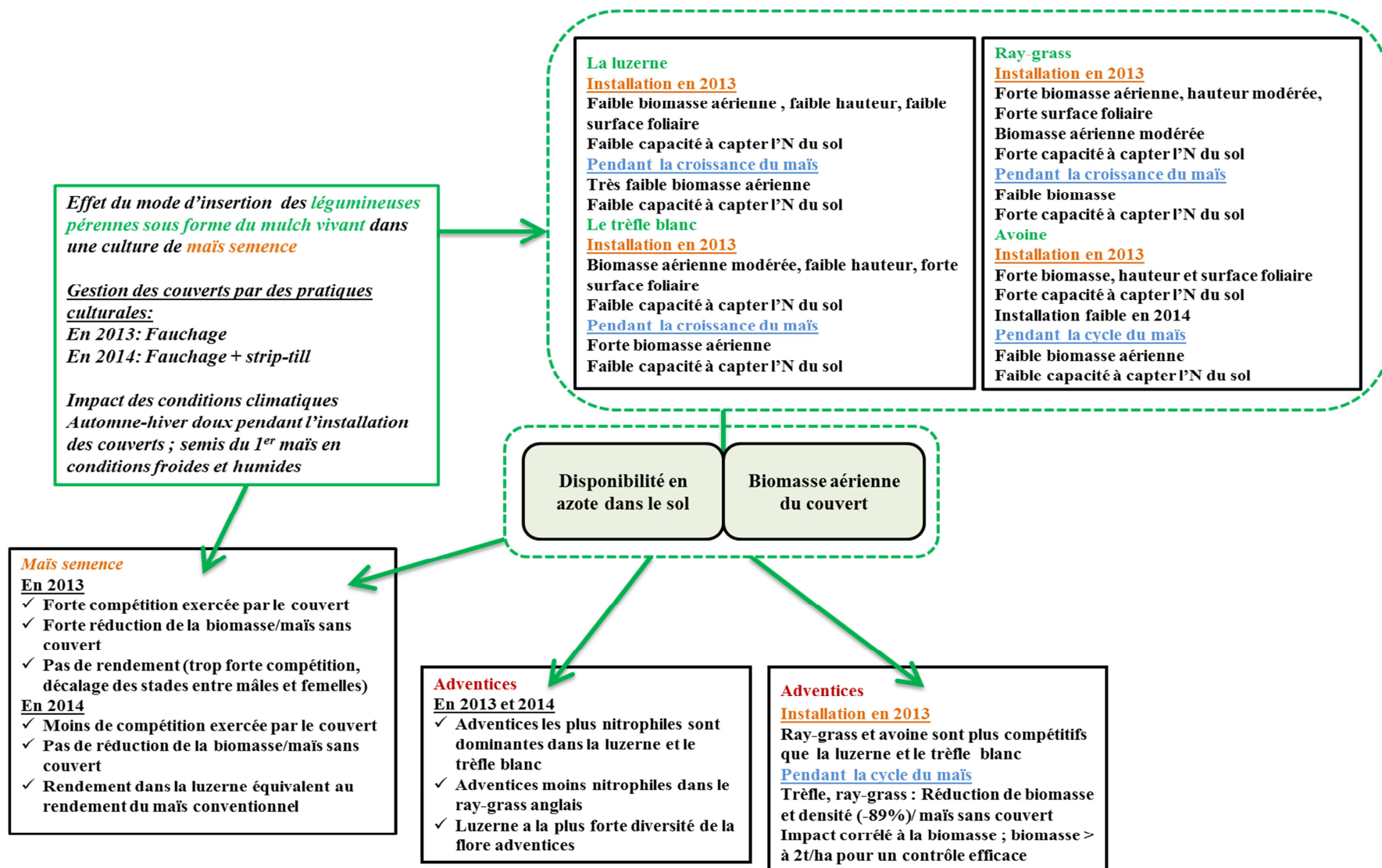


Figure 8.3. Schéma résumant les résultats obtenus dans l'essai du mulch vivant dans une culture du maïs semence

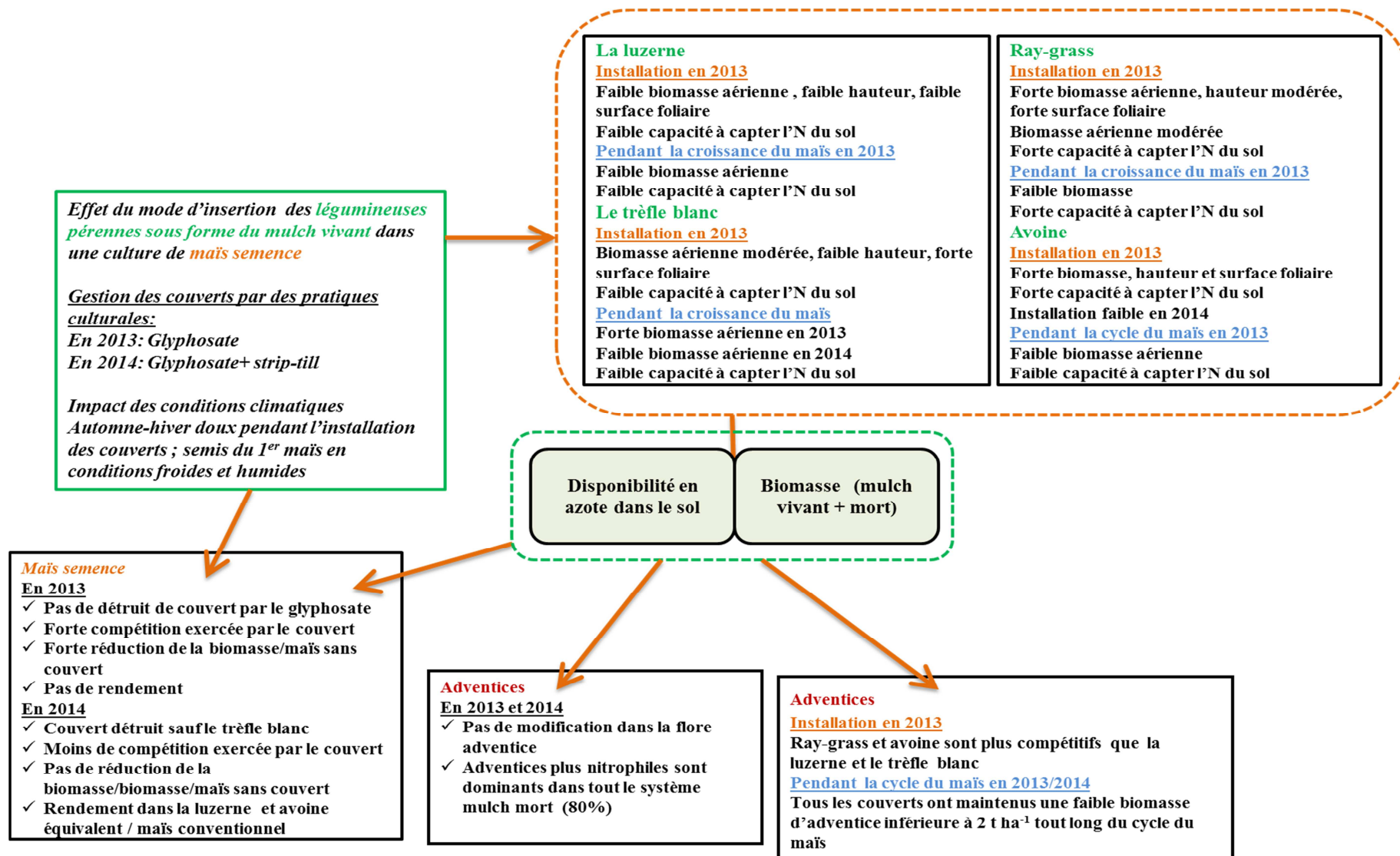


Figure 8.4. Schéma résumant les résultats obtenus dans l'essai du mulch mort dans une culture du maïs semence

8.4. Le rôle clé des légumineuses dans les systèmes de culture

Les légumineuses dans les systèmes de culture sont surtout étudiées pour l'apport d'azote qu'elles peuvent fournir aux cultures compagnes et/ou cultures suivantes. L'effet de l'insertion de légumineuse sur la gestion des adventices est moins étudié. Dans nos travaux nous montrons l'intérêt d'étudier conjointement l'effet des légumineuses sur la gestion des adventices et leurs effets sur la disponibilité en N dans le milieu, ces effets étant en partie liés. Nous observons aussi que la faible compétitivité des légumineuses vis-à-vis de l'azote du sol (processus central étudié dans nos travaux) a des conséquences à différents niveaux.

- Les légumineuses ont une faible capacité de capture d'N du sol en début de cycle, ce qui contribue à leur faible compétitivité vis-à-vis des adventices.
- Cette faible capacité de capture d'N couplée à la capacité à fixer à l' N_2 de l'air dans des stades plus avancés que ceux de la croissance précoce peut néanmoins devenir un atout quand ces légumineuses sont insérées comme plante de couverture en association avec une culture principale. Elles exercent moins de compétition sur la culture principale par rapport à des couverts graminées. En effet, les graminées ont une forte capacité à maîtriser les adventices mais elles peuvent aussi réduire la croissance de la culture principale par leur forte capacité à réduire l'azote du sol disponible.
- Les légumineuses ont une faible de capture de l'N du sol et leur capacité à fixer l' N_2 de l'air offre, notamment dans des associations substitutives, une complémentarité intéressante avec une espèce associée à forte besoins en N qui entraîne à une meilleure croissance et un meilleur rendement comparativement aux cultures pures. Ces gains de croissance et de capture d'N observés dans l'association colza-féverole contribuent aussi à accroître la compétitivité vis-à-vis des adventices et assurer une productivité de la culture principale satisfaisante.
- La faible capacité des légumineuses à capter l'N du sol, associée à d'autres processus que sont la fixation de N_2 , la restitution au sol d'une partie de cet azote accumulé via la rhizodéposition et la minéralisation de ces résidus si elle est détruite au cours du cycle (Fustec et al., 2011; Høgh-Jensen and Schjoerring, 2001) contribuent à augmenter la disponibilité en azote pour les cultures associées et les cultures suivantes dans le système de culture. Dans le cas d'un système pérenne, l'augmentation de la disponibilité en azote est forte et impacte la composition de la flore d'adventices en favorisant les espèces fortement nitrophiliques.
- Nous montrons qu'il existe des différences entre légumineuses et nous mettons en avant certaines espèces tout à fait intéressantes dans les systèmes étudiés.

