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# Synthèse bibliographique

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## 1 Le contexte des cultures intermédiaires

### 1.1 La pollution nitrique : un vieil enjeu toujours actuel

Depuis les années 1950, afin d'anticiper la demande grandissante en nourriture en France et en Europe, les rendements par unité de temps et par unité d'espace des principales cultures agricoles ont été significativement augmentés par la modification des techniques culturales. L'intensification de l'agriculture a été rendue possible par une utilisation massive d'intrants chimiques comme les produits phytosanitaires et les fertilisants dont les engrais azotés (Cassman, 1999). Peu réglementée après la Seconde Guerre Mondiale, la consommation européenne d'engrais azotés n'a cessé d'augmenter jusqu'aux années 1990 jusqu'à atteindre à l'heure actuelle un niveau stable aux alentours de 12Mt/an consommés. Au niveau mondial, l'arrivée de pays émergents n'a pas permis d'atteindre une consommation stable puisque celle-ci ne cesse d'augmenter jusqu'à atteindre aujourd'hui un niveau maximal de près de 108Mt pour l'année 2011 (Figure 1). Des scénarios projettent que la consommation mondiale d'engrais azoté risque d'encore tripler d'ici 2050 (Tilman et al., 2002).

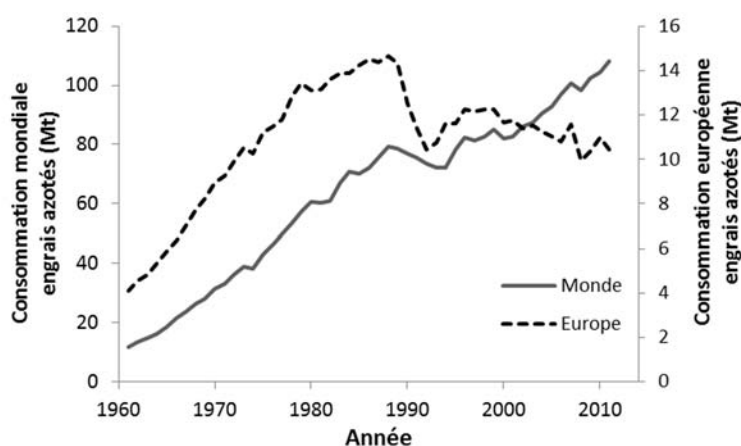


Figure 1: Consommation annuelle mondiale et européenne d'engrais azotés au cours du temps (Source : IFA, 2014)

Par ailleurs, environ 50% de la quantité d'azote synthétique épandue dans le but de maximiser les rendements sont assimilés par les cultures au cours de leur année d'épandage. La quantité restante est, à court terme, soit organisée sous forme de matière organique du sol, soit perdue dans l'environnement sous forme de gaz dans l'atmosphère ( $\text{NH}_3$  et  $\text{N}_2\text{O}$ ). Elle peut également, à moyen terme, être re-minéralisée à partir de la matière organique du sol et, en période d'interculture, être perdue par lixiviation nitrique (transport des solutés azotés présents dans le sol par transfert vertical) vers les nappes phréatiques ou encore par le biais d'écoulement dans les eaux superficielles (Cassman et al., 2002; Ladha et al., 2005; Tilman et al., 2002).

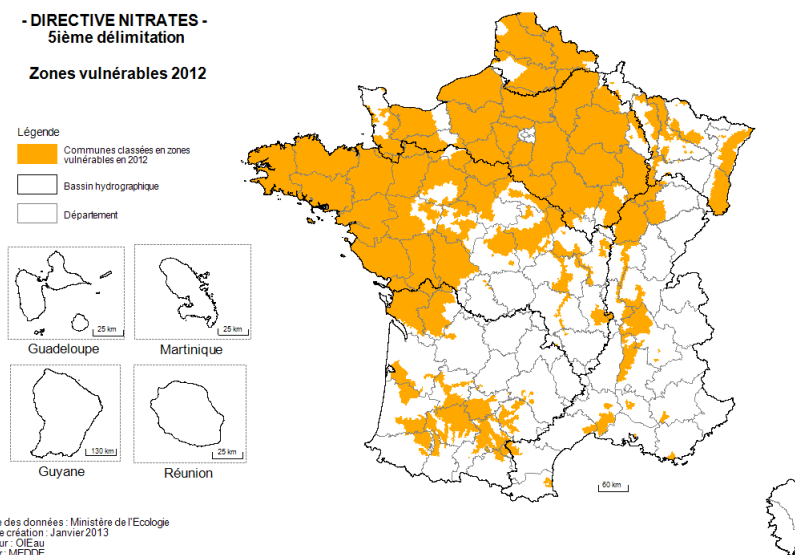
La pollution des eaux souterraines et des eaux de surface par des teneurs élevées en ions nitrate est principalement imputable aux activités agricoles et notamment aux pratiques d'agricultures intensives (Wit et al., 2002). Cette pollution peut parfois être un enjeu de santé publique mais représente surtout un risque environnemental. Pour induire des problèmes de santé humaine, il est nécessaire que le nitrate soit absorbé en quantité importante. Les nitrates, transformés en ions nitrite, peuvent être responsables d'une maladie potentiellement mortelle chez les nourrissons: la méthémoglobine, également appelée syndrome du « bébé bleu ». Ainsi, il est recommandé aux femmes enceintes de ne pas consommer des eaux trop riches en nitrate (norme de potabilité de l'eau de 50 mg  $\text{NO}_3^-/\text{L}$ ). Les ions nitrite se lient à l'hémoglobine et provoquent une diminution du transport de l'oxygène. Chez l'adulte, les ions nitrate sont soupçonnés d'induire des cancers de l'estomac bien que cela soit controversé par plusieurs études (Addiscott and Benjamin, 2004).

La pollution nitrique a également un impact environnemental. Lorsque la teneur en nitrate dans les eaux de surface est trop élevée, l'azote induit une amplification des phénomènes d'eutrophisation lorsque le phosphore n'est pas un facteur limitant dans les hydro-systèmes (Howarth and Marino, 2006); c'est le cas notamment dans les lacs ou en zone côtière où des développements d'algues peuvent se produire (cas des côtes bretonnes en France). L'eau s'enrichit en éléments nutritifs tels que le phosphore et le nitrate, provoquant une prolifération importante de végétaux pouvant entraîner la mort d'une grande partie d'un biotope (Justes et al., 2012a).

Ces phénomènes de perte d'azote sont susceptibles d'être importants dans les zones où l'agriculture intensive est pratiquée depuis longtemps, avec une utilisation parfois excessive de fertilisants chimiques par manque d'ajustement des doses et du fractionnement des apports aux besoins des cultures. C'est aussi, et surtout le cas, dans les zones de concentration d'élevages hors-sol (poulets et porcs) qui peuvent se trouver en excédent structurel d'azote avec une surface d'épandage trop faible pour bien valoriser l'azote des produits organiques recyclés. Les fertilisants chimiques sont soit concentrés en nitrate, soit rapidement convertis sous forme d'ions nitrate dans le sol. Quand cet apport d'azote minéral excède les besoins de la plante et la capacité du sol à organiser et à dénitrifier l'azote minéral non utilisé par la plante, l'azote nitrique en excès peut être lixivié hors de la zone racinaire par la percolation de l'eau à travers le profil du sol. Plusieurs facteurs tels que la texture du sol, la disponibilité en azote minéral du sol, l'excédent pluviométrique, la conductivité hydraulique mais aussi les espèces implantées vont influencer les quantités d'ions nitrate lixiviés vers les nappes phréatiques (Köhler et al., 2006; Singh and Sekhon, 1977).

## 1.2 Contexte réglementaire français et européen

La directive européenne 91/676/CEE dite « Directive Nitrates » a été mise en place dès 1991 afin de limiter les impacts environnementaux et les impacts sur la santé humaine des excès de nitrates d'origine agricole dans les eaux (CEE, 1991). Elle fixe notamment la limite acceptable en ions nitrate dans les eaux à 50 mg NO<sub>3</sub><sup>-</sup>/L pour les eaux de surfaces et à 100 mg NO<sub>3</sub><sup>-</sup>/L dans le cas d'eaux souterraines (Arrêté du 11 janvier 2007) bien qu'une valeur indicative de 25 mg NO<sub>3</sub><sup>-</sup>/L subsiste pour évaluer la qualité des eaux de surfaces et qui correspond à une valeur seuil d'alerte. La « Directive Nitrates » impose aux Etats membres de l'Union Européenne d'identifier sur leur territoire les zones vulnérables c'est-à-dire les zones ayant une concentration en nitrate supérieure à la norme et/ou sujettes au phénomène d'eutrophisation. Chaque nouvelle campagne de surveillance (au moins tous les 4 ans) donne lieu à une révision du zonage. La cinquième et dernière révision des zones vulnérables a eu lieu en 2012 et a défini près de 55% du territoire français comme étant en zone vulnérable, représentant plus des deux-tiers de la SAU (surface agricole utile) occupée par les grandes cultures (Ministère de l'Ecologie et du Développement Durable et de l'Energie, 2013), comme le montre la carte ci-dessous (Figure 2).



**Figure 2: Carte des zones vulnérables françaises en 2012 (Source :Ministère de l'Ecologie et du Développement Durable et de l'Energie, 2013)**

Dans ces zones « vulnérables nitrates » un certain nombre de programmes d'actions ont déjà été mis en œuvre depuis plus de 20 ans et, en dehors de ces zones, l'application du Code des Bonnes Pratiques Agricoles adapté régionalement est recommandée. Les mesures portent notamment sur la gestion des effluents (conditions d'épandage, capacité de stockage), la

limitation de l'utilisation des fertilisants azotés à la juste dose d'engrais basée sur la méthode du bilan prévisionnel, la mise en place d'un plan de fertilisation et d'un cahier d'épandage. La mise en place d'une couverture automnale du sol durant l'interculture longue précèdent une culture de printemps est également une mesure réglementaire. La circulaire du 26 mars 2008 fixant les modalités de mise en œuvre du 4<sup>ème</sup> programme de la « Directive Nitrates », entré en vigueur en juillet 2009, implique le prolongement de la couverture des sols pendant les périodes de risque de lixiviation (périodes pluvieuses) dans l'ensemble des zones vulnérables. Cette couverture du sol peut être une prairie, une culture d'hiver, des repousses de colza, ou une culture intermédiaire. Cette couverture devait atteindre 100% des surfaces cultivées à l'automne 2012, ce qui n'a pas été le cas. Les cinquièmes « programmes d'actions nitrates » entrés en vigueur en novembre 2013 sont définis par l'arrêté du 19 décembre 2011 modifié par l'arrêté du 23 octobre 2013 qui fixe un socle réglementaire national commun, applicable sur l'ensemble des zones vulnérables françaises. Ces 5<sup>èmes</sup> programmes d'action fixent également des mesures supplémentaires dans des zones d'actions renforcées au niveau régional. A titre d'exemple, au niveau de la région Midi-Pyrénées, 34% des surfaces agricoles sont concernées par cette réglementation. Le programme régional précise notamment les règles de mise en place obligatoire des couverts végétaux tels que :

- Date de limite d'implantation du couvert au 20 septembre pour une durée minimale de deux mois,
- Date de destruction possible à partir du 1<sup>er</sup> novembre ou 1<sup>er</sup> octobre en terrain argileux,
- Exemption d'implantation de cultures intermédiaires piège à nitrate (CIPAN) en zone argileuse si en interculture longue au moins 20% des surfaces sont couvertes.

Dans certaines régions il peut également être précisé que les cultures pures de légumineuses en interculture sont interdites ou bien un pourcentage seuil de légumineuse en mélange avec une CIPAN est fixé.

Au-delà de l'aspect strictement réglementaire, la maîtrise de la gestion de l'azote représente un enjeu important pour réduire les pollutions nitriques, y compris en zones de grandes cultures sans élevage lorsque la fertilisation azotée est correctement ajustée. En effet, certains systèmes de cultures induisent de très longues périodes d'interculture sans plante pour absorber l'azote minéral produit par la minéralisation en azote des matières organiques humifiées du sol, ce qui va contribuer aux pertes d'azote hivernales et printanières par lixiviation de l'azote nitrique et ainsi induire la pollution des eaux souterraines (Justes et al., 2012a).



### 1.3 Le cycle de l'azote et les sources de nitrate

La pollution nitrique des eaux de surface et souterraines est principalement due à un transfert en profondeur, au-delà de la portée des racines des ions nitrate dissous dans l'eau percolée. La quantité d'ions nitrate susceptibles d'être ainsi lixiviés est dépendante du pool d'azote organique et minéral dans le sol, des processus biochimiques de transformation de l'azote ainsi que des entrées (résidus, fertilisants...) et des sorties d'azote dans le système (lixiviation, volatilisation...) (Figure 3).

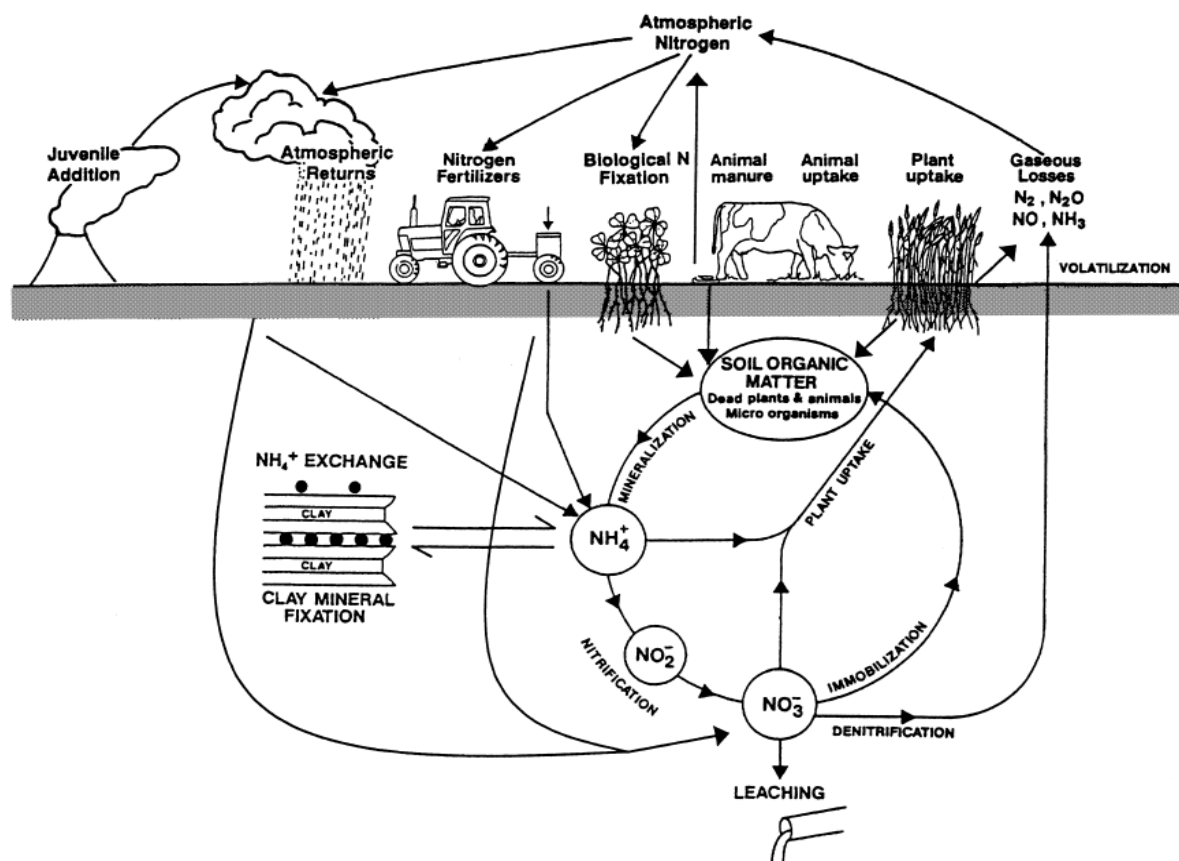


Figure 3 Schéma du cycle de l'azote (Di and Cameron 2002 d'après McLaren and Cameron 1990)

Dans le sol, l'azote est un élément sous différentes formes chimiques qui sont en perpétuel changement ; la très grande majorité de l'azote du sol est sous forme d'azote organique (plus de 95%) et le reste sous forme d'azote minéral, qui est labile et sujet aux pertes. Ce pool d'azote organique du sol n'est pas directement assimilable par les plantes, il est susceptible de se transformer en azote minéral par les processus de minéralisation. Les apports d'engrais azotés et les amendements organiques, les résidus de cultures participent à la formation de la matière organique humifiée du sol via la macrofaune et la biomasse microbienne de l'écosystème du sol. L'azote minéral est principalement constitué d'azote sous forme ammoniacale ( $NH_4^+$  et  $NH_3$  pour sa forme gazeuse qui contribue à la volatilisation) et sous

forme nitrique ( $\text{NO}_3^-$ ), passant d'un état à l'autre par les processus d'ammonification et de nitrification. Les bactéries nitrificatrices du sol oxydent rapidement les cations ammonium en anions nitrate ou azote nitrique ( $\text{NO}_3^-$ ). C'est pourquoi, les ions ammonium sont «transitoires» en conditions de sol aérobie. L'azote nitrique est très soluble et donc facilement transporté par les mouvements d'eau dans le sol (Di and Cameron, 2002). L'eau chargée en ions nitrate percole ensuite jusqu'aux nappes phréatiques et rejoint les eaux de surface (Bundy and Andraski, 2005; Howarth, 1988). L'azote absorbé par les plantes se présente sous forme minérale, sous forme de nitrates ( $\text{NO}_3^-$ ) et sous forme d'ions ammonium ( $\text{NH}_4^+$ ). La cinétique d'absorption des plantes varie en fonction de l'espèce et du stade végétatif : faible au moment de la germination puis maximale en période végétative puis réduite en phase de maturation (e.g. Turpin, Vernier and Joncour 1997).

Ces différents processus sont également régis par des facteurs environnementaux tels que la température, l'humidité du sol ou encore le pH et certaines propriétés physico-chimiques. Au cours de l'automne, la minéralisation de l'azote organique du sol peut être forte (sol chaud et humide), et par conséquent de fortes quantités d'ions nitrate peuvent être présents dans les sols en fin d'automne et au début de la période de drainage (Meisinger et al., 1991). Lorsque le sol contient de grandes quantités de nitrate au cours de la période avec de fortes précipitations et qu'aucune plante n'est présente pour intercepter cet azote minéral, il se produit une lixiviation des ions nitrate vers les aquifères (Justes et al., 2012a). Cette lixiviation est influencée par les pratiques culturales et les processus du sol tels que la fertilisation, la minéralisation, l'acquisition des nitrates par les plantes et les transferts (notamment verticaux) des éléments du sol (Addiscott and Whitmore, 1991; Beaudoin, 2006; Hoffmann and Johnsson, 1999).

## **2 Les cultures intermédiaires et les services écosystémiques**

### **2.1 Cultures intermédiaires : définitions et terminologie**

L'interculture est définie comme la période entre deux cultures principales, ou cultures de vente (Figure 4). Elle correspond à l'intervalle de temps entre la récolte d'une culture principale précédente et le semis de la suivante. Sa durée peut varier de quelques jours jusqu'à 9 mois (Machet et al., 1997) et s'étend généralement entre la fin de l'été, l'automne et parfois l'hiver. Durant cette période le sol peut être laissé nu (ou sans plante) ou peut être couvert avec une culture d'hiver semée (ex. colza), des repousses de la culture précédente ou encore une « culture intermédiaire ». Les cultures intermédiaires n'ont pas vocation à être récoltées ni

exportées. Elles sont détruites puis incorporées au sol avant la culture suivante pour produire des fonctions écosystémiques.

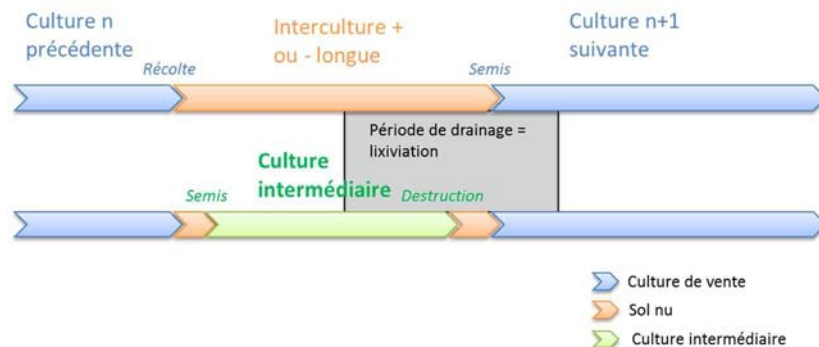


Figure 4 Schéma de la gestion de l'interculture avec et sans culture intermédiaire (adapté Justes et al 2012)

Les cultures intermédiaires sont souvent désignées par plusieurs termes en fonction de l'objectif recherché, il est donc judicieux de définir chacun de ces termes afin d'éviter toute confusion. En effet, parmi les cultures intermédiaires on distingue notamment la notion de « cultures intermédiaires pièges à nitrate » (CIPAN ou *catch crop* en anglais), de culture de couverture (terme souvent générique de *cover crop* en anglais), ou encore d'« engrais verts » (*green manure* en anglais) (review de Thorup-Kristensen et al., 2003). Une CIPAN est définie comme une culture dont le rôle premier est de piéger l'azote minéral présent dans le sol afin d'éviter les pertes de nitrate par lixiviation. Elles sont donc généralement implantées dans des zones où la fertilisation s'est révélée excédentaire et le potentiel de minéralisation du sol en interculture est élevé. Par ailleurs, une culture intermédiaire dont l'objectif majeur est de fournir de l'azote à la culture suivante sera plutôt qualifiée d'« engrais vert ». Ce type de culture est souvent composé de légumineuses (plantes fixatrices d'azote atmosphérique) afin d'enrichir le couvert végétal en azote et ainsi accroître la quantité d'azote qui sera disponible pour la culture suivante après décomposition et minéralisation des résidus incorporés ou laissés à la surface du sol (Justes et al., 2012a). Les couverts d'engrais verts sont donc généralement mis en place dans des systèmes de production à bas niveau d'intrants comme en situation d'Agriculture biologique afin d'utiliser le plus possible de ressources naturelles en azote. Enfin, les « couverts végétaux » sont souvent utilisés en agriculture de conservation des sols. Une des fonctions de ces couverts végétaux est de produire une protection physique des sols afin de lutter contre l'érosion et d'améliorer les propriétés physiques et hydrodynamiques des sols (e.g. Folorunso et al., 1992).