La féverole se distinguait déjà dans les expérimentations en conditions contrôlées par sa capacité à démarrer plus rapidement que d'autres espèces à la fois l'acquisition de l'N du sol et la fixation de N_2 en tout début de cycle. Elle s'est aussi distinguée au champ par ses traits morphologiques aériens

(forte hauteur, surface foliaire et biomasse aérienne) et sa capacité à capter l'azote du sol plus forte que la vesce commune. Nous avons mis en évidence l'intérêt d'associer des espèces aux traits contrastés mais nous montrons aussi que chez certaines légumineuses comme la féverole, on trouve différents traits favorables à la compétitivité vis-à-vis des adventices ; ceci peut constituer un avantage par rapport à d'autres espèces qui aurait un trait tout à fait favorable mais unique.

Par ailleurs, la luzerne est apparue peu compétitive lors de son installation (faible croissance et faible capture d'N du sol) à la fois en conditions contrôlées et au champ. Elle est apparue moins compétitive vis-à-vis des adventices que d'autres couverts, mais elle a réussi à réduire la croissance des adventices par rapport une modalité sans couvert tout en maintenant la diversité des espèces et la richesse spécifique dans la communauté d'adventices et en produisant un rendement de maïs équivalent à celui du maïs conventionnel.

8.5. Le choix des espèces en fonction de leurs traits

L'approche visant à choisir des espèces en fonction de leurs traits fonctionnels est un levier essentiel à recommander dans la conception d'associations d'espèces. En effet, l'augmentation de la diversité d'un système conduit à augmenter aussi la diversité dans les traits fonctionnels présents permettant une plus grande compétitivité vis-à-vis des adventices par rapport aux cultures pures. Afin d'obtenir une complémentarité dans l'utilisation des ressources entre deux espèces et donc accroître la compétitivité vis-à-vis des adventices, il est conseillé de choisir des espèces ayant des traits contrastés au niveau aérien mais aussi racinaire pour favoriser à la fois la capture de la lumière mais aussi l'azote du sol. Dans nos travaux, la féverole a présenté des valeurs des traits particuliers dont une grande hauteur, une forte biomasse aérienne et une assez forte capacité de capture de l'azote du sol. Le colza est moins haut et produit une plus faible biomasse aérienne que la féverole mais avec une forte capacité à capter l'azote du sol par rapport à la biomasse aérienne produite. Par conséquent, le mélange des deux espèces peut contribuer à diversifier les traits et augmenter les valeurs de certains traits comme la capacité à capter les ressources. La combinaison des espèces ayant une gamme des traits contrastés permettrait d'améliorer des services comme la gestion des adventices et le rendement de la culture principale. Des travaux en écologie proposent des méthodes pour l'assemblage d'espèces en fonction de leurs traits fonctionnels (Barot et al., 2017) et proposent d'analyser la variance entre traits pour caractériser les combinaisons de variétés ou d'espèces. L'atteinte d'un service peut être caractérisée en fonction des traits des espèces associées. Par ailleurs l'atteinte de plusieurs services peut faire appel à des traits antagonistes. Associer des espèces aux traits contrastés peut aider à atteindre plusieurs services qui peuvent être plus difficiles à concilier en cultures pures.

Même si la motivation centrale des agriculteurs pratiquant ces associations concerne la gestion des adventices, les associations étudiées peuvent être cultivées aussi pour d'autres objectifs (restitution d'N, régulation des ravageurs et maladies...). La caractérisation de traits pour différents services et la recherche de compromis entre traits seraient aussi à travailler en fonction des objectifs recherchés.

Pour guider le choix des espèces pour accroître la compétitivité vis-à-vis les adventices, les relations mises en évidence entre la biomasse de la culture et la biomasse des adventices peuvent être utilisées pour identifier des seuils de biomasse nécessaire pour maîtriser suffisamment les adventices. Dans cette étude, nous mettons en évidence des seuils de biomasse aérienne (2 t ha^{-1}) et de d'azote accumulé (80 kg ha^{-1}) en production de colza qui sont nécessaires pour atteindre une forte réduction de la biomasse des adventices. En production de maïs semence, nous avons observé qu'un niveau de biomasse de couvert maintenu (vivant ou mort) en dessous de 2 t ha^{-1} pendant la croissance du maïs est suffisant pour assurer un bon contrôle des adventices sans pénaliser le rendement du maïs. Ces seuils seraient toutefois à confirmer avec davantage d'observations dans une plus grande gamme de conditions via de nouvelles expérimentations ou la mutualisation de différentes données publiées existantes.

Le choix des espèces peut être adapté aussi en fonction de la flore adventice présente dans le système. Par exemple, si le système de culture est infesté par des adventices connues pour leur forte nitrophilie, l'insertion de couverts plus compétitifs pour l'azote du sol peut-être préférable dans telles conditions.

8.6. Complexité de la gestion de système de culture

La gestion du système de culture peut être plus ou moins complexe en fonction de la culture principale, le type de couvert à insérer et aussi le mode d'insertion. La gestion de l'association annuelle colza-légumineuse est beaucoup plus simple que la gestion du système pluriannuel maïs semence-légumineuse. Dans le système pluri-annuel, on peut considérer que le raisonnement des pratiques de gestion du couvert et du maïs a un impact plus fort que le raisonnement du choix des couverts. Travailler sur ces systèmes demande aussi d'expérimenter de façon différente : en conditions agricoles sur de grandes surfaces pour adapter la conduite à chaque modalité, adapter les pratiques en cours d'essai en fonction de l'évolution du couvert en interaction étroite avec l'agriculteur, formuler des règles de décision pour l'adaptation des pratiques malgré le niveau d'incertitude sur les conséquences des choix. Dans l'association annuelle, testée avec un dispositif expérimental plus simple en station expérimentale en petites parcelles, nous mettons aussi en évidence des difficultés notamment la forte dépendance aux conditions météorologiques. Ces associations colza-légumineuse visent à couvrir mieux le sol pendant l'automne-hiver, et réduire ainsi la croissance des adventices mais l'insertion de légumineuse vise aussi à fournir de l'azote après sa destruction par le gel. Les

conditions hivernales ne sont pas toujours favorables pour le gel de la légumineuse et la sensibilité au gel est aussi un critère de choix des espèces à prendre en compte.

8.7. Perspectives de recherche

Nous avons montré l'importance de certains traits dans la mise en place de la compétition (légumineuse-adventice) pendant la croissance précoce. Notre travail a porté sur quelques espèces contrastées pour lesquelles nous avons approfondi les connaissances sur les traits influençant la croissance et l'acquisition précoce de l'azote. Ce travail pourrait être étendu à davantage d'espèces ou à une gamme de génotypes au sein d'une espèce en s'appuyant sur nos travaux qui ont permis de repérer des traits déterminants à bien caractériser notamment la vitesse d'enracinement en latéral et le niveau d'utilisation des réserves azotés. L'utilisation des rhizotrons est tout à fait pertinente pour ce type d'investigation. Des dispositifs expérimentaux permettant un phénotypage haut-débit pourraient être utilisés pour caractériser quelques traits clés sur une gamme importante de génotypes.

Des travaux précédents montrent qu'il peut exister aussi une variabilité intraspécifique sur les traits racinaires (Chen et al., 2012; McPhee, 2005) bien qu'elle soit probablement inférieure à la variabilité interspécifique. Ces travaux ne se sont toutefois pas focalisés sur la croissance précoce.

Certains processus particuliers identifiés pendant la phase précoce pourraient être approfondis à l'aide d'expérimentations spécifiques. Ils concernent la remobilisation des réserves azotées de la semence (avec un suivi dynamique depuis la germination tout au long de la vidange) et son impact sur le démarrage de capture de l'azote exogène. Les facteurs influençant la mise en place de l'appareil fixateur pourraient être davantage étudiés. Cette étude nécessiterait des méthodes plus fines de mesures de l'activité fixatrice à différents stades. La détection précise du démarrage de la fixation de N_2 à des stades aussi précoces et le suivi de son évolution nécessiteraient des méthodes plus fines de mesure de l'activité fixatrice (méthodes de marquage isotopique adaptées).

Des travaux supplémentaires pourraient être conduits pour identifier si les différences identifiées entre espèces au début du cycle se maintiennent à des stades ultérieurs. Ces travaux pourraient aussi étudier des relations entre des traits racinaires et aériens et les interactions entre la capture de l'azote du sol et le capture de la lumière pendant la phase de croissance précoce et surtout dans la suite de cycle, ces processus étant fortement connectés.

Nous avons montré l'intérêt de l'approche basée sur les traits des espèces pour le choix des espèces à associer. Il s'agit d'un levier essentiel dans la conception d'associations d'espèces.

Il serait intéressant de tester cette approche sur une gamme de combinaisons d'espèces afin d'évaluer la complémentarité des traits des espèces et les conséquences sur la productivité et le contrôle des adventices. Nous pourrions aussi identifier des niveaux de complémentarité à atteindre sur tel ou tel trait déterminant pour maximiser la complémentarité quelle que soient les espèces travaillées. Le travail peut être fait sur des associations innovantes déjà pratiquées par des agriculteurs pour mieux comprendre leurs intérêts et favoriser leur déploiement. Cette approche peut aussi être utilisée pour guider la conception de nouvelles associations à partir des connaissances existantes sur les traits de différentes espèces.