## **2.2 Les services écosystémiques rendus par les cultures intermédiaires**

Le Grenelle de l'Environnement de 2005 a mis en exergue la notion d'agriculture écologiquement intensive, qui fait écho à l'agroécologie, afin de s'inspirer des écosystèmes pour concevoir des agrosystèmes innovants utilisant moins d'intrants de synthèse (Griffon, 2006). Cela se traduit par la nécessité de produire en quantité et qualité des produits agricoles tout en préservant l'environnement ; cela veut dire qu'il faut valoriser au mieux les ressources naturelles disponibles et renouvelables pour produire des services écosystémiques. Les services écosystémiques sont définis comme des bénéfices que tire la société de la biodiversité et du fonctionnement des écosystèmes (Daily, 1997; Ehrlich and Mooney, 1983; Millennium Ecosystem Assessment, 2005). Les services peuvent être répertoriés sous trois catégories (Zhang et al., 2007):

- les services intrants qui participent à la fourniture de ressources, au maintien des supports de la production agricole et qui assurent la régulation des interactions biologiques,
- les services d'approvisionnement contribuant au revenu agricole,
- les externalités positives qui incluent le contrôle de la qualité des eaux, la séquestration du carbone ou la valeur paysagère, et qui ne contribuent généralement pas au revenu agricole direct.

De par cette définition, les cultures intermédiaires peuvent fournir différents types de services écosystémiques tels que des services intrants contribuant à la fourniture de ressources pour la culture suivante, le maintien voire l'amélioration de la fertilité des sols par un recyclage des éléments minéraux, la stabilité de la structure des sols et des services de préservation de la qualité des eaux et de séquestration du carbone dans les sols (Schipanski et al., 2014). Ainsi on peut considérer que le piégeage de l'azote par les cultures intermédiaires permettant une réduction de l'azote lixivié et donc une amélioration de la qualité de l'eau est un service de type production hors revenu agricole. L'effet engrais vert correspond à une meilleure disponibilité en azote pour la culture principale et peut donc être considéré comme un service de type intrant.

### **2.2.1 Les services écosystémiques liés à la gestion de l'azote**

La mise en place de ces couverts végétaux en interculture influe donc sur le cycle de l'azote au sein de l'agroécosystème en modifiant deux flux (Figure 5):

- l'absorption d'une partie de l'azote minéral par les plantes avant les périodes de drainage (diminution de la lixiviation), et donc la sauvegarde et le recyclage de l'azote nitrique ;

- la restitution partielle d'azote à la culture suivante par minéralisation des résidus après destruction du couvert et le stockage de l'autre partie sous forme d'azote organique dans la matière organique du sol, permettant un entretien de la fertilité du sol.

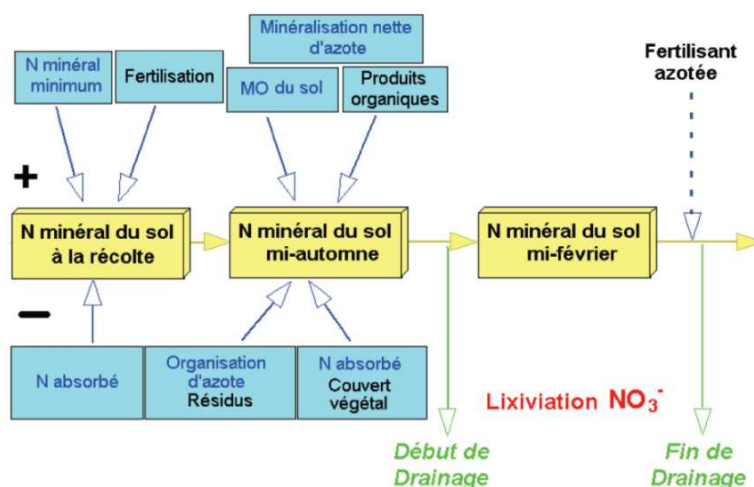


Figure 5 Schéma de l'évolution du stock d'azote minéral du sol (source: Justes et al., 2012 d'après Machet et al., 1997)

### 2.2.1.1 Les CIPAN et le piégeage des ions nitrate

En France, et dans les autres pays à climat tempéré, la période automnale se caractérise généralement par une baisse des températures induisant un ralentissement de la croissance des plantes, et un excédent pluviométrique produisant du drainage et donc de la lixiviation nitrique (Meisinger et al., 1991). Ainsi, pour que le piégeage de l'azote nitrique soit optimal, il est nécessaire d'implanter la culture intermédiaire suffisamment tôt en été afin qu'elle puisse absorber et donc piéger l'azote minéral du sol avant la période de drainage. Par exemple, dans la moitié Nord de la France, un semis entre le 05 août et le 20 août permet une acquisition maximale de l'azote (Dorsainvil, 2002). Ainsi, un couvert semé en fin d'été, après la récolte de céréale ou de colza, sera capable d'absorber une partie de l'azote minéral non absorbé par la culture principale et celui provenant de la minéralisation du sol. A titre d'illustration, Vos and van der Putten (2004) montrent que des couverts installés en août au Pays-Bas et détruits en fin d'automne peuvent piéger jusqu'à 180 kg N ha<sup>-1</sup>. De nombreuses études ont montré que les cultures intermédiaires sont un moyen efficace de réduire l'azote minéral du sol pendant la période de drainage et qu'elles ont un effet significatif sur la réduction de la lixiviation (Aronsson and Tortensson, 1998; Beaudoin et al., 2005; Constantin et al., 2011; Justes et al., 2012a; Thomsen and Hansen, 2014; Thorup-Kristensen et al., 2003; Tonitto et al., 2006; Vos and van der Putten, 2004). Leur efficacité en termes de piégeage de l'azote nitrique a souvent été étudiée dans la littérature à travers la quantité d'azote acquis dans le sol par les plantes (Kristensen and Thorup-Kristensen, 2004; Munkholm and Hansen,

2012; Thorup-kristensen and Nielsen, 1998). De nombreuses espèces, notamment de la famille des Poacées ou des Brassicacées ou encore la phacélie (Hydrophyllacée), ont démontré pouvoir acquérir des quantités importantes d'azote en quelques mois en fin d'été et durant l'automne, également lorsque les reliquats d'azote au moment du semis des couverts étaient élevés (Toffoli et al., 2010). Certaines études ont également quantifié la capacité de certaines espèces à réduire la lixiviation de nitrate jusqu'à plus de 70% par rapport à un sol nu sur du court terme (Hansen et al., 2007; Tonitto et al., 2006; Wyland et al., 1996) et jusqu'à 36% sur du long-terme à l'échelle de la rotation (Constantin et al., 2012). Certaines espèces telles que l'avoine, la phacélie, la moutarde blanche ou encore le radis fourrager peuvent capter l'azote minéral en profondeur dans le sol, même au-delà d'un mètre de profondeur grâce à de puissantes racines qui se développent rapidement (Thorup-Kristensen, 1994). Ainsi, la précocité et la rapidité de croissance, notamment racinaire, sont deux critères importants puisque c'est l'azote présent dans les horizons les plus profonds qui est le plus susceptible d'être lixivié (Thorup-kristensen and Nielsen, 1998).

Par ailleurs, bien qu'elles aient la capacité d'acquérir de l'azote via la fixation symbiotique, les légumineuses peuvent également avoir un effet non négligeable sur le piégeage de l'azote minéral ainsi que sur la lixiviation nitrique (Askegaard and Eriksen, 2008; Meisinger et al., 1991; Thorup-Kristensen, 1994; Tonitto et al., 2006). En revanche, il semble que, globalement, les légumineuses restent moins efficaces que les non-légumineuses en termes de piégeage du nitrate (Askegaard and Eriksen, 2008) et qu'elles peuvent même dans certains cas conduire à une augmentation de la teneur en azote nitrique du sol après incorporation précoce et donc augmenter le risque de lixiviation nitrique (Muñoz-Carpena et al., 2008).

Outre la fonction de piégeage des ions nitrate, l'implantation de cultures intermédiaires peut favoriser la restitution d'azote pour la culture principale suivante par un recyclage efficace de l'azote et la production d'un effet « engrais vert ».

### **2.2.1.2 L'effet « engrais vert »**

Après la destruction des cultures intermédiaires, leurs résidus peuvent être laissés en surface ou incorporés dans le sol. Les processus de décomposition des résidus par la microfaune du sol et leur minéralisation permettent la libération d'azote minéral dans le sol qui sera disponible pour la culture suivante. Une partie de l'azote des résidus est rapidement minéralisée tandis que l'autre partie est humifiée et ne sera minéralisée qu'après plusieurs années. La quantité d'azote minéralisé à partir des résidus de culture sera d'autant plus rapide que les résidus possèdent un rapport C/N faible (Trinsoutrot et al., 2000). La fraction de

l'azote rapidement minéralisé après la destruction de la culture intermédiaire pourra être utilisée par la culture principale suivante et ainsi induire des augmentations de rendements (Askegaard and Eriksen, 2007; Thomsen, 1993; Thorup-Kristensen, 1994; Thorup-Kristensen et al., 2003). Par ailleurs, les légumineuses semblent particulièrement efficaces comme « engrais vert » car elles peuvent acquérir de grandes quantités d'azote via la fixation symbiotique, réduire le C/N du couvert par amélioration du niveau de nutrition azotée puisqu'elles ne seront généralement pas en déficience en azote pour la croissance et qu'elles auront donc une teneur élevée en azote dans leurs tissus. Ceci va permettre une libération importante et plus rapide de l'azote pour la culture suivante (Peoples et al., 1995; Stopes et al., 1996; Tonitto et al., 2006; Touchton et al., 1984). Certaines espèces telles que les trèfles et les vesces peuvent induire une restitution de 50 à 370 kg N ha<sup>-1</sup> (Peoples et al., 1995; Tonitto et al., 2006). De plus, la culture suivante peut acquérir près de 32% de l'azote total acquis provenant de l'azote minéralisé à partir des résidus de culture intermédiaire (Peoples et al., 2004). Bien que les non-légumineuses puissent acquérir d'importantes quantités d'azote, elles restent généralement moins efficaces que les légumineuses comme « engrais vert » car leur C/N est généralement plus élevé (Justes et al., 2009). Par exemple, il a été montré qu'un couvert de trèfle peut induire un rendement de la culture principale suivante presque 3 fois supérieur à un couvert de raygrass (Stopes et al., 1996).

En effet, les quantités d'azote acquises puis restituées à la culture suivante sont fortement liées la quantité d'azote contenue dans le couvert végétal mais aussi au rapport C/N de la culture intermédiaire. Le ratio C/N de la culture intermédiaire au moment de sa destruction est un paramètre clé contrôlant la minéralisation des résidus organiques après leur incorporation dans le sol, il contrôle ainsi la libération plus ou moins rapide de l'azote sous forme minérale qui sera susceptible d'être acquis par la culture principale suivante (Kumar and Goh, 2002; Quemada and Cabrera, 1995; Tejada et al., 2008a). Ainsi, plus le C/N des résidus est faible et plus la minéralisation est rapide. En revanche si le C/N est élevé, des phénomènes d'organisation nette (ou immobilisation) de l'azote peuvent conduire à une diminution de la quantité d'azote disponible pour la culture suivante (Francis et al., 1998; Justes et al., 2009; Trinsoutrot et al., 2000). Cependant, les facteurs pédoclimatiques, notamment la température et l'humidité, ainsi que les techniques culturales (travail du sol) peuvent également influencer la minéralisation des résidus (Valé, 2006).

Ainsi, la restitution d'une partie de l'azote acquis par les cultures intermédiaires peut réduire les apports de fertilisants de synthèse ce qui correspond à un service intrant qui peut devenir intéressant dans le contexte actuel d'augmentation du prix de l'énergie et de diminution de la

dépendance de l'agriculture aux intrants de synthèse. De plus, les systèmes de culture comprenant régulièrement l'implantation de cultures intermédiaires semblent être moins sensibles aux variations annuelles de rendement, notamment dans les systèmes en agriculture biologique (Olesen et al., 2007).

### 2.2.2 Les autres services écosystémiques

Lorsqu'elles sont bien conduites, l'intérêt des cultures intermédiaires ne se restreint pas au piégeage des ions nitrate et à l'effet « engrais vert » pour la culture suivante. On compte de nombreux autres services écosystémiques potentiellement produits.

Les cultures intermédiaires peuvent notamment avoir un impact positif sur la protection et la structure du sol. D'une part grâce à leurs parties aériennes qui couvrent le sol et peuvent intercepter les gouttes de pluies avant qu'elles n'atteignent le sol. Ainsi, l'érosion des sols peut être limitée (Kaspar et al., 2001; Malik et al., 2000; Ryder and Fares, 2008). Par ailleurs, l'enracinement des cultures intermédiaires permet également de structurer le sol et d'éviter des phénomènes de compaction ce qui peut permettre de faciliter l'exploration racinaire de la culture suivante (De Baets et al., 2011; Williams and Weil, 2004).

Par ailleurs il a également été reporté des effets des cultures intermédiaires sur la vie du sol puisque la biomasse microbienne du sol semble favorisée (Dinesh et al., 2006; Tejada et al., 2008b). On note également une augmentation du stockage de matières organiques (azote et carbone) sur le long terme (Destain et al., 2010; Mutegi et al., 2013).

Quelques études ont également mis en avant la capacité des cultures intermédiaires à réduire légèrement les émissions de gaz à effet de serre, notamment le N<sub>2</sub>O (Baggs et al., 2003; McSwiney et al., 2010; Parkin et al., 2006). Cependant d'autres études ne montrent pas d'effet significatif sur la réduction des émissions de N<sub>2</sub>O (Petersen et al., 2011; Sarkodie-Addo et al., 2003).

Enfin, les cultures intermédiaires peuvent également avoir un impact sur les bio-agresseurs et notamment sur les adventices. La réduction de la pression des adventices par les couverts peut s'effectuer par un effet compétitif, un effet allélopathique ou encore en réduisant le stock semencier ou l'aptitude à la germination des adventices (Campiglia et al., 2010; Dhima et al., 2006; Hertwig Bittencourt et al., 2013; Pullaro et al., 2006; Webster et al., 2013). Certaines études montrent également une réduction des ravageurs, notamment les nématodes par des espèces et des variétés *ad hoc* de crucifères, et des maladies par l'implantation de cultures intermédiaires (Snapp et al., 2005). Néanmoins les cultures intermédiaires peuvent aussi produire des dis-services en favorisant certains ravageurs (ex. limaces) si l'espèce et



l'itinéraire technique sont mal adaptés ; ces effets restent néanmoins insuffisamment documentés (Justes et al., 2012a).

### **3 Les mélanges bispécifiques de cultures intermédiaires**

Aujourd'hui, bien que la culture monospécifique ou « pure » reste le modèle dominant en France et en Europe, notamment avec des couverts de crucifères (ex. moutarde blanche dans la moitié Nord de la France en système betteravier) des associations d'espèces avec des légumineuses sont utilisés, notamment en agriculture de conservation, afin d'économiser des intrants comme l'azote et d'augmenter les services écosystémiques produits simultanément par les couverts de cultures intermédiaires.

#### **3.1 Généralités sur les associations d'espèces**

La culture en association ou en mélange d'espèces se définit comme une culture simultanée de deux espèces ou plus, sur la même parcelle et pendant une durée significative de leur cycle de croissance (Willey, 1979). Cette pratique est basée sur l'hypothèse que lorsqu'on cultive plusieurs espèces en association, certains avantages se produisent par rapport aux cultures monospécifiques « pures » de ces mêmes espèces. L'association d'espèces permettrait notamment d'optimiser l'utilisation des ressources naturelles comme l'azote, la lumière, le carbone et l'eau (Hauggaard-Nielsen et al., 2003). En effet, dans les systèmes de productions vivrières, de nombreux avantages ont été démontrés tels qu'une augmentation de la productivité et des rendements des cultures en association (Hauggaard-Nielsen and Jensen, 2001; Hauggaard-Nielsen et al., 2003), une augmentation de la teneur en protéines des graines (Bedoussac and Justes, 2010a) et une diminution de la pression des maladies et des ravageurs (Hauggaard-Nielsen and Jensen, 2001; Sekamatte et al., 2003; Trenbath, 1993) et des adventices (Corre-Hellou and Crozat, 2005; Fenández-Aparicio et al., 2007; Hauggaard-nielsen et al., 2001).

L'objectif d'un mélange d'espèces est d'améliorer le partage des ressources entre les espèces par des phénomènes d'interactions interspécifiques régissant le développement des espèces telle que la complémentarité de niche, ou encore la facilitation, tout en limitant les effets négatifs de fortes compétitions (Vandermeer, 1989). La compétition intervient quand l'une des espèces modifie l'environnement de façon négative pour l'autre, ce qui diminue les chances de survie, la croissance ou la reproduction d'au moins une des espèces (Crawley, 1997). La complémentarité de niche peut être définie comme une utilisation complémentaire

des ressources par les deux espèces, leur permettant de ne pas entrer en compétition au niveau des mêmes niches de ressources (Ofori and Stern, 1987). Enfin, la facilitation est définie comme étant la modification de l'environnement par une espèce qui profite à l'autre espèce associée, ce qui permet une meilleure production globale du couvert (Høgh-Jensen and Schjoerring, 2010).

Les mélanges les plus étudiés sont principalement composés d'une espèce légumineuse associée à une espèce non-légumineuse. Pour les cultures de vente, c'est généralement une céréale associée à une légumineuse à graine en raison de la complémentarité pour l'utilisation de l'azote (Hauggaard-Nielsen et al., 2001; Jensen, 1996b; Ofori and Stern, 1987). En effet, la légumineuse possède la faculté de fixer l'azote atmosphérique (N<sub>2</sub>) grâce à la fixation symbiotique, laissant ainsi l'azote minéral du sol disponible pour la non-légumineuse. De plus, il a aussi été démontré que les racines de la non-légumineuse induisent une compétition pour l'azote minéral dans les horizons superficiels, « obligeant » la légumineuse à capter l'azote atmosphérique, induisant ainsi une augmentation du taux de fixation symbiotique (Ghaley et al., 2005; Tofinga et al., 2009). Par ailleurs, des phénomènes de facilitation ont également été démontrés dans certaines études, notamment un effet positif de la légumineuse pour l'absorption de l'azote par la céréale grâce à une production d'exsudats racinaires riches en azote assimilable par la céréale (Jensen, 1996a; Stern, 1993; Xiao et al., 2004).

Afin de maximiser les services écosystémiques rendus par les mélanges d'espèces ou associations, il est nécessaire que les différentes espèces associées puissent se développer de manière suffisante et en complémentarité (Kramberger et al., 2013). En cultures associées il est communément observé qu'une des espèces se développe plus rapidement que l'autre (ou les autres). L'environnement dans lequel se développent les espèces se modifie au cours de la saison de culture et les compétitions entre les espèces peuvent évoluer au cours de leur croissance (Ofori and Stern, 1987). Les associations sont généralement plus productives lorsque les cultures ont des durées de croissance grandement différentes car leurs demandes maximales en ressources sont décalées dans le temps. Pour une forte productivité de l'association, les plantes ayant une maturité précoce se développent avec peu d'interférence sur le développement d'une culture à maturité tardive. L'espèce tardive peut être affectée d'une certaine manière par l'autre espèce mais une période suffisamment longue après la destruction de la première espèce peut permettre une bonne récupération ainsi qu'une utilisation entière des ressources disponibles (Fukai and Trenbath, 1993). Dans le cas d'une association d'une céréale avec une légumineuse, l'espèce dominante est généralement la céréale car elle possède un système racinaire et un système aérien à croissance rapide.

L'espèce se développe donc plus rapidement que la légumineuse -dont la fixation symbiotique de l'azote atmosphérique se produit plus tardivement, lui donnant, ainsi un avantage compétitif sur l'autre espèce (Andersen et al., 2004; Jensen, 1996b). Cependant, la dominance de la céréale qui s'exerce au début du développement n'empêche pas pour autant la légumineuse de se développer si la densité de la céréale est bien adaptée, ce qui indique que les différences physiologiques jouent un rôle dans la dynamique de dominance. Cette complémentarité dans l'espace est expliquée par une différence du type d'enracinement et des mécanismes de prélèvements de ressources du sol (eau, minéraux) entre la céréale et la légumineuse, on observe notamment une utilisation plus efficace de l'azote minéral disponible (Hauggaard-Nielsen et al., 2006).