Concernant les systèmes testés dans cette thèse, nous montrons l'importance de certaines pratiques sur la réussite de ces systèmes. Des expérimentations supplémentaires pourraient permettre de tester l'effet de quelques leviers techniques tels que la date de semis, l'utilisation de *strip-till* et les combinaisons de plusieurs méthodes de gestion des adventices (effet du couvert combiné à du désherbage mécanique et chimique). L'intégration de plusieurs techniques est considérée aujourd'hui essentielle pour développer des systèmes de culture plus durables et moins dépendants des herbicides.

Ce travail de thèse a été financé par le Gouvernement Syrien. Dans cette étude, nous nous intéressons aux espèces de légumineuse comme la lentille, l'arachide ainsi que le pois chiche qui sont des cultures principales dans les assolements et des ressources alimentaires essentielles riches en protéines en Syrie. Ces légumineuses ont d'ailleurs beaucoup plus d'importance qu'en Europe.

D'autre part, *Solanum* spp. (espèce nitrophile étudiée dans cette thèse) notamment *Solanum elaeagnifolium* L. est une problématique majeure dans une grande gamme de cultures en Syrie telles que le maïs et le blé depuis quelques années. Cette adventice est devenue invasive et répandue dans la région et des projets sont lancés par la FAO afin de réduire la propagation de cette adventice depuis 2015. Les résultats de ce travail serviront aussi à impulser de nouvelles stratégies dans la gestion des adventices, ainsi que des systèmes de culture innovants moins dépendants des herbicides en Syrie.

9. Références bibliographiques

- Abdin OA, Zhou XM, Cloutier D, Coulman DC, Faris MA, Smith DL (2000) Cover crops and interrow tillage for weed control in short season maize (*Zea mays*). *European Journal of Agronomy* 12:93-102.
- Adler MJ, Chase CA (2007) Comparison of the allelopathic potential of leguminous summer cover crops: cowpea, sunn hemp, and velvetbean. *HortScience* 42:289-293
- Akemo MC, Regnier EE, Bennett MA (2000) Weed Suppression in Spring-Sown Rye (*Secale cereale*)–Pea (*Pisum sativum*) Cover Crop Mixes 1. *Weed technology* 14:545-549.
- Amarger N, Mariotti A, Mariotti F, Durr J, Bourguignon C, Lagacherie B (1979) Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in ¹⁵N Natural abundance. *Plant and Soil* 52:269-280.
- Andersen MK, Hauggaard-Nielsen H, Ambus P, Jensen ES (2005) Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. *Plant and Soil* 266:273-287.
- Andersen MK, Hauggaard-Nielsen H, Weiner J, Jensen ES (2007) Competitive dynamics in two-and three-component intercrops. *Journal of Applied Ecology* 44:545-551.
- Andrews D, Kassam A (1976) The importance of multiple cropping in increasing world food supplies: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America
- Asif M, Iqbal M, Randhawa H, Spaner D (2014) Managing and breeding wheat for organic systems: enhancing competitiveness against weeds: Springer Science & Business Media.
- Avola G, Tuttobene R, Gresta F, Abbate V (2008) Weed control strategies for grain legumes. *Agronomy for sustainable development* 28:389-395.
- Ayub M, Shehzad M, Siddique MS (2013) Allelopathic effect of legumes leachates on seed germination and seedling growth of maize (*Zea mays* L.). *Journal of Agricultural Technology* 9:863-875
- Banik P, Midya A, Sarkar BK, Ghose SS (2006) Wheat and chickpea intercropping systems in an additive series experiment: Advantages and weed smothering. *European Journal of Agronomy* 24:325-332.
- Banik P, Sasmal T, Ghosal P, Bagchi D (2000) Evaluation of Mustard (*Brassica campestris* Var. Toria) and Legume Intercropping under 1: 1 and 2: 1 Row-Replacement Series Systems. *Journal of Agronomy and Crop Science* 185:9-14.
- Barberi P, Lo Cascio B (2001) Long-term tillage and crop rotation effects on weed seedbank size and composition. *Weed Research* 41:325-340.
- Barot S, Allard V, Cantarel A, Enjalbert J, Gauffreteau A, Goldringer I, Lata J-C, Le Roux X, Niboyet A, Porcher E (2017) Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review. *Agronomy for sustainable development* 37:13
- Beckie HJ, Johnson EN, Blackshaw RE, Gan Y (2008) Weed suppression by canola and mustard cultivars. *Weed Technology* 22:182-185.
- Bedford I, Kelly A, Banks G, Briddon R, Cenis J, Markham P (1998) *Solanum nigrum*: an indigenous weed reservoir for a tomato yellow leaf curl geminivirus in southern Spain. *European Journal of Plant Pathology* 104:221-222.
- Begna S, Fielding D, Tsegaye T, Van Veldhuizen R, Angadi S, Smith D (2011) Intercropping of oat and field pea in Alaska: An alternative approach to quality forage production and weed control. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science* 61:235-244.
- Bellostas N, Hauggaard-Nielsen H, Andersen MK, Jensen ES (2003) Early Interference Dynamics in Intercrops of Pea, Barley and Oilseed Rape. *Biological Agriculture and Horticulture* 21:337-348.

- Belz RG (2007) Allelopathy in crop/weed interactions—an update. *Pest Management Science* 63:308-326.
- Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Science* 51:37-43.
- Berendse F (1979) Competition between plant populations with different rooting depths. *Oecologia* 43:19-26.
- Bhowmik PC (2003) Challenges and opportunities in implementing allelopathy for natural weed management. *Crop Protection* 22:661-671.
- Blackshaw R, Anderson R, Lemerle D (2007) Cultural Weed Management. *Non-Chemical Weed Management: Principles, Concepts and Technology*:35.
- Blackshaw RE, Brandt RN, Janzen HH, Entz T, Grant CA, Derksen DA (2003) Differential response of weed species to added nitrogen. *Weed Science* 51:532-539.
- Blackshaw RE, O'Donovan JT, Harker KN, Clayton GW, Stougaard RN (2006) Reduced herbicide doses in field crops: A review. *Weed Biology and Management* 6:10-17.
- Booth BD, Swanton CJ (2002) Assembly theory applied to weed communities. *Weed Science* 50:2-13.
- Bourion V, Rizvi SMH, Fournier S, de Larambergue H, Galmiche F, Marget P, Duc G, Burstin J (2010) Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability. *Theoretical and Applied Genetics* 121:71-86.
- Bvenura C, Afolayan AJ (2014) Growth and physiological response of *Solanum nigrum* L. to organic and/or inorganic fertilisers. *Journal of Applied Botany and Food Quality* 87.
- Cadoux S, Sauzet G, Valantin-Morison M, Pontet C, Champolivier L, Céline R, Lieven J, Flénet F, Mangenot O, Fauvin P (2015) Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency. *Oilseeds and fats, Crops and Lipids*: 22(3) D302.
- Campiglia E, Mancinelli R, Radicetti E, Caporali F (2010) Effect of cover crops and mulches on weed control and nitrogen fertilization in tomato (*Lycopersicon esculentum* Mill.). *Crop Protection* 29:354-363.
- Campiglia E, Radicetti E, Mancinelli R (2012) Weed control strategies and yield response in a pepper crop (*Capsicum annuum* L.) mulched with hairy vetch (*Vicia villosa* Roth.) and oat (*Avena sativa* L.) residues. *Crop Protection* 33:65-73.
- Cardina J, Herms CP, Doohan DJ (2002) Crop rotation and tillage system effects on weed seedbanks. *Weed Science* 50:448-460.
- Carr PM, Gardner JJ, Schatz BG, Zwinger SW, Guldan SJ (1995) Grain yield and weed biomass of a wheat-lentil intercrop. *Agronomy Journal* 87:574-579.
- Carruthers K, Fe Q, Cloutier D, Smith D (1998) Intercropping corn with soybean, lupin and forages: weed control by intercrops combined with interrow cultivation. *European Journal of Agronomy* 8:225-238.
- Casper BB, Jackson RB (1997) Plant competition underground. *Annual Review of Ecology and Systematics* 28:545-570.
- Chang C-F, Suzuki A, Kumai S, Tamura S (1969) Chemical studies on “clover sickness” part II. Biological functions of isoflavonoids and their related compounds. *Agricultural and Biological Chemistry* 33:398-408.
- Chauvel B, Dessaint F, Lonchamp J, Gasquez J Enquête sur les mauvaises herbes envahissantes en France
- Chauvel B, Guillemin J, Colbach N, Gasquez J (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). *Crop Protection* 20:127-137.