### **3.2 Mutualisation des services de la gestion de l'azote par les mélanges**

#### **bispécifiques de cultures intermédiaires**

Les associations d'espèces comprenant une légumineuse appliquées aux cultures intermédiaires sont beaucoup moins étudiées dans la littérature scientifique que les mélanges de cultures de vente, tout au moins quant au fonctionnement du couvert et aux phénomènes de compétition et de complémentarité, voire de facilitation. Néanmoins quelques études, portant sur un nombre restreint de mélanges, permettent de mettre en évidence certains avantages. En effet, associer une espèce légumineuse et une espèce non-légumineuse comme culture intermédiaire permettrait de mutualiser les effets de piégeage des nitrates et de réduction de la lixiviation tout en ayant un effet engrais vert pour la culture suivante (Kuo and Sainju, 1998; Möller and Reents, 2009). Cette mutualisation des deux types de services écosystémique est possible grâce à la complémentarité de niche pour l'azote (Jensen, 1996b). En effet, l'espèce de non-légumineuse serait plutôt attendue pour piéger l'azote minéral du sol et ainsi réduire la lixiviation (Meisinger et al., 1991; Thomsen and Hansen, 2014), et la légumineuse associée serait plutôt performante pour produire un effet engrais vert en introduisant davantage d'azote ( $N_2$ ) grâce à la fixation symbiotique (Ranells and Wagger, 1997; Wortman et al., 2012a). Cependant, chacune des deux espèces peut contribuer à fournir les deux types de services.

Plusieurs études ont montré que les mélanges de cultures intermédiaires comprenant une légumineuse permettaient une augmentation de productivité mais aussi de production de services écosystémiques (Kramberger et al., 2013; Kuo and Sainju, 1998; Möller et al., 2008; Ranells and Wagger, 1997; Summers et al., 2014; Tosti et al., 2012b). En effet, certains mélanges bispécifiques de type légumineuse/non-légumineuse utilisés comme cultures

intermédiaires permettent un effet engrais vert proche de celui des cultures pures de non-légumineuse. Par exemple, selon Ranells and Wagger (1996), un mélange de seigle avec de la vesce velue permettrait de restituer une quantité proche de la culture pure de vesce velue : 132 kg ha<sup>-1</sup> pour le mélange, 108 kg ha<sup>-1</sup> pour la vesce en pure et 41 kg ha<sup>-1</sup> pour le seigle en pure, 8 semaines après la destruction des couverts. De plus, l'introduction de légumineuse dans le mélange permet de diminuer le ratio C/N comparé à la culture pure de non-légumineuse, notamment pour les mélanges ray-grass/ trèfle incarnat, seigle/ vesce velue ou trèfle incarnat... (Kramberger et al., 2013; Ranells and Wagger, 1996; Sullivan et al., 1991; Tosti et al., 2012a). Ainsi réduire le C/N induit une meilleure minéralisation des résidus et une quantité d'azote plus rapidement assimilable pour la culture suivante. En effet, les cultures pures de non-légumineuses présentent un risque de préemption d'azote en raison de leur C/N souvent élevé limitant la minéralisation nette de l'azote des résidus. Cependant, l'effet sur la culture suivante, notamment l'impact sur son rendement, est très variable et dépend des conditions pédoclimatiques mais aussi de l'itinéraire technique tel que la date et la méthode de destruction des couverts (Wortman et al., 2012a). Globalement, les mélanges de culture intermédiaire comprenant une légumineuse et une non-légumineuse ont un impact neutre ou positif sur les rendements de la culture suivante (Clark et al., 1997, 1994; Kramberger et al., 2014) alors que les cultures intermédiaires « pures » non-légumineuses produisent souvent un effet négatif sur les rendements de la culture suivante (Kramberger et al., 2009; Rüegg et al., 1998; Salmerón et al., 2010; Thorup-Kristensen and Dresbøll, 2010).

Par ailleurs, ce type de mélange peut également s'avérer efficace pour réduire l'azote minéral résiduel dans le sol (Ranells and Wagger, 1997) et la lixiviation de l'azote nitrique. Ces mélanges peuvent parfois même présenter la même aptitude à piéger l'azote que les cultures pures de non-légumineuses, principalement lorsque les conditions du milieu sont relativement pauvres en azote minéral. C'est notamment le cas pour les mélanges de radis/vesce ou pois et d'orge/vesce velue (Möller and Reents, 2009; Tosti et al., 2012b). Bien que les conditions du milieu influencent grandement la lixiviation de l'azote nitrique (Di and Cameron, 2002), dans le cas du mélange orge/vesce velue, l'effet de réduction la quantité d'azote lixivié a été démontré au cours du cycle de végétation mais aussi après la destruction et l'incorporation des couverts (Tosti et al., 2012b). Cela peut notamment être expliqué par un enracinement des deux espèces plus profond en mélange qu'en cultures pures (Li et al., 2006; Tosti and Thorup-Kristensen, 2010).

### **3.3 La problématique du choix des espèces utilisées en culture intermédiaire**

Il existe une large gamme d'espèces susceptibles d'être utilisées comme cultures intermédiaires appartenant à divers familles botaniques : voir Annexe 1 pour une liste de 34 espèces, en plus de ces espèces, le lin (*Linum usitatissimum*), le radis chinois (*Raphanus sativus longipinnatus*), le millet perlé fourrager (*Pennisetum glaucum*) ou encore le trèfle de Perse (*Trifolium resupinatum*) et le trèfle souterrain (*T. subterraneanum*) peuvent également être semées comme cultures intermédiaires (Arvalis, 2011; Ingels et al., 1998). Ces espèces ont des propriétés différentes en termes de vitesse de développement et d'aptitude à acquérir l'azote minéral bien qu'une description détaillée des différentes espèces soit encore manquante dans la littérature (Bodner et al., 2010). Ainsi, le choix des espèces à implanter est à réfléchir en fonction de la durée de l'interculture, de la disponibilité en azote résiduel après la récolte de la culture précédente, du pédoclimat (sol filtrant, fortes précipitations, température élevée au semis..) mais aussi de l'aptitude des espèces à explorer les différents horizons du sol (Kristensen and Thorup-Kristensen, 2004; Thorup-Kristensen et al., 2003). La rotation des cultures est également à prendre en compte pour limiter l'utilisation de cultures intermédiaires de la même famille que les cultures principales afin de rompre les cycles d'organismes nuisibles. La phacélie (*Phacelia tanacetifolia*), est un exemple d'espèce de culture intermédiaire utilisable à cet effet car elle appartient à la famille des Hydrophyllacées dont aucune autre espèce n'est cultivée dans les systèmes de production de France et d'Europe et a donc très peu de chance d'être une plante hôte de maladies ou de ravageurs des cultures principales. Les principales espèces de type non-légumineuses utilisées en cultures intermédiaires sont principalement des crucifères (ou Brassicées) et des graminées (ou Poacées). Les graminées, telles que l'avoine rude ou le ray-grass sont reconnues pour avoir une croissance plutôt lente et être résistantes au froid, leur permettant ainsi de maintenir un couvert dans des intercultures longues (Fageria et al., 2005). Cependant cette famille, présentent également des espèces de type C<sub>4</sub> tels que le moha ou le sorgho fourrager qui sont, elles, plus rapides pour se développer mais très sensibles au gel (gel dès -1°C). Par ailleurs, les crucifères telles que la moutarde blanche ou la navette fourragère sont généralement mises en place pour leur rapidité d'installation tant au niveau aérien que souterrain leur permettant, ainsi de capter rapidement de grandes quantités d'azote minéral (Thorup-Kristensen, 2001). De plus, certaines espèces et variétés sont également connues pour avoir des effets contre les nématodes, soit en limitant leur multiplication, soit en limitant les dégâts occasionnés par les nématodes nuisibles (Liébanas and Castillo, 2004). Les crucifères possèdent également des propriétés d'allélopathie pouvant réduire la pression des adventices par des effets biocides

(Dam et al., 2008; Kirkegaard and Sarwar, 1998). En revanche cette propriété peut également induire un effet négatif sur une espèce associée, il faudra donc veiller à limiter les effets allélopathiques en association d'espèces afin que l'espèce associée puisse croître correctement.

Pour qu'un mélange d'espèces soit performant pour produire des services écosystémiques donnés, il est important de choisir des espèces dont les périodes de semis et la vitesse de croissance ainsi que la hauteur de couvert n'induisent pas une compétition excessive pour l'accès à la lumière d'une des espèces. De plus, pour limiter les fortes compétitions que peuvent exercer les non-légumineuses sur les légumineuses, dues à des vitesses de développement plus rapides, ces dernières doivent établir leur fixation symbiotique de l'azote atmosphérique rapidement et avoir une capacité importante d'absorption des rayonnements lumineux pénétrant à travers le couvert végétal de l'espèce associée (Hauggaard-Nielsen and Jensen, 2001), ce qui est d'autant plus important que leur vitesse d'implantation étant généralement assez lente. Le choix des espèces à associer doit donc finalement être raisonné en fonction des caractéristiques des espèces et des services attendus.

## **4 Influence de l'implantation et de la destruction des cultures intermédiaires sur le niveau de service attendu**

La mise en place de cultures intermédiaires durant l'interculture de fin d'été et d'automne peut apporter divers services écosystémiques. Pour cela, il est nécessaire de prêter attention à leur implantation, afin de garantir un couvert homogène, et leur destruction, afin de limiter d'éventuels effets négatifs (« pre-emptive competition » pour l'azote et l'eau) sur la culture principale suivante.

### **4.1 La levée des cultures intermédiaires**

Pour obtenir un couvert dense et homogène permettant une croissance correcte des cultures intermédiaires, il est nécessaire d'avoir un nombre suffisant de graines germées puis levées en conditions de semis de fin d'été. Selon Cohan et al. (2011), les conditions de levée des cultures intermédiaires sont rarement idéales car leur semis est effectué à la suite de la culture précédente avec peu ou pas travail du sol, ceci couplé avec des températures souvent élevées, ce qui entraîne souvent un lit de semence du sol asséché. Par ailleurs, le temps pour lever est généralement plus variable en fonction de l'état hydrique et de la température, que le taux de levée lui-même (Dorsainvil et al., 2005). La disponibilité en eau et la température sont les

principaux facteurs qui influencent la levée car ils interviennent directement sur l'aptitude à la germination des espèces (Baskin and Baskin, 1988). En effet, chaque espèce peut être caractérisée par trois températures cardinales de germination : une température minimale au-dessous de laquelle les graines ne germeront pas, une température maximale au-dessus de laquelle les graines ne germeront pas non plus et une température optimale pour laquelle la vitesse de germination sera la plus rapide (Bewley and Black, 1994). En condition de semis de fin d'été, particulièrement dans le Sud de la France où les températures dans les premiers centimètres du sol peuvent être très élevées cela peut induire des taux de levées très faibles par déficit hydrique (Constantin et al. Submitted), il faudra donc veiller à privilégier des espèces de cultures intermédiaires ayant les aptitudes nécessaires en termes de germination notamment en situation de température élevée et de déficit hydrique dans le lit de semences.