- Chen YL, Dunbabin VM, Diggle AJ, Siddique KH, Rengel Z (2012) Assessing variability in root traits of wild *Lupinus angustifolius* germplasm: basis for modelling root system structure. *Plant and Soil* 354:141-155.
- Corre-Hellou G, Brisson N, Launay M, Fustec J, Crozat Y (2007) Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea–barley intercrops given different soil nitrogen supplies. *Field Crops Research* 103:76-85.
- Corre-Hellou G, Crozat Y (2005) N₂ fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems as affected by weeds and pea weevil (*Sitona lineatus* L.). *European Journal of Agronomy* 22:449-458.
- Corre-Hellou G, Dibet A, Hauggaard-Nielsen H, Crozat Y, Gooding M, Ambus P, Dahlmann C, von Fragstein P, Pristeri A, Monti M (2011) The competitive ability of pea–barley intercrops against weeds and the interactions with crop productivity and soil N availability. *Field Crops Research* 122:264-272.
- Corre-Hellou G, Fustec J, Crozat Y (2006) Interspecific competition for soil N and its interaction with N₂ fixation, leaf expansion and crop growth in pea–barley intercrops. *Plant and Soil* 282:195-208.
- Croster MP, Masiunas JB (1998) The effect of weed-free period and nitrogen on eastern black nightshade competition with English pea. *HortScience* 33:88-91.
- Davis AS, Anderson KI, Hallett SG, Renner KA (2006) Weed seed mortality in soils with contrasting agricultural management histories. *Weed Science* 54:291-297.
- Dayoub E, Naudin C, Piva G, Shirtliffe SJ, Fustec J, Corre-Hellou G (2017) Traits affecting early season nitrogen uptake in nine legume species. *Heliyon* 3:Article e00244.
- De Tourdonnet S, Shili I, Scopel E (2008) Utilisation des mulchs vivants pour la maîtrise des flores adventices. *Innovations agronomiques* 3:43-48.
- Defelice MS (2003) The Black Nightshades, *Solanum nigrum* L. et al.—Poison, Poultry, and Pie. *Weed Technology* 17:421-427.
- Den Hollander N, Bastiaans L, Kropff M (2007) Clover as a cover crop for weed suppression in an intercropping design: I. Characteristics of several clover species. *European Journal of Agronomy* 26:92-103.
- Diepenbrock W (2000) Yield analysis of winter oilseed rape (*Brassica napus* L.): a review. *Field Crops Research* 67:35-49.
- Dixon GR (2007) *Vegetable Brassicas and related crucifers*: CABI.
- Doberst RC, Blevins DG (1993) Effect of seed size and plant growth on nodulation and nodule development in lima bean (*Phaseolus lunatus* L.). *Plant and Soil* 148:11-19.
- Donald C (1968) The breeding of crop ideotypes. *Euphytica* 17:385-403.
- Duc G, Mignolet C, Carrouée B, Huyghe C (2010) Importance économique passée et présente des légumineuses: Rôle historique dans les assolements et les facteurs d'évolution. *Innovations agronomiques* 11:1-24.
- Dunbabin V (2007) Simulating the role of rooting traits in crop-weed competition. *Field Crops Research* 104:44-51.
- Dürr C, Boiffin J (1995) Sugarbeet seedling growth from germination to first leaf stage. *Journal of Agricultural Science* 124:427-427.
- Dusabumuremyi P, Niyibigira C, Mashingaidze A (2014) Narrow row planting increases yield and suppresses weeds in common bean (*Phaseolus vulgaris* L.) in a semi-arid agro-ecology of Nyagatare, Rwanda. *Crop Protection* 64:13-18.
- Dyck E, Liebman M (1994) Soil fertility management as a factor in weed control: the effect of crimson clover residue, synthetic nitrogen fertilizer, and their interaction on emergence and early growth of lambsquarters and sweet corn. *Plant and Soil* 167:227-237.

- Ellenberg H (1974) Indicator values of vascular plants in central Europe. Indicator values of vascular plants in central Europe. 9.
- Enache AJ, Ilnicki RD (1990) Weed Control by Subterranean Clover (*Trifolium subterraneum*) Used as a Living Mulch. *Weed technology* 4:534-538.
- Ercoli L, Masoni A, Pampana S, Arduini I (2007) Allelopathic effects of rye, brown mustard and hairy vetch on redroot pigweed, common lambsquarter and knotweed. *Allelopathy Journal* 19:249.
- Evans LT (1998) Feeding the ten billion: plants and population growth: Cambridge University Press.
- Evers GW (1982) Seedling growth and nodulation of arrow-leaf, crimson and subterranean clover. *Agronomy Journal* 74: 629–632.
- Fargione J, Tilman D (2006) Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. *Functional Ecology* 20:533-540.
- Fayaud B, Coste F, Corre-Hellou G, Gardarin A, Dürr C (2014) Modelling early growth under different sowing conditions: A tool to predict variations in intercrop early stages. *European Journal of Agronomy* 52:180-190.
- Ferrero R, Lima M, Davis AS, Gonzalez-Andujar JL (2017) Weed Diversity Affects Soybean and Maize Yield in a Long Term Experiment in Michigan, USA. *Frontiers in Plant Science* 8:236.
- Fisher RA, Yates F (1938) Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, Edinburg.
- Fisk JW, Hesterman OB, Shrestha A, Kells JJ, Harwood RR, Squire JM, Sheaffer CC (2001) Weed suppression by annual legume cover crops in no-tillage corn. *Agronomy Journal* 93:319-325.
- Fustec J, Lesuffleur F, Mahieu S, Cliquet J-B (2011) Nitrogen Rhizodeposition of Legumes. Pages 869-881. *in* Lichtfouse E, Hamelin M, Navarrete M, Debaeke P, eds. Sustainable Agriculture Volume 2. Dordrecht: Springer Netherlands.
- Gardarin A, Durr C, Colbach N (2010) Effects of seed depth and soil aggregates on the emergence of weeds with contrasting seed traits. *Weed Res* 50:91-101.
- Gharineh M, Telavat MRM (2009) Investigation of ecological relationship and density acceptance of canola in canola-field bean intercropping. *Asian Journal of Agricultural Research* 1:11-17.
- Ghosheh HZ, Bsoul EY, Abdullah AY (2005) Utilization of Alfalfa (*Medicago sativa* L.) as a Smother Crop in Field Corn (*Zea mays* L.). *Journal of Sustainable Agriculture* 25:5-17.
- Gibson KD, Fischer AJ, Foin TC, Hill JE (2003) Crop traits related to weed suppression in water-seeded rice (*Oryza sativa* L). *Weed Science* 51:87-93.
- Goldberg DE (1990) Components of resource competition in plant communities. *Perspectives on plant competition*:27-49.
- Gómez R, Liebman M, Munkvold G (2014) Weed seed decay in conventional and diversified cropping systems. *Weed Research* 54:13-25.
- Greenwood DJ, Gerwitz A, Stone DA, Barnes A (1982) Root development of vegetable crops. *Plant and Soil* 68:75-96.
- Gregory P (1988) Root growth of chickpea, faba bean, lentil, and pea and effects of water and salt stresses. Pages 857-867. *World crops: Cool season food legumes*: Springer.
- Gronle A, Böhm H, Heß J (2014) Effect of intercropping winter peas of differing leaf type and time of flowering on annual weed infestation in deep and shallow ploughed soils and on pea pests. *Landbauforsch Agriculture and Forestry Research* 64:31-44.

- Gross KL (1984) Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *Journal of Ecology*:369-387.
- Gunton RM, Petit S, Gaba S (2011) Functional traits relating arable weed communities to crop characteristics. *Journal of Vegetation Science* 22:541-550.
- Hall MR, Swanton CJ, Anderson GW (1992) The critical period of weed control in grain corn (*Zea mays*). *Weed Science*:441-447.
- Hamblin A, Tennant D (1987) Root length density and water uptake in cereals and grain legumes: How well are they correlated?[spring wheat; barley; *Lupinus angustifolius*; *Pisum sativum*; morphology]. *Australian Journal of Agricultural Research*.
- Hamblin AP, Hamblin J (1985) Root characteristics of some temperate legume species and varieties on deep, free-draining entisols. *Crop and Pasture Science* 36:63-72.
- Hans SR, Johnson WG (2002) Influence of Shattercane [*Sorghum bicolor* (L.) Moench.] Interference on Corn (*Zea mays* L.) Yield and Nitrogen Accumulation 1. *Weed technology* 16:787-791.
- Harker KN, Blackshaw RE, Clayton GW (2001) Timing Weed Removal in Field Pea (*Pisum sativum*) 1. *Weed technology* 15:277-283.
- Harper JE, Gibson AH (1984) Differential nodulation tolerance to nitrate among legume species. *Crop Science* 24:797-801.
- Hartwig NL, Ammon HU (2002) Cover crops and living mulches. *Weed Science* 50:688-699.
- Hauggaard-Nielsen H, Ambus P, Jensen E (2001a) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops: a field study employing 32P technique. *Plant and Soil* 236:63-74.
- Hauggaard-Nielsen H, Ambus P, Jensen ES (2001b) Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research* 70:101-109.
- Hauggaard-Nielsen H, Gooding M, Ambus P, Corre-Hellou G, Crozat Y, Dahlmann C, Dibet A, Von Fragstein P, Pristeri A, Monti M (2009) Pea-barley intercropping for efficient symbiotic N₂-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crops Research* 113:64-71.
- Heap I (2014) Global perspective of herbicide-resistant weeds. *Pest Management Science* 70:1306-1315.
- Hebinger H (2013) *Le colza*. Paris: Editions France Agricoles.
- Henson JF, Jordan LS (1982) Wild oat (*Avena fatua*) competition with wheat (*Triticum aestivum* and *T. turgidum durum*) for nitrate. *Weed Science*:297-300.
- Herdina, Silsbury JH (1990) Growth, nitrogen accumulation and partitioning, and N₂ fixation in faba bean (*Vicia faba* cv. Fiord) and pea (*Pisum sativum* cv. Early Dun). *Field Crops Research* 24:173-188.
- Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* 311:1-18.
- Hill EC, Ngouajio M, Nair MG (2007) Allelopathic potential of hairy vetch (*Vicia villosa*) and cowpea (*Vigna unguiculata*) methanol and ethyl acetate extracts on weeds and vegetables. *Weed technology* 21:437-444.
- Hiltbrunner J, Jeanneret P, Liedgens M, Stamp P, Streit B (2007a) Response of weed communities to legume living mulches in winter wheat. *Journal of Agronomy and Crop Science* 193:93-102.
- Hiltbrunner J, Liedgens M, Bloch L, Stamp P, Streit B (2007b) Legume cover crops as living mulches for winter wheat: Components of biomass and the control of weeds. *European Journal of Agronomy* 26:21-29.
- Høgh-Jensen H, Schjoerring JK (2001) Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. *Soil Biology and Biochemistry* 33:439-448.