#### **4.2 La date de destruction et la durée d'interculture**

La date de destruction des couverts est à adapter en premier lieu à la durée de l'interculture. En effet, celle-ci peut varier de quelques jours (après une récolte tardive suivie d'une culture d'hiver) à près de 9 mois (dans le cas d'une culture de printemps). Pour être efficace, la culture intermédiaire doit rester en place suffisamment longtemps et principalement avant la période de drainage afin de réduire au maximum l'azote minéral du sol avant drainage et donc diminuer les risques de lixiviation de l'azote nitrique. En revanche, il n'est généralement pas conseillé d'attendre la reprise de végétation au printemps pour détruire le couvert. En effet, il semble qu'il n'y ait que peu de différences entre une incorporation plutôt précoce à 3 ou 4 mois après le semis et une incorporation tardive, à 7 ou 8 mois en termes d'azote acquis (Thorup-Kristensen and Dresbøll, 2010). De plus, une destruction trop tardive (au printemps) peut réduire la minéralisation des résidus en augmentant le rapport C/N et donc diminuer la quantité d'azote disponible pour la culture suivante. Par ailleurs, pour toutes les durées d'interculture, il est recommandé de laisser au moins 15 jours entre la date de destruction des couverts, en cas d'incorporation dans le sol, et la date de semis de la culture suivante afin d'éviter le phénomène de préemption de l'azote (Justes et al., 2012a). En effet, l'incorporation des résidus s'accompagne, quand le C/N est élevé (>15), d'une phase d'immobilisation nette de l'azote minéral, réduisant temporairement la quantité d'azote minéral sur la profondeur d'incorporation des résidus et donc susceptible de créer une faim en azote pour la culture suivante (Nicolardot et al., 2001).

Le choix de la date de destruction, notamment dans le cadre d'interculture longue (jusqu'au printemps suivant), est également fonction du type de services écosystémiques attendus. En

effet, si une protection physique du sol est recherchée, tout en gardant un objectif exigeant une réduction de la lixiviation nitrique, il peut s'avérer nécessaire de garder un couvert pendant tout l'hiver (Stopes et al., 1996; Van Schöll et al., 1997). En revanche, on peut également rechercher une destruction naturelle par le gel ou la sénescence des couverts afin de réduire le nombre d'interventions sur la parcelle, réduisant ainsi les coûts et le temps de travail, mais aussi les intrants chimiques pour détruire le couvert et les émissions de gaz à effet de serre dues aux passages du tracteur. En effet, l'utilisation d'espèce gélives (dès -1°C) peut permettre une destruction par le froid hivernal. La sensibilité au gel des cultures intermédiaires varie grandement en fonction des espèces mais aussi des variétés et du stade développement où le gel se produit (Besnard et al., 2011).

Pour conclure, la date de destruction est à réfléchir en fonction de la durée de l'interculture mais aussi des services écosystémiques visés et du compromis acceptable entre les services écosystémiques. Afin de maximiser les services apportés dans le laps de temps de l'interculture, une solution est d'associer deux ou plusieurs espèces, notamment une légumineuse et une non-légumineuse en mélange dans le cas de services liés à la gestion de l'azote.

## **5 L'approche par les traits fonctionnels pour caractériser les espèces**

L'écologie fonctionnelle suppose qu'un individu (espèce, variété...) peut être représenté par un ensemble de valeurs de traits (ou « attributs ») qui sont des indicateurs de leur fonctionnement comme, par exemple, la croissance ou l'acquisition des nutriments. Cette approche basée sur les traits fonctionnels est largement utilisée en écologie (e.g. Garnier and Navas, 2012; Violle et al., 2007; Wilson et al., 1999) pour les communautés naturelles mais aussi pour le cas des prairies (e.g. Al Haj Khaled et al. 2005; Ansquer et al. 2009).

### **5.1 Généralités sur les traits fonctionnels**

Violle et al. (2007) définissent la notion de « trait » comme : « toute caractéristique morphologique, physiologique ou phénologique mesurable au niveau de l'individu, de la cellule jusqu'à l'individu en entier, sans référence à l'environnement (facteurs environnementaux) ou à tout autre niveau d'organisation (population, communauté ou écosystème) ». Un trait fonctionnel est donc une caractéristique d'un individu qui a démontré posséder des liens, directs ou indirects, avec une fonction de l'individu. Par ailleurs, il existe pour chaque espèce une gamme de variation de valeurs de trait qui traduit la variabilité intra-



spécifique due à la diversité génétique entre individus et à leur plasticité en réponse aux variations du milieu (Cornelissen et al., 2003; Garnier et al., 2001; Roche et al., 2004). Les traits fonctionnels permettent donc d'apporter des informations sur une fonction d'une large gamme d'espèces grâce à leur facilité et rapidité de mesures (Cornelissen et al., 2003; Lavorel et al., 2007). En effet, dans certaines études des corrélations entre des traits fonctionnels et la production de services écosystémiques (Lavorel and Garnier, 2002) dont notamment la production primaire de biomasse (Pontes et al., 2007) ou encore la décomposition de la litière (Fortunel et al., 2009) ont été démontrées.

L'approche par les traits permet également de classifier les espèces par groupes fonctionnels afin de simplifier la diversité des êtres vivants et de généraliser leur fonctionnement (Gitay and Noble, 1997; Lavorel et al., 2007). Les groupes fonctionnels sont des collections d'individus possédant des syndromes de traits fonctionnels similaires et que l'on regroupe indépendamment de leurs liens historiques et/ou phylogénétiques (classification taxonomique). Les espèces appartenant au même groupe fonctionnel présentent des réponses similaires aux conditions environnementales et/ou des effets similaires sur les processus dominants des écosystèmes (Noble and Gitay, 1996; Walker, 1992). L'objectif de cette démarche de regroupement est de réduire la complexité des écosystèmes afin de faciliter leur description et/ou leur prédictibilité (Smith et al., 1997). Les génotypes sont ainsi réunis en fonction de leur mode d'utilisation des ressources (Chapin, 1980; Chapin et al., 1990; Diaz et al., 2004), des attributs biologiques qu'elles partagent (Lavorel and Garnier, 2002) ou de leur rôle dans l'écosystème (Walker, 1992). Ces groupes sont ainsi généralement basés sur les différentes stratégies adaptatives des individus (Harrington et al., 2010; Lavorel et al., 1997).

## **5.2 Les stratégies fonctionnelles des plantes**

Dans le cas d'une étude sur les cultures intermédiaires, il semble intéressant d'étudier les traits fonctionnels et les stratégies associées relevant de l'aptitude à acquérir et utiliser les ressources. Il s'agit notamment d'un des axes de spécialisation des plantes décrit par Westoby (1998) et se focalisant principalement sur les traits foliaires. Cet axe est largement utilisé par les écologues pour décrire les communautés végétales (Diaz et al., 2004). Il s'exprime par un compromis entre la stratégie d'utilisation des ressources se caractérisant par une croissance rapide des plantes, et la stratégie de conservation des ressources se caractérisant par une croissance plus lente (Grime et al., 1997; Wright et al., 2004). Ce gradient entre ces deux stratégies est généralement liée aux traits fonctionnels foliaires tels que : i) la surface foliaire spécifique (Specific Leaf Area) ou SLA qui est la surface d'une feuille divisée par sa masse,

ii) la teneur en matière sèche d'une feuille (leaf dry matter content) ou LDMC, qui est la biomasse sèche d'une feuille divisée par sa biomasse fraîche et saturée en eau, ou encore iii) la teneur en azote d'une feuille (leaf nitrogen content) ou LNC. Au niveau de la feuille, la SLA, la LDMC et la LNC ont été proposées comme des indicateurs de ressources des plantes, il s'agit de traits fonctionnels relevant de l'aptitude à acquérir et à utiliser les ressources (Westoby, 1998).

Le « Leaf Economics Spetrum » ou LES (Wright et al., 2004) représente ce gradient dans lequel les espèces à croissance rapide sont capables d'acquérir rapidement les ressources telles que les éléments nutritifs et la lumière. Au niveau de la feuille, il existe notamment une corrélation positive entre SLA, LNC et le taux de photosynthèse net par unité de masse (Reich et al., 1997; Wright et al., 2004). Ces espèces à stratégie d'acquisition, possèdent également une durée de vie des feuilles courte et à l'opposé du gradient, les espèces ayant une croissance lente, présentent un taux de photosynthèse net moins élevé mais une durée de vie des feuilles plus longue (Wright et al., 2004). De plus, la SLA et la LDMC sont négativement corrélées et expriment également cet axe de stratégie d'utilisation des ressources (Garnier et al., 2001). En effet, les plantes ayant une SLA élevée et une LDMC faible peuvent être considérées comme ayant une stratégie d'acquisition rapide des ressources et, à l'opposé les plantes avec une SLA faible et une LDMC élevée auront plutôt une stratégie de conservation des ressources (Cruz et al., 2010; Garnier et al., 2007; Poorter and Bergkotte, 1992).

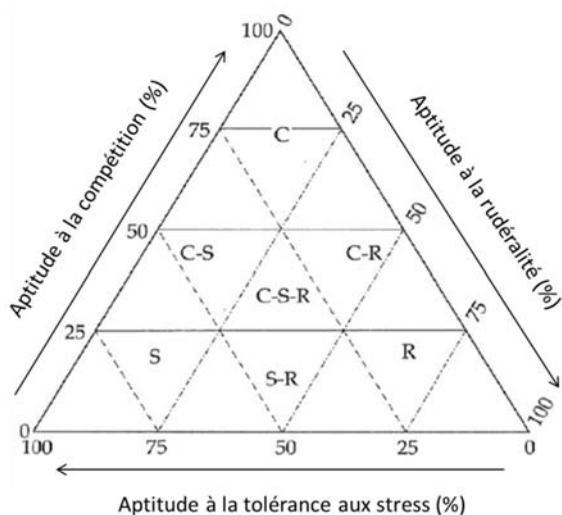


Figure 6 Triangle CSR (Competitiveness-Stress tolerance-Ruderality) de Grime, adapté de (Gibson, 2009)

Par ailleurs, Grime (1977) a identifié trois autres types de stratégie fonctionnelle des plantes, relatifs à leur croissance et toutes reliées entre elles ; il s'agit du triangle CSR (Figure 6). Les espèces sont ainsi classées par leur aptitude à être i) compétitrices (C), ii) tolérantes aux stress (S) et iii) rudérales (R) : plante résistante aux perturbations du milieu (feu, herbivorie...).

Les espèces classées comme compétitrices selon Grime (1977), sont capables de mobiliser rapidement les ressources grâce à une croissance rapide alors que celles classées comme tolérantes au stress possèdent une croissance plus faible mais sont susceptibles de résister à des stress importants notamment grâce à un renforcement des tissus. Enfin, les espèces

considérées comme rudérales peuvent résister à de fortes perturbations du milieu (herbivorie, feu...). Afin de positionner facilement un grand nombre d'espèces selon ces trois stratégies, Pierce et al. (2013a) propose une méthode basée sur seulement trois traits fonctionnels foliaires : i) la SLA pour exprimer la tolérance aux perturbations, ii) la LDMC pour la résistance aux stress et iii) la surface foliaire (LA) pour l'aptitude à la compétition.