- Jamont M, Piva G, Fustec J (2013) Sharing N resources in the early growth of rapeseed intercropped with faba bean: does N transfer matter? *Plant Soil*. 371:641-653.
- Jensen ES (1996) Grain yield, symbiotic N₂ fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil* 182:25-38.
- Jensen ES, Andersen AJ, Thomsen JD (1985) The Influence of Seed-borne N in ¹⁵N Isotope Dilution Studies with Legumes. *Acta Agriculture Scandinavica, Section B Soil and Plant Science* 35:438-443.
- Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems. *Field Crops Research* 115:203-216.
- JKI O (2010) Growth stages of mono-and dicotyledonous plants. *BBCH Skala*.
- Justes E, Bedoussac L, Corre-Hellou G, Fustec J, Hinsinger P, Jeuffroy M-H, Journet E-P, Louarn G, Naudin C, Pelzer E (2014) Les processus de complémentarité de niche et de facilitation déterminent le fonctionnement des associations végétales et leur efficacité pour l'acquisition des ressources abiotiques. *Innovations agronomiques* 40:1-24.
- Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V, Serraj R (2006) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). *Euphytica* 146:213-222.
- Khan M, Afaq S, Afridi R (1995) Response of black nightshade (*Solanum nigrum* L.) to nitrogen application. *Journal of Agronomy and Crop Science* 174:91-98.
- Kour R, Sharma B, Kumar A, Kour P (2013) Nutrient uptake by chickpea+ mustard intercropping system as influenced by weed management. *Indian Journal of Weed Science* 45:183-188.
- Kruidhof HM, Bastiaans L, Kropff MJ (2008) Ecological weed management by cover cropping: effects on weed growth in autumn and weed establishment in spring. *Weed Research* 48:492-502.
- Kurdali F, Sharabi NE, Arslan A (1996) Rainfed vetch-barley mixed cropping in the Syrian semi-arid conditions. *Plant and Soil* 183:137-148.
- Lagarde F, Champolivier L (2006) Le raisonnement et les avancées techniques permettent de réduire la fertilisation azotée: le cas de Farmstar-colza®. *Oilseeds and fats, Crops and Lipids* 13:384-387.
- Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. *Journal of statistical software* 25:1-18.
- Lemerle D, Gill G, Murphy C, Walker S, Cousens R, Mokhtari S, Peltzer S, Coleman R, Luckett D (2001) Genetic improvement and agronomy for enhanced wheat competitiveness with weeds. *Crop and Pasture Science* 52:527-548
- Lemerle D, Verbeek B, Coombes N (1995) Losses in grain yield of winter crops from *Lolium rigidum* competition depend on crop species, cultivar and season. *Weed Research* 35:503-509.
- Lemerle D, Verbeek B, Cousens R, Coombes N (1996) The potential for selecting wheat varieties strongly competitive against weeds. *Weed Research* 36:505-513
- Li H, Ma Q, Li H, Zhang F, Rengel Z, Shen J (2014) Root morphological responses to localized nutrient supply differ among crop species with contrasting root traits. *Plant and Soil* 376:151-163.
- Liebman M, Dyck E (1993) Crop rotation and intercropping strategies for weed management. *Ecol Appl*. 3:92-122.
- Liebman M, Staver CP (2001) Crop diversification for weed management. *Ecological management of agricultural weeds*. Cambridge Univ. Press, Cambridge, UK:322-374
- Lindquist JL, Mortensen DA (1998) Tolerance and velvetleaf (*Abutilon theophrasti*) suppressive ability of two old and two modern corn (*Zea mays*) hybrids. *Weed Science*:569-574.

- Lins RD, Boerboom CM Effect of soybean row spacing on weed competition
- Liu L, Gan Y, Bueckert R, Van Rees K (2011) Rooting systems of oilseed and pulse crops. II: Vertical distribution patterns across the soil profile. *Field Crops Research* 122:248-255.
- López-Bellido FJ, López-Bellido RJ, Redondo R, López-Bellido L (2010) B value and isotopic fractionation in N₂ fixation by chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.). *Plant and Soil* 337:425-434.
- Lorin M, Jeuffroy MH, Butier A, Valantin-Morison M (2015) Undersowing winter oilseed rape with frost-sensitive legume living mulches to improve weed control. *European Journal of Agronomy* 71:96-105.
- Louarn G, Corre-Hellou G, Fustec J, Lô-Pelzer E, Julier B, Litrico I, Hinsinger P, Lecomte C (2010) Déterminants écologiques et physiologiques de la productivité et de la stabilité des associations graminées-légumineuses. *Innovations agronomiques* 11:79-99.
- Macé K, Morlon P, Munier-Jolain N, Quéré L (2007) Time scales as a factor in decision-making by French farmers on weed management in annual crops. *Agricultural Systems* 93:115-142.
- Magurran AE (1988) Why diversity? Pages 1-5. *Ecological diversity and its measurement*: Springer
- Malagoli P, Laine P, Rossato L, Ourry A (2005) Dynamics of nitrogen uptake and mobilization in field-grown winter oilseed rape (*Brassica napus*) from stem extension to harvest I. Global N flows between vegetative and reproductive tissues in relation to leaf fall and their residual N. *Ann Bot-London*. 95:853-861.
- Marinov-Serafimov P (2010) Determination of Allelopathic Effect of Some Invasive Weed Species on Germination and Initial Development of Grain Legume Crops. *Pesticidi i Fitomedicina*
- Masinde PW, Wesonga JM, Ojiewo CO, Agong SG, Masuda M (2009) Plant Growth and Leaf N Content of *Solanum villosum* Genotypes in Response to Nitrogen Supply. *Dynamic Soil, Dynamic Plant* 3:36-47.
- McDonald GK (2003) Competitiveness against grass weeds in field pea genotypes. *Weed Research* 43:48-58.
- McPhee K (2005) Variation for seedling root architecture in the core collection of pea germplasm. *Crop Science* 45:1758-1763.
- Meiss H, Médiène S, Waldhardt R, Caneill J, Munier-Jolain N (2010) Contrasting weed species composition in perennial alfalfas and six annual crops: implications for integrated weed management. *Agronomy for sustainable development* 30:657-666.
- Meiss H, Munier-Jolain N, Henriot F, Caneill J (2008) Effects of biomass, age and functional traits on regrowth of arable weeds after cutting. *Journal of Plant Diseases and Protection* 21:493-499.
- Mikić A, Čupina B, Rubiales D, Mihailović V, Šarūnaitė L, Fustec J, Antanasović S, Krstić Đ, Bedoussac L, Zorić L, Đorđević V, Perić V, Srebrić M (2015) Chapter Six - Models, Developments, and Perspectives of Mutual Legume Intercropping. Pages 337-419. *in* Donald LS, ed. *Advances in Agronomy*: Academic Press
- Mohler CL (2001) Enhancing the competitive ability of crops. *Ecological management of agricultural weeds*:269-321.
- Mohler CL, Dykeman C, Nelson EB, Ditommaso A (2012) Reduction in weed seedling emergence by pathogens following the incorporation of green crop residue. *Weed Research* 52:467-477
- Moreau D, Abiven F, Busset H, Matejcek A (2017) Effects of species and soil-nitrogen availability on root system architecture traits—study on a set of weed and crop species. *Annals of Applied Biology* 171:103-116.

- Moreau D, Milard G, Munier-Jolain N (2013) A plant nitrophily index based on plant leaf area response to soil nitrogen availability. *Agronomy for sustainable development* 33:809-815.
- Munier-Jolain N, Deytieux V, Guillemain J-P, Granger S, Gaba S (2008) Conception et évaluation multicritères de prototypes de systèmes de culture dans le cadre de la Protection Intégrée contre la flore adventice en grandes cultures. *Innovations agronomiques* 3:75-88.
- Naudin C, Corre-Hellou G, Pineau S, Crozat Y, Jeuffroy MH (2010) The effect of various dynamics of N availability on winter pea-wheat intercrops: Crop growth, N partitioning and symbiotic N₂ fixation. *Field Crops Research* 119:2-11.
- Naudin C, Corre-Hellou G, Voisin A-S, Oury V, Salon C, Crozat Y, Jeuffroy M-H (2011) Inhibition and recovery of symbiotic N₂ fixation by peas (*Pisum sativum* L.) in response to short-term nitrate exposure. *Plant and Soil* 346:275-287.
- Oerke E-C (2006) Crop losses to pests. *The Journal of Agricultural Science* 144:31-43.
- Ofori F, Stern WR (1987) Cereal-legume intercropping systems. *Advances in Agronomy* 41:41-90.
- Ogg Jr AG, Rogers BS, Schilling EE (1981) Characterization of black nightshade (*Solanum nigrum*) and related species in the United States. *Weed Science*:27-32.
- Ohno T, Doolan KL (2001) Effects of red clover decomposition on phytotoxicity to wild mustard seedling growth. *Applied Soil Ecology* 16:187-192.
- Opiyo A (2004) Effect of nitrogen application on leaf yield and nutritive quality of black nightshade (*Solanum nigrum* L.). *Outlook on agriculture* 33:209-214.
- Pakeman RJ, Karley AJ, Newton AC, Morcillo L, Brooker RW, Schöb C (2015) A trait-based approach to crop-weed interactions. *European Journal of Agronomy* 70:22-32.
- Poggio SL (2005) Structure of weed communities occurring in monoculture and intercropping of field pea and barley. *Agriculture, Ecosystems & Environment* 109:48-58.
- Pridham JC, Entz MH (2008) Intercropping spring wheat with cereal grains, legumes, and oilseeds fails to improve productivity under organic management. *Agron J.* 100:1436-1442.
- Primot S, Valantin-Morison M, Makowski D (2006) Predicting the risk of weed infestation in winter oilseed rape crops. *Weed Research* 46:22-33.
- Pullaro TC, Marino PC, Jackson DM, Harrison HF, Keinath AP (2006) Effects of killed cover crop mulch on weeds, weed seeds, and herbivores. *Agriculture, ecosystems & environment* 115:97-104.
- Pyšek P, Lepš J (1991) Response of a weed community to nitrogen fertilization: a multivariate analysis. *J Veg Sci.* 2:237-244.
- Qasem J (1992) Nutrient accumulation by weeds and their associated vegetable crops. *Journal of Horticultural Science* 67:189-195.
- Radicetti E, Mancinelli R, Campiglia E (2012) Combined effect of genotype and inter-row tillage on yield and weed control of chickpea (*Cicer arietinum* L.) in a rainfed Mediterranean environment. *Field Crops Research* 127:161-169.
- RCore T (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.: ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Reddy KN, Koger CH (2004) Live and Killed Hairy Vetch Cover Crop Effects on Weeds and Yield in Glyphosate-Resistant Corn 1. *Weed technology* 18:835-840.
- Reeves T, Brooke H (1977) The effect of genotype and phenotype on the competition between wheat and annual ryegrass (*Lolium rigidum* Gaud.). Pages 167-172.
- Ross SM, King JR, Izaurralde RC, O'Donovan JT (2001) Weed suppression by seven clover species. *Agronomy Journal* 93:820-827.

- Saia S, Urso V, Amato G, Frenda AS, Giambalvo D, Ruisi P, Di Miceli G (2016) Mediterranean forage legumes grown alone or in mixture with annual ryegrass: biomass production, N₂ fixation, and indices of intercrop efficiency. *Plant and Soil* 402:395-407.
- Sarker A, Erskine W, Singh M (2005) Variation in shoot and root characteristics and their association with drought tolerance in lentil landraces. *Genetic Resources and Crop Evolution* 52:89-97.
- Sarrantonio M, Gallandt E (2003) The Role of Cover Crops in North American Cropping Systems. *Journal of Crop Production* 8:53-74.
- Satorre E, Snaydon R (1992) A comparison of root and shoot competition between spring cereals and *Avena fatua* L. *Weed Research* 32:45-55.
- Saucke H, Ackermann K (2006) Weed suppression in mixed cropped grain peas and false flax (*Camelina sativa*). *Weed Research* 46:453-461.
- Schneider A, Huyghe C (2015) Les légumineuses pour des systèmes agricoles et alimentaires: Editions Quae
- Schott C, Mignolet C, Meynard J-M (2010) Les oléoprotéagineux dans les systèmes de culture: évolution des assolements et des successions culturales depuis les années 1970 dans le bassin de la Seine. *Oléagineux, Corps Gras, Lipides* 17:276-291.
- Schröder D, Köpke U (2012) Faba bean (*Vicia faba* L.) intercropped with oil crops—a strategy to enhance rooting density and to optimize nitrogen use and grain production? *Field Crops Research* 135:74-81.
- Shaner DL (2000) The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. *Pest Management Science* 56:320-326.
- Sheaffer CC, Gunsolus JL, Grimsbo Jewett J, Lee SH (2002) Annual Medicago as a Smother Crop in Soybean. *Journal of Agronomy and Crop Science* 188:408-416.
- Smith RS, Ellis MA (1980) Soybean Nodulation as Influenced by Seedling Vigor1. *Agronomy Journal* 72:605-608.
- Spitters C, Van Den Bergh J (1982) Competition between crop and weeds: a system approach. Pages 137-148. *Biology and ecology of weeds.*: Springer
- Sprent JI, Thomas RJ (1984) Nitrogen nutrition of seedling grain legumes: some taxonomic, morphological and physiological constraints. *Plant, Cell & Environment* 7:637-645.
- Stevanato P, Trebbi D, Bertaggia M, Colombo M, Broccanello C, Concheri G, Saccomani M (2011) Root traits and competitiveness against weeds in sugar beet. *International Sugar Journal* 113:497.
- Supasilapa S, Steer B, Milroy S (1992) Competition between lupin (*Lupinus angustifolius* L.) and great brome (*Bromus diandrus* Roth.): development of leaf area, light interception and yields. *Australian Journal of Experimental Agriculture (Australia)*.
- Swanton CJ, Nkoa R, Blackshaw RE (2015) Experimental methods for crop-weed competition studies. *Weed Science* 63:2-11.
- Szumigalski A, Van Acker R (2005) Weed suppression and crop production in annual intercrops. *Weed Science* 53:813-825.
- Szumigalski AR, Van Acker RC (2006) Nitrogen yield and land use efficiency in annual sole crops and intercrops. *Agron J.* 98:1030-1040.
- Tamet V, Boiffin J, Dürr C, Souty N (1996) Emergence and early growth of an epigeal seedling (*Daucus carota* L.): influence of soil temperature, sowing depth, soil crusting and seed weight. *Soil and Tillage Research* 40:25-38.
- Tang C, Unkovich MJ, Bowden JW (1999) Factors affecting soil acidification under legumes. III. Acid production by N₂-fixing legumes as influenced by nitrate supply. *New Phytologist* 143:513-521.

- Teasdale JR (1993) Interaction of light, soil moisture, and temperature with weed suppression by hairy vetch residue. *Weed Science*:46-51.
- Teasdale JR (1996) Contribution of cover crops to weed management in sustainable agricultural systems. *Journal of Production Agriculture* 9:475-479.
- Teasdale JR, Beste CE, Potts WE (1991) Response of Weeds to Tillage and Cover Crop Residue. *Weed Science* 39:195-199.
- Teasdale JR, Daughtry CS (1993) Weed suppression by live and desiccated hairy vetch (*Vicia villosa*). *Weed Science*:207-212.
- Teasdale JR, Mohler CL (2000) The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Science* 48:385-392.
- Tepe I, Erman M, Yazlik A, Levent R, Ipek K (2005) Comparison of some winter lentil cultivars in weed–crop competition. *Crop Protection* 24:585-589.
- Thorsted MD, Olesen JE, Weiner J (2006) Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. *Field Crops Research* 95:280-290.
- Thorup-Kristensen K (1994) The effect of nitrogen catch crop species on the nitrogen nutrition of succeeding crops. *Fertilizer Research* 37:227-234.
- Thorup-Kristensen K (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? *Plant and Soil* 230:185-195.
- Thurling N (1974) Morphophysiological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*). II.* Yield components. *Crop and Pasture Science* 25:711-721.
- Townley-Smith L, Wright AT (1994) Field pea cultivar and weed response to crop seed rate in western Canada. *Canadian Journal of Plant Science* 74:387-393.
- Turpin J, Herridge D, Robertson M (2002) Nitrogen fixation and soil nitrate interactions in field-grown chickpea (*Cicer arietinum*) and fababean (*Vicia faba*). *Crop Pasture Science* 53:599-608.
- Uchino H, Iwama K, Jitsuyama Y, Ichiyama K, Sugiura E, Yudate T (2011) Stable characteristics of cover crops for weed suppression in organic farming systems. *Plant Production Science* 14:75-85.
- Valantin-Morison M, Ferre F, Quere L (2004) Améliorer la compétitivité du colza d'hiver vis-à-vis des adventices et réduire l'utilisation d'herbicides. *Oléoscope*:17-19.
- Valantin-Morison M, Guichard L, Jeuffroy M (2008) Comment maîtriser la flore adventice des grandes cultures à travers les éléments de l'itinéraire technique. *Carrefour de l'Innovation Agronomique* 3:27-41.
- Valantin-Morison M, Meynard J (2008) Diagnosis of limiting factors of organic oilseed rape yield. A survey of farmers' fields. *Agronomy for sustainable development* 28:527-539.
- Verma M, Rao P (2006) Allelopathic effect of four weed species extracts on germination, growth and protein in different varieties of *Glycine max* (L.) Merrill. *Journal of Environmental Biology* 27:571.
- Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional! *Oikos* 116:882-892.
- Voisin A-S, Munier-Jolain NG, Salon C (2010) The nodulation process is tightly adjusted to plant growth. An analysis using environmentally and genetically induced variation of nodule number and biomass in pea. *Plant Soil* 337:399-412.
- Voisin A-S, Salon C, Crozat Y (2005) La mise en place des racines et des nodosités. *Agro-physiologie du pois protéagineux* Inra Editions, pp. 86-94.

- Voisin A-S, Salon C, Munier-Jolain NG, Ney B (2002) Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil* 243:31-42.
- Wall DA, Friesen GH, Bhati TK (1991) Wild mustard interference in traditional and semi-leafless field peas. *Canadian Journal of Plant Science* 71:473-480.
- Waterer JG, Vessey JK, Stobbe EH, Soper RJ (1994) Yield and symbiotic nitrogen fixation in a pea-mustard intercrop as influenced by N fertilizer addition. *Soil Biology and Biochemistry* 26:447-453.
- Webster TM, Scully BT, Grey TL, Culpepper AS (2013) Winter cover crops influence *Amaranthus palmeri* establishment. *Crop Protection* 52:130-135.
- Weihner E, Werf A, Thompson K, Roderick M, Garnier E, Eriksson O (1999) Challenging Theophrastus: a common core list of plant traits for functional ecology. *Journal of Vegetation Science* 10:609-620.
- Weston LA (1996) Utilization of Allelopathy for Weed Management in Agroecosystems. *Agronomy Journal* 88:860-866.
- Whish J, Sindel B, Jessop R, Felton W (2002) The effect of row spacing and weed density on yield loss of chickpea. *Crop and Pasture Science* 53:1335-1340.
- White RH, Worsham AD, Blum U (1989) Allelopathic Potential of Legume Debris and Aqueous Extracts. *Weed Science* 37:674-679.
- Willey RW (1979) Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. *Field Crop Abstracts* 32:1-10.
- Xuan TD, Tsuzuki E (2002) Varietal Differences in Allelopathic Potential of Alfalfa. *Journal of Agronomy and Crop Science* 188:2-7.
- Xuan TD, Tsuzuki E, Terao H, Matsuo M, Khanh TD, Murayama S, Hong NH (2003) Alfalfa, rice by-products and their incorporation for weed control in rice. *Weed Biology and Management* 3:137-144.
- Yoneyama T, Fujita K, Yoshida T, Matsumoto T, Kambayashi I, Yazaki J (1986) Variation in natural abundance of ^{15}N among plant parts and in $^{15}\text{N}/^{14}\text{N}$ fractionation during N_2 fixation in the legume-rhizobia symbiotic system. *Plant Cell Physiology* 27:791-799.
- Zerner MC, Gill GS, Vandeleur RK (2008) Effect of height on the competitive ability of wheat with oats. *Agronomy Journal* 100:1729-1734.
- Zhang D, Zhang C, Tang X, Li H, Zhang F, Rengel Z, Whalley WR, Davies WJ, Shen J (2016) Increased soil phosphorus availability induced by faba bean root exudation stimulates root growth and phosphorus uptake in neighbouring maize. *New Phytologist* 209:823-831.