## **6 Conclusion partielle**

La littérature met en exergue des avantages certains des cultures intermédiaires sur la gestion de l'azote dans les agrosystèmes. Les services écosystémiques produits permettent de sauvegarder et recycler l'azote minéral durant l'interculture automnale et hivernale, principale période de drainage, mais aussi de produire un effet engrais vert pour la culture suivante. De nombreuses espèces peuvent être implantées et sont utilisées en culture intermédiaire car elles ont des aptitudes différentes à produire ces services, notamment en fonction de la durée de l'interculture. Cependant, il n'existe que peu d'informations « validées » disponibles et peu d'études ont réalisé une caractérisation fine d'un grand nombre de ces espèces, alors qu'il nous semble essentiel d'avoir ces informations pour adapter le choix en fonction i) des services attendus, ii) la durée de l'interculture et du précédent cultural, ou encore iii) des conditions pédoclimatiques. L'approche par les traits fonctionnels semble une approche pertinente a priori pour caractériser de manière simple et robuste un grand nombre d'espèces, comme celles utilisées en culture intermédiaire. Par ailleurs, les mélanges bispécifique de type légumineuse/non-légumineuse semblent pouvoir produire des compromis entre les services écosystémiques. Certains résultats présentés dans la littérature sont prometteurs quant à la mobilisation de cette approche en agronomie, mais les études ne portent généralement que sur un nombre limité de mélanges et d'espèces étudiées. Bien que de nombreuses études aient mis en exergue les effets d'interactions interspécifiques dans les mélanges, celles-ci portent principalement sur les associations de cultures de vente, ou vivrières, et peu d'informations sont disponibles pour les cultures intermédiaires.



## Objectifs et démarche de la thèse

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## 1 Objectifs et questions de recherche

L'objectif principal de la thèse est d'étudier les performances de différentes espèces de cultures intermédiaires utilisées en mélanges bispécifiques de type légumineuse/non-légumineuse. Ces mélanges ont pour objectif de délivrer un compromis entre services écosystémiques liés à la gestion de l'azote, maximisant simultanément et autant que possible, la fonction de piégeage de l'azote minéral (effet CIPAN) et l'effet « engrais vert » (restitution d'azote à la culture principale suivante).

### 1.1 Caractérisation des espèces utilisées en culture intermédiaire

Pour cela, le premier objectif de ce travail de thèse est de caractériser un grand nombre d'espèces, issues de différentes familles botaniques, qui sont aujourd'hui disponibles et potentiellement utilisables comme cultures intermédiaires. En effet, ces espèces peuvent produire les services écosystémiques recherchés de manière plus ou moins importante et plus ou moins précoce durant l'interculture. Il est donc nécessaire d'évaluer leur aptitude et ainsi pouvoir les discriminer afin d'avoir des informations objectives pour adapter leur choix et l'association bispécifique a priori *ad hoc* en fonction de la date de destruction des couverts et des objectifs de gestion de l'interculture. Par exemple, dans le cas d'une interculture courte avec une destruction automnale, le choix d'espèce à mettre en place se portera prioritairement sur une espèce rapide à s'installer et avec des capacités de croissance aérienne et racinaire permettant de capter rapidement l'azote minéral du sol. A l'opposé, pour une interculture longue et dont l'objectif est le maintien du couvert pendant l'hiver, on privilégiera une ou des espèces au développement plus tardif mais résistant au niveau de gel habituel dans la région.

Afin de répondre à cet objectif, nous avons fait l'hypothèse que le concept de traits fonctionnels pouvait permettre cette caractérisation des espèces. En effet, à l'instar des méthodes « classiques » généralement utilisées en agronomie qui visent à caractériser un nombre limité d'espèces mais de manière fine, nous avons privilégié l'approche par les traits fonctionnels empruntée à l'écologie permettant de caractériser simplement et rapidement un grand nombre d'espèces différentes. La première question de recherche à laquelle nous apporterons des éléments de réponse est donc :

*Q1 : Les traits fonctionnels et les méthodes écologiques associées permettent-ils de caractériser et différencier les diverses espèces utilisées en cultures intermédiaires en qualifiant leur stratégie d'acquisition des ressources ?*

La phase semis-levée étant critique en semis d'été où les températures dans le sol peuvent être élevées, particulièrement dans le Sud de la France, il nous a semblé nécessaire de caractériser les mêmes espèces par rapport à leur aptitude germinative, notamment en conditions de températures élevées. Nous avons donc fait l'hypothèse que les caractéristiques de germination peuvent déterminer la mise en place plus ou moins rapide du couvert, pouvant ainsi, dans le cas d'un mélange d'espèces, donner un avantage compétitif d'une espèce sur l'autre. De plus, la détermination des températures cardinales de germination pourrait servir de première caractérisation des températures minimales, optimales et maximales de croissance de ces espèces, selon l'hypothèse que la réponse à la température est quasiment identique pour les différents processus de développement et de croissance des plantes pour un génotype donné (Parent and Tardieu, 2012). La seconde question de recherche traitée est la suivante :

*Q2 : La réponse de la germination des espèces utilisées en culture intermédiaire à une large gamme de températures, notamment élevées, peut-elle discriminer les espèces pour leur aptitude germinative en conditions de semis d'été ?*

## **1.2 Les performances et les interactions des espèces de cultures intermédiaires en mélanges bispécifiques pour gérer l'azote dans le cas d'une destruction automnale des couverts**

Un second objectif de cette thèse est d'évaluer les performances des mélanges bispécifiques pour capter l'azote minéral du sol et donc réduire la quantité d'azote lixivié, mais aussi pour produire un effet engrais vert c'est-à-dire pour restituer de l'azote à la culture principale suivante. Cela revient à étudier un nombre relativement important de mélanges composés de divers espèces issues de familles botaniques variées et présentant des architectures, vitesses de croissances ou encore systèmes racinaires différents. La performance de ces mélanges pour produire simultanément les deux services écosystémiques de gestion de l'azote avec le même couvert doit être évaluée par rapport aux cultures pures mais aussi au sol nu (sans culture intermédiaire) qui est généralement la situation de référence. Les interactions entre les deux espèces au sein du mélange qui peuvent s'exprimer sous forme de compétition ou de complémentarité, voire de facilitation, seront également étudiées et mises en relation avec les performances des mélanges pour produire les services attendus. Afin d'évaluer la généralité des performances des mélanges, cette analyse devra être réalisée sur plusieurs sites pédoclimatiques contrastés. Pour répondre à cet objectif nous nous attacherons à apporter des éléments de réponse aux questions de recherche suivantes :



*Q3.1 : Les mélanges sont-ils aussi performants que les cultures pures pour simultanément piéger l'azote minéral et produire un effet « engrais-vert » quelles que soient les conditions pédoclimatiques ?*

*Q3.2 : Les interactions entre les espèces en mélange favorisent-elles la mutualisation des services écosystémiques visés ?*

### **1.3 Proposition d'un modèle conceptuel pour estimer les fonctions écosystémiques de couverts de cultures intermédiaires en mélange bispécifique**

Enfin, un des objectifs du travail de thèse est de proposer un modèle conceptuel simple et générique pour prédire des fonctions ou indicateurs d'agroécosystème tels que la quantité d'azote acquise, la biomasse produite ou encore le C/N du couvert, qui sont reliés aux services écosystémiques attendus pour la gestion de l'azote. Dans un premier temps, dans le cadre de cette thèse, nous nous intéresserons à des situations de destruction avant hiver avec une durée de croissance des couverts courte (environ 3 mois). En effet, cela permettrait d'estimer les performances d'un mélange en fonction des espèces associées, et donc des interactions qui peuvent s'opérer entre elles, mais aussi en fonction des facteurs environnementaux afin de l'adapter à différentes espèces et à différents sites pédoclimatiques.

*Q4 : Est-il possible de concevoir un modèle générique pour prédire les fonctions écosystémiques à partir de variables simples ?*

Pour répondre à cette question, plusieurs sous-questions seront traitées :

*Q4.1 : Les traits fonctionnels sont-ils pertinents pour modéliser les fonctions écosystémiques ?*

*Q4.2 : Les variables « agronomiques » comme les traits de couvert sont-elles nécessaires pour obtenir une prédiction fiable ?*

*Q4.3 : Quelle est la généricité du modèle conceptuel et son domaine de validité, notamment pour une large gamme d'espèces cultivées en mélange bispécifique ?*

### **1.4 Evaluation des performances des mélanges bispécifiques de cultures intermédiaires en fonction du mode de gestion de l'interculture**

Le choix des espèces à associer, et donc des mélanges, peut également être adapté en fonction de la date de destruction mais aussi des services écosystémiques attendus prioritairement ou du niveau de compromis entre services visé. Par exemple, si l'on recherche une couverture du sol durant tout l'hiver ou bien que l'on cherche à privilégier un effet engrais vert pour la

culture suivante, la date de destruction de la culture intermédiaire pourra être différente. Par ailleurs, afin d'appréhender le fonctionnement des mélanges et les aptitudes des deux espèces à fournir une partie des services recherchés, il est intéressant d'analyser les phénomènes d'interactions interspécifiques pouvant induire des compétitions ou de la complémentarité de niche. Ainsi, on peut faire l'hypothèse que les interactions entre les espèces en mélange évoluent au cours du temps conduisant à adapter le choix des mélanges au mode de gestion de l'interculture, notamment à la date de destruction et d'incorporation des couverts ou au service écosystémique privilégié.

*Q5 : Comment évoluent la production des services de gestion de l'azote et les interactions entre les deux espèces en fonction de la date de destruction des couverts ?*

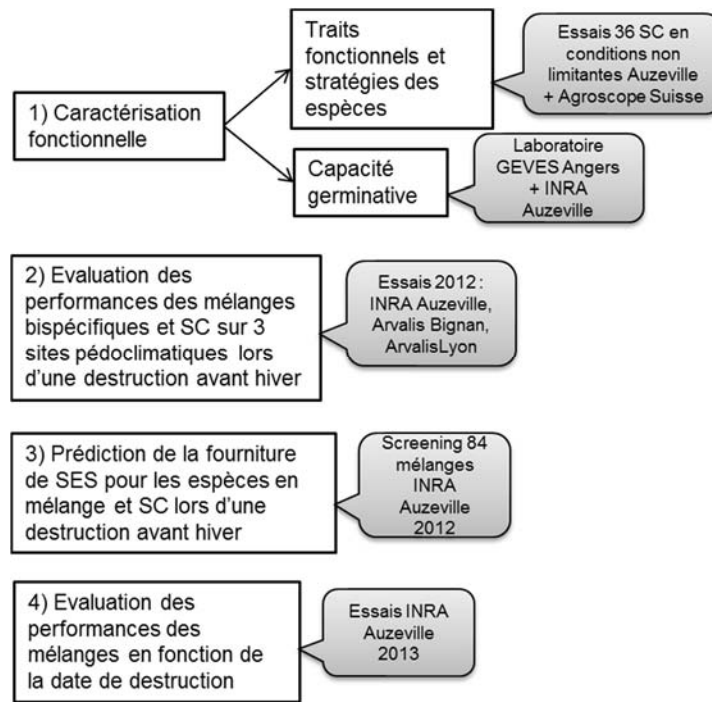
*Q5.1 : Quels mélanges sont les mieux adaptés en fonction du mode de gestion de l'interculture ?*

*Q5.2 : Les interactions entre les espèces en mélanges permettent-elles une complémentarité pour le partage des ressources dans le temps ?*

## **2 Démarche de la thèse et description générale des expérimentations**

La démarche mise en place (Figure 7) pour répondre aux objectifs et aux questions de recherche est composée d'expérimentations au champ, d'expériences en laboratoire et de modélisation.