Table des illustrations

Figures

| | |
|--|----|
| Figure 1.1. Le nombre d'espèces adventices résistantes aux herbicides dans différentes cultures, d'après Heap (2014)..... | 5 |
| Figure 1.2. Augmentation au cours du temps du nombre d'espèces adventices résistantes à plusieurs classes d'herbicides. D'après Heap (2014)..... | 5 |
| Figure 1.3. Modes d'insertion des légumineuses dans les systèmes de culture étudiés dans le cadre de la thèse | 10 |
| Figure 2.1. Questions abordées dans l'axe 1 | 16 |
| Figure 2.2. La disposition des rhizotrons dans la serre du LEVA pendant la première expérimentation..... | 22 |
| Figure 2.3. Á gauche : photographie montrant le suivi racinaire chez le pois chiche cultivé en rhizotron. Au milieu : démolage du rhizotron pour prélever le système racinaire en fin d'expérimentation. Á droite : nodules isolés pour leur comptage | 23 |
| Figure 2.4. Questions et hypothèses abordées dans l'axe 2..... | 18 |
| Figure 2.5. Photos présentant les 5 modalités de l'essai (colza-légumineuse) au champ | 26 |
| Figure 2.6. Illustration des systèmes étudiés dans l'essai en maïs semence | 28 |
| Figure 4.1. Plant N (a), the percentage of N derived from seed, air and soil (b) in the total plant N and remaining N-NO ₃ ⁻ in soil (c) for nine legume species grown without N supply. Values are means (n=4, except for fenugreek, where n=3) ± SE (standard error). Means with different letters are significantly different (P<0.05). | 44 |
| Figure 4.2. a Factor loadings for the variables measured on nine legume species grown under low soil N availability, for the first two axes of the principal component (PC) analysis. The percentage of the total variance explained by the first two principal components is shown in parentheses. b Projections of the different species on the first two axes of the principal component analysis. Variables: Shoot dry matter (Sdm), root dry matter (Rdm), total dry matter (Tdm), root:shoot ratio (R.S), initial seed dry matter (isdm), initial seed N (isN), N content in seeds (%sN), seed reserve depletion (srd), root lateral expansion rate (Rlr), root depth penetration rate (Rdr), nodule number (Nnb), nodule dry matter (Ndm), plant N (N), percentage of plant N derived from seeds (%Ns), N ₂ fixed (N ₂), percentage of exogenous N derived from air (%Ndfa), soil N uptake (Nso), N content in shoot dry matter (%NSdm), N content in root dry matter (%Nrdm), remaining N-NO ₃ ⁻ in soil (NO ₃ ⁻). Species: Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb)..... | 45 |
| Figure 4.3. Dendrograms of relationships among species, calculated using Ward's hierarchical clustering of species, which accounts for the distance matrix comparing all variables (used in PCA analysis) of each species grown under low N availability (a) or high soil N availability (b)..... | 46 |
| Figure 4.4. Dry matter (a) and root:shoot ratio (b), root depth penetration (c) and lateral root expansion (d), nodule number (e) nodule dry matter (f), plant N (g), soil N uptake (h) with (N) and without N supply (0N) for nine legume species: Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb). ***, **, * indicate significant differences between the two levels of soil N availability at p<0.001, p<0.005, p<0.01, respectively. | 48 |
| Figure 4.5. a Factor loadings for the variables measured on nine legume species grown under high soil N availability for the first two axes of the principal component (PC) analysis. The percentage of the total variance explained by the first two principal components is shown in parentheses. b Projection of the different species on the first two axes of the principal component analysis. Variables: Shoot dry matter (Sdm), root dry matter (Rdm), total dry matter (Tdm), root:shoot ratio (R.S), initial seed dry matter (isdm), initial seed N (isN), N content in seeds (%sN), seed reserve depletion (srd), root lateral expansion rate (Rlr), root depth penetration rate (Rdr), nodule number (Nnb), nodule dry matter (Ndm), plant N (N), percentage of plant N derived from seeds (%Ns), Soil N uptake | |

(Nso), N content in shoot dry matter (%NSdm), N content in root dry matter (%Nrdm), remaining N-NO₃- in soil (NO₃-). **Species:** Alfalfa (Al), Fenugreek (Fe), Lentil (Le), Common vetch (Cv), Soybean (So), Pea (Pe), Chickpea (Cp), Peanut (Pn) and Faba bean (Fb)49

Figure 5.1. Pure stands: Plant biomass (a), root: shoot biomass ratio (b), root depth (c), lateral root expansion (d), plant N and soil N uptake (e) for black nightshade and legume species chickpea, faba bean and common vetch without N supply (0N). Values represent mean ($n=5$, except for chickpea and weed where $n=4$) \pm standard error. Values followed by the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$): capital letters and small letters for total plant N and N derived from soil, respectively.65

Figure 5.2. Pure stands: Contribution of the three N sources; seed N, N₂ atmospheric and soil N uptake to the total plant N for chickpea, faba bean and common vetch: a) without (0N) and b) under (N) N supply. Values represent mean ($n=5$, except for chickpea and weed where $n=4$) \pm standard error. Values followed by the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$).66

Figure 5.3. Effects of legume species on total biomass (a), root: shoot ratio (b), root depth (c), lateral root expansion (d) and total N of black nightshade when grown in pure stand (Bn) or in mixed stands with legume species; faba bean (Fb Bn), chickpea (Cp Bn), and common vetch (Cv Bn) without N supply (0N). Values represent mean ($n=5$, except for chickpea and weed where, $n=4$) \pm standard error. Treatments with the same letters are not significantly different (Tukey's HSD; $\alpha=0.05$).68

Figure 5.4. Root depth penetration and lateral root expansion of black nightshade grown in pure stand (Bn) or in mixed stands with legume species; faba bean (Fb Bn), chickpea (Cp Bn), and common vetch (Cv Bn) without (0N) and under N supply (N) at 19 days after seed germination. Values represent mean ($n=4$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare weed at each date. Vertical bars represent Tukey's HSD ($\alpha=0.05$).69

Figure 5.5. Soil N sharing between legume species and black nightshade when weed grown in mixed stands with chickpea (Cp Bn), faba bean (Fb Bn) and common vetch (Cv Bn) without (0N, a) and under (N, b) N supply. Values represent mean ($n=4$) \pm standard error.70

Figure 5.6. Plant biomass (a), root: shoot biomass ratio (b), root depth penetration (c), lateral root expansion (d), plant N (e) and soil N uptake (f) with (N) and without N supply (0N) for legumes and weed species: chickpea (Cp), faba bean (Fb), common vetch (Cv) and black nightshade (Bn) grown in pure stands or in mixed stands. ***, **, * indicate significant differences between the two levels of soil N availability at $p<0.001$, $p<0.005$, $p<0.01$, respectively.72

Figure 6.1. Effects of legume species on intercropped oilseed rape (OR) with faba bean (OR F IC) or common vetch (OR V IC) before winter (BW) in December and after winter (AW) in February. Values are mean ($n=4$) \pm standard error for the two experimental years (2013/14 and 2015/16), except for leaf area after winter, which was measured only in 2013/14. Columns with the different letters are significantly different using Tukey's studentized range test (Tukey's HSD; $\alpha=0.05$)90

Figure 6.2. Total and partial Land Equivalent Ratio (LER) of oilseed rape (OR), faba bean (F) and common vetch (V) using aboveground biomass (DW) and soil N uptake (Ndfs) before winter (BW) in December and after winter (AW) in February when oilseed rape is intercropped using a replacement design with faba bean (OR F IC) or common vetch (OR V IC). Values are mean ($n=4$) \pm standard error for the two experimental years 2013/14 and 2015/16. "*"above the histogram indicates that a total LER > 1 was significant at $P=0.05$, whereas "*" on the histogram indicates that a partial LER > 0.5 was significant at $P=0.05$ 91

Figure 6.3. Weed aboveground biomass ($t\ ha^{-1}$) as a function of the crop aboveground biomass (a) and crop soil N uptake (b), and weed density as a function of crop aboveground biomass (c) for all treatments. Linear regression was carried out for the combined two years and included both the sole and intercropped treatments after winter in February. "****", "***", "**" indicate that a regression is significant at $p<0.001$, $p<0.005$, $p<0.01$, respectively, according to the table proposed by Fisher and Yates (1938).94

| | |
|--|-----|
| Figure 6.4. Comparison between the sole crops and intercropping simultaneously for their studied traits and their effects on weed suppression after winter in February by using radar plots for each experimental year in 2013/14 and 2015/16. Oilseed rape sole crop (OR SC), faba bean sole crop (F SC), common vetch sole crop (V SC), and oilseed rape intercropped with faba bean (OR F IC) or common vetch (OR V IC). Weed biomass (%) and weed N (%) indicate the proportion of weed aboveground biomass and weed aboveground N in the total (weeds + crops), respectively. | 95 |
| Figure 7.1. Evolution of living mulch aboveground biomass (a), weed density (b) and weed aboveground biomass (c) for five cover crop treatments compared with no cover treatment from maize sowing to maize flowering stage in both 2013 and 2014. Values are mean ($n=3$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date. | 119 |
| Figure 7.2. Weed aboveground biomass ($t\ ha^{-1}$) as a function of the aboveground biomass of cover crops (a) and of cover crops and maize (b). Linear and logarithmic regressions were carried out for the two years separately under living mulch at maize flowering stage. “*”, “**”, “***” indicate that a regression is significant at $p<0.001$, $p<0.005$, $p<0.01$, respectively, according to the table proposed by Fisher and Yates (1938) | 120 |
| Figure 7.3. The Shannon-Wiener index (H'), species richness (S) and the Simpson evenness index (D) corresponding to the treatments of cover crops compared to no cover treatment under the living mulch system using the means ($n=3$) \pm standard error in both 2013 and 2014. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date. | 122 |
| Figure 7.4. The percentage of weed species categories (%) relatively to the total weed species density under living mulch and no cover treatment. The four categories are: A= high nitrophilic and upright weeds, C= high nitrophilic and creeping weeds, D= low nitrophilic and creeping weeds, E= high nitrophilic and monocotyledons and F= low nitrophilic and monocotyledons weeds for two sampling dates at 3-leaf and at flowering maize stages in both 2013 and 2014. | 124 |
| Figure 7.5. Effect of the mineral soil N availability on the percentage of low nitrophilic weed species (%) for each cover crop at 3-leaf maize stage for combined 2013 and 2014. The percentage of low nitrophilic weed species (%) was calculated relatively to the total weed species density. | 125 |
| Figure 7.6. Evolution of cover crop aboveground biomass (a), weed density (b) and weed aboveground biomass (c) for five cover crops treatments compared with no cover treatment under the dead mulch system from maize sowing to maize flowering stage in both 2013 and 2014. Values are mean ($n=3$) \pm standard error. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at each date. | 127 |
| Figure 8.1. Schéma résumant les résultats obtenus sur la compétitivité des légumineuses vis-à-vis des adventices pendant la croissance précoce, en fonction des traits contrastés pour la capture de l’azote minéral du sol | 143 |
| Figure 8.2. Schéma résumant les résultats obtenus dans les associations légumineuses-colza | 150 |
| Figure 8.3. Schéma résumant les résultats obtenus dans l’essai du mulch vivant dans une culture du maïs semence | 151 |
| Figure 8.4. Schéma résumant les résultats obtenus dans l’essai du mulch mort dans une culture du maïs semence | 152 |

Tableaux

| | |
|--|-----|
| Tableau 1.1 Le pourcentage de réduction de la biomasse des adventices par différents types d'association légumineuse-non-légumineuses. Exemples issus de la littérature. | 12 |
| Table 2.1. Les plantes de couverture étudiées dans l'expérimentation conduite au champ du maïs semence | 28 |
| Table 4.1. List of studied species | 38 |
| Table 4.2. Shoot and root dry matter, root: shoot ratio and initial seed dry matter for nine legume species grown without N supply. Values are means (n=4, except for fenugreek, where n=3). CV: coefficient of variation. <i>P</i> values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$). ***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively. Means on the same line with different letters are significantly different ($P<0.05$) | 42 |
| Table 4.3. Root exploration and nodules establishment for nine legume species grown without N supply. Values are means (n=4, except for fenugreek, where n=3). CV: coefficient of variation. <i>P</i> values (analysis of variance ANOVA Type III, sum of squares, $\alpha=0.05$). ***, **, *, indicate significant differences among species at $p<0.001$, $p<0.005$, $p<0.01$, respectively. Means on the same line with different letters are significantly different ($P<0.05$) | 42 |
| Table 5.1. Description of the studied legume and weeds species..... | 60 |
| Table 5.2. Quantity of Soil N-NO ₃ ⁻ remained in the soil (mg kg ⁻¹) for mixed stands of black nightshade with chickpea (Cp Bn) or faba bean (Fb Bn) or common vetch (Cv Bn) and for pure stands of black nightshade (Bn), chickpea (Cp), faba bean (Fb) and common vetch (Cv) without N supply (8.65: 0N) and with N supply (36.48: N) N-NO ₃ mg Kg ⁻¹ of dry soil at 33 days after seed germination | 64 |
| Table 6.1. Characteristics of the measured topsoil (0 to 30 cm) before crop establishment in 2013/14 and 2015/16 | 83 |
| Table 6.2. Mean air temperature and precipitation during the 2013/14 and 2015/16 experimental years | 83 |
| Table 6.3. Dates of weed and crop biomass samplings with the corresponding crop growth stages (BBCH) in 2013/14 and 2015/16 | 85 |
| Table 6.4. Comparison between oilseed rape (OR), faba bean (F) and common vetch (V) growth and soil N uptake as sole crops (SC) before winter in December and after winter in February. Values are mean ($n=4$) \pm standard error for 2013/14 and 2015/16. Crop leaf area index was measured only before winter in 2015/16 | 88 |
| Table 6.5. Effects of oilseed rape (OR), faba bean (F) and common vetch (V) grown as sole crops (SC) or as intercrops (IC) on weed infestation before winter (BW) and after winter (AW) in 2013/14 and 2015/16. Values are mean ($n=4$) \pm standard error for the two separate experimental years | 93 |
| Table 6.6. The Shannon-Wiener index (<i>H'</i>), species richness (<i>S</i>) and the Simpson evenness index (<i>D</i>) corresponding to the treatments of oilseed rape (OR), faba bean (F) and common vetch (V) grown as sole crops (SC) or as intercrops (IC) carried out using the means ($n=4$) \pm standard error in 2013/14 and 2015/16. | 96 |
| Table 6.7. The grain yield components of oilseed rape grown as a sole crop (OR SC) or intercropped with faba bean (OR F IC) or common vetch (OR V IC) in 2013/14 and 2015/16. Values are mean ($n=3$) \pm standard error. | 97 |
| Table 7.1. Mean air temperature and amount of precipitation during the experimental years from November 2012 to November 2014 | 111 |
| Table 7.2. Characteristics of the soil (0 to 90 cm) at cover crop sowing in November 2012..... | 111 |
| Table 7.3. Characteristics of the cover crops sown in November 2012..... | 112 |

| | |
|---|-----|
| Table 7.4. The characteristics of the cover crops and their effects on weed density, biomass, species diversity of weed communities (H'), weed species richness(S) and weed species evenness (D) before maize sowing in 2013 | 116 |
| Table 7.5. The percentage of weed species categories (%) relatively to the total weed species density before maize sowing in 2013. Values are mean ($n=6$) | 116 |
| Table 7.6. The quantity of mineral nitrogen (N) remaining in the soil (kg ha^{-1}) at both sampling date for 3-leaf and flowering maize stage under living mulch in both experimental years 2013 and 2014 | 120 |
| Table 7.7. The quantity of mineral nitrogen (N) remaining in the soil (kg ha^{-1}) at 3-leaf and flowering maize stages under the dead mulch system in both experimental years 2013 and 2014 | 128 |
| Table 7.8. The Shannon-Wiener index (H'), species richness (S) and the Simpson evenness index (D) corresponding to the treatments of cover crops compared to no cover treatment under dead mulch system carried out using the means ($n=3$) \pm standard error in both 2013 and 2014. Analysis of variance (ANOVA Type III, sum of squares, $\alpha=0.05$) was carried out to compare treatments at 3-leaf and flowering of maize stage | 129 |
| Table 7.9. Maize biomass at flowering stage and maize grain yield under living and dead mulches in both 2013 and 2014. | 131 |

ENGAGEMENT DE NON PLAGIAT

Je, soussigné(e) Elana DAYOUB

déclare être pleinement conscient(e) que le plagiat de documents ou d'une partie d'un document publiée sur toutes formes de support, y compris l'internet, constitue une violation des droits d'auteur ainsi qu'une fraude caractérisée.

Cet engagement de non plagiat doit être signé et joint

à tous les rapports, dossiers, mémoires.

Thèse de Doctorat

Elana DAYOUB

Compétitivité des légumineuses vis-à-vis des adventices : traits impliqués dans la capture précoce de l'azote minéral du sol et complémentarité des traits entre espèces dans des associations légumineuse-non légumineuse

Competitiveness of legume species against weeds: traits involved in the early soil nitrogen uptake and complementarity of traits in legume-non legume intercropping systems

Résumé

Accroître la compétitivité des cultures vis-à-vis des adventices est un levier essentiel pour concevoir des systèmes de culture moins dépendants des herbicides. Les légumineuses sont des espèces majeures pour diversifier les systèmes mais elles sont réputées peu compétitives vis-à-vis des adventices. Cependant la variabilité entre espèces en particulier pendant la phase précoce a été peu explorée. Ce travail vise à i) étudier, via deux expérimentations en rhizotrons en serre, les traits impliqués dans la croissance et l'acquisition d'azote (N) en début de cycle chez une gamme d'espèces de légumineuse et les conséquences sur la compétition légumineuse-adventice et à ii) quantifier au champ l'intérêt d'associations légumineuse-non légumineuse (colza-légumineuses, maïs-légumineuses pérennes, la légumineuse étant plante de service) sur la croissance et la composition de la flore adventice. Nous montrons qu'il existe une variabilité entre espèces de capture d'N en début de cycle en lien avec les réserves azotées de la semence et l'exploration racinaire. La croissance et l'acquisition d'N de l'adventice sont impactées différemment selon l'espèce de légumineuse pendant la phase précoce. Les essais au champ montrent l'intérêt de combiner des espèces aux traits complémentaires pour l'utilisation des ressources conduisant à un meilleur contrôle des adventices tout en maintenant la productivité de la culture. Cette étude montre aussi que la composition de la flore est modifiée en fonction de la légumineuse insérée dans le système en lien à la fois avec les traits des légumineuses et les traits de réponse des adventices.

Mots clés : Légumineuses, croissance précoce, acquisition d'azote, cultures associées, gestion des adventices

Abstract

Enhancing crop competitiveness against weeds is a relevant way in order to design cropping system less dependent on herbicides. Legumes are key species in diversified cropping systems but they are known to be low competitive against weeds. However, inter-specific variability especially in the early growth had been little studied. Our work aims to i) study, via two greenhouse experiments in rhizotrons, the traits involved in the growth and nitrogen (N) acquisition for a range of legume species and the impacts on weed-legume competition at the beginning of crop cycle and to ii) quantify under field experiments the interest of legume-non legume intercropping (oilseed rape-legumes, maize-perennial legumes, where legume species were grown as service plant) on weed growth and weed species composition. Our results highlighted the inter-specific variability for soil N uptake in the early growth in relation with seed reserves and rooting exploration. Weed growth and N acquisition are influenced differently as a function of legume species in the early growth. Field experiments show the advantage of species combinations with complementary traits for using resources, leading to better weed control while maintaining crop productivity. This study shows also that weed species composition was modified as a function of legume species in relation with both legume traits and weed traits response.

Key Words: Legume species, early growth nitrogen acquisition, intercrops, weed suppression