Ce travail de thèse est centré sur les cultures intermédiaires pour produire des services écosystémiques liés à la gestion de l'azote : la réduction de la lixiviation par le piégeage de l'azote minéral et l'effet engrais vert par la quantité d'azote disponible pour la culture principale suivante. Pour évaluer ces services de manière directe au champ, une méthodologie lourde est souvent nécessaire (installation de plaques lysimétriques, suivi des effets sur la culture suivante...). Nous avons donc privilégié l'étude de fonctions et d'indicateurs agro-écologiques liés à la production de ces services tels que la production de biomasse, l'acquisition de l'azote ou encore le ratio C/N que nous avons couplé avec des simulations pour notamment estimer la quantité d'azote lixivié et la quantité d'azote minéralisé à partir des résidus de cultures intermédiaires.



**Figure 7** Schéma de la démarche globale de la thèse en quatre parties et des expérimentations mises en place. SC : culture pure (« sole crop » en anglais), SES : services écosystémiques.

Ainsi, en premier lieu, nous avons évalué la rapidité des espèces pour se développer et acquérir l'azote grâce aux mesures « crop growth rate » (CGR) et « crop N acquisition rate » (CNR). Ces deux taux peuvent donc permettre de caractériser les espèces par leur rapidité à se développer en interculture et ainsi, à produire les services attendus. Afin de caractériser les principales espèces pouvant être utilisées en cultures intermédiaires, nous avons sélectionné un grand nombre d'espèces représentant la diversité en termes de familles botaniques, d'architectures de plantes et d'aptitude à se développer rapidement à l'automne pour produire les services attendus en quelques semaines. Nous avons donc caractérisé 34 espèces et 2 variétés pour 2 espèces (fêverole et pois), soit au total 36 taxons différents. Nous les avons caractérisés en cultures pures et en conditions non-limitantes afin d'évaluer leur potentiel de croissance et de développement (aérien et racinaire) mais aussi d'acquisition de l'azote. Cela a aussi permis de mesurer des traits fonctionnels sans stress hydrique ni azoté. La mesure des traits fonctionnels s'effectue au niveau de l'individu, et particulièrement de la feuille. La caractérisation de ces espèces par les traits fonctionnels foliaires a été réalisée en automne 2012 sur deux sites expérimentaux : un à l'INRA d'Auzeville dans le Sud-Ouest de la France, et un autre à Agroscope à Nyon en Suisse, en collaboration avec les collègues d'Agroscope Changins. Cela nous a permis d'évaluer la robustesse et la généricité de l'approche par les traits fonctionnels foliaires qui ont été mesurés dans des conditions environnementales différentes, bien qu'en conditions d'eau et d'azote non-limitantes.

Par ailleurs, pour la caractérisation de l'aptitude germinative de ces mêmes 36 taxons de cultures intermédiaires, celle-ci a été réalisée en grande partie au laboratoire du GEVES d'Angers sur des tables de Jacobsen, en conditions d'eau et de températures contrôlées. L'autre partie de ces expérimentations a été réalisée à l'INRA d'Auzeville en incubateurs où la température était également contrôlée. La germination des espèces a été testée sous huit températures, de 4.5°C à 39.5°C, voire 43°C pour certaines d'entre elles ayant germé à 39.5°C.

Enfin, l'évaluation des performances agronomiques des mélanges bispécifiques de cultures intermédiaires a été réalisée à partir de plusieurs expérimentations au champ, à l'échelle de la micro-parcelle. Une première campagne d'essais a été conduite en automne 2012 sur trois sites expérimentaux : un sur le site de l'INRA d'Auzeville, un autre sur un site Arvalis de Bignan et le troisième sur le site Arvalis de Lyon (Figure 8). Le site d'Auzeville présentait un sol de type limon-argileux, des reliquats après récolte du précédent de 53kg ha<sup>-1</sup>, des températures plutôt élevées et peu de pluviométrie. Le site de Lyon présentait un sol limoneux et caillouteux, une faible disponibilité initiale d'azote minéral (31kg ha<sup>-1</sup>), des températures plutôt élevées et une pluviométrie importante sur la période de croissance. Enfin, le site de Bignan comportait un sol de limon fin, avec une quantité d'azote initiale élevée (112kg ha<sup>-1</sup>), des températures plutôt faibles et une pluviométrie importante.



Figure 8 Positionnement des trois sites expérimentaux des essais de mélanges de cultures intermédiaires de 2012.

Sur chacun des trois sites le même dispositif expérimental a été mis en place. Cinq espèces de légumineuses ont été choisies pour leur rapidité de croissance et cinq espèces de non-légumineuses ont été choisies pour leur diversité de famille botanique et de vitesse de croissance et présentant des hauteurs relativement peu importantes afin d'éviter une trop forte

compétition pour l'accès à la lumière sur la légumineuse associée. Chaque légumineuse a été associée à chaque non-légumineuse afin d'évaluer 25 mélanges différents par rapport aux 10 cultures correspondantes et aux sols nus, c'est-à-dire sans semis de culture intermédiaire. Chacune des espèces en mélange a été semée en demi-densité par rapport à leur densité respective en culture pure, les mélanges sont donc de type substitutif. Les densités en cultures pures ont été choisies par dire d'experts (semenciers ou instituts). Les semis ont été faits entre début et mi-août en fonction des sites. Les densités de semis des espèces évaluées lors des différentes expérimentations sont disponibles en Annexe A1, les densités étaient les mêmes pour les différents sites expérimentaux.

Pour évaluer la performance ces mélanges, un prélèvement destructeur a été réalisé entre fin octobre et fin novembre sur chaque site, où ont été mesurés : la biomasse, la quantité d'azote acquis, le C/N et les reliquats d'azote minéral.

Enfin, une seconde campagne d'essai a été réalisée, en 2013, sur les sites expérimentaux de l'INRA d'Auzeville et d'Arvalis à Bignan. Cependant, le site d'Arvalis Bignan n'a pas été exploité dans ce manuscrit en raison d'une très forte pression adventive. Le choix des mélanges testés a été basé sur les résultats de la campagne de 2012 et notre expertise. Au total, 16 mélanges et 14 cultures pures correspondantes ont été analysés à Auzeville. Des sols nus ont également été suivis comme référence sans culture intermédiaire. Les prélèvements ont été effectués à trois dates, ces différentes dates correspondant à des dates potentielles de destruction des couverts. Les essais comportaient trois répétitions. Par ailleurs, sur le site d'Auzeville, les cultures pures et quelques mélanges ont été équipés de capteurs de rayonnement solaire, et des tubes en Plexiglass transparent ont été implantés dans le sol afin d'y mesurer la dynamique d'enracinement grâce à un scanner circulaire introduit dans les tubes. Les dynamiques de hauteur et de taux de couverture du sol ont également été mesurées. Pour les deux campagnes d'essais, les services écosystémiques ont été simulés avec le modèle de culture STICS (Brisson et al., 2008) à partir des fonctions et indicateurs d'agroécosystèmes mesurés au champ. Ce modèle a été choisi car il a déjà été évalué comme performant pour l'estimation de la lixiviation nitrique et de la minéralisation de l'azote à partir des résidus de cultures intermédiaires (Beaudoin et al., 2008; Constantin et al., 2012; Justes et al., 2009).

Les différentes variables et les traits fonctionnels mesurés sur les différents essais ont également servi de support pour la construction des modèles conceptuels. Leur calibration a été faite à partir des données pour les mélanges testés dans les expérimentations d'Auzeville 2012 et 2013 et de Bignan 2012 dans le cas d'une destruction automnale des couverts. Pour la validation de leur généralité applicable à d'autres espèces en mélanges, celle-ci a été réalisée à

partir d'un dispositif annexe, mis en place en 2012 sur le site d'Auzeville, qui avait pour objectif de réaliser un screening de mélanges bispécifiques sur un grand nombre d'espèces et de mélanges différents.

### **3 Organisation du manuscrit**

Les résultats issus de ce travail de thèse sont constitués de quatre chapitres écrits sous forme de manuscrit pour publication en anglais. Deux articles ont été soumis et trois correspondent à un premier draft qu'il conviendra d'améliorer avant soumission.

Le **Chapitre 1** regroupe deux articles dont l'objectif est de caractériser une large gamme d'espèces pouvant être utilisées en cultures intermédiaires. Le premier concerne une caractérisation par les traits fonctionnels foliaires et les stratégies écologiques associées. Le second porte sur la caractérisation de la germination des espèces en fonction de la température.

L'objectif du **Chapitre 2** est d'évaluer les performances agronomiques des mélanges bispécifiques de cultures intermédiaires pour produire les services relatifs à la gestion de l'azote dans le cas d'une interculture courte et sur trois sites pédoclimatiques contrastés. Les performances des mélanges sont évaluées par rapport aux cultures pures, et au sol nu. Les interactions interspécifiques au sein des mélanges ont également été évaluées et mises en relation avec les performances des mélanges.

Dans le **Chapitre 3**, nous proposons des modèles conceptuels couplant des traits fonctionnels et des variables agronomiques pour estimer la production des fonctions et indicateurs agro-écologiques déterminant la production de services écosystémiques relatifs à la gestion de l'azote des espèces en cultures pures ou en mélanges.

Le **Chapitre 4** porte sur l'évolution en dynamique des interactions interspécifiques entre les espèces en mélange et sur la production des services en fonction du mode de gestion de l'interculture et notamment de la date de destruction.

Enfin, une dernière partie correspond à une synthèse des principaux résultats avec une discussion générale et des perspectives de recherche possibles à ce travail.

# **Chapitre 1 : Caractérisation fonctionnelle et capacité germinative de 36 taxons de cultures intermédiaires**

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## **1 A functional characterisation of a wide range of cover crop species: growth and nitrogen acquisition rates, leaf traits and ecological strategies**

*Submitted to PlosOne.*

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### **Abstract**

Cover crops produce various ecosystem services in agriculture during the fallow period, such as nitrate leaching reduction and N green manuring by N recycling. To produce these services a wide range of species differing in their crop growth rates (CGRs) and crop nitrogen acquisition rates (CNRs) are used. We investigated the use of leaf functional traits to characterise 36 cover crops growth strategies as an approach to rapidly characterise their ability to produce ecosystem services related to N management. We measured specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) and leaf area (LA) and we evaluated their relevance for characterising CGRs and CNRs. Based on these traits, we positioned cover crop species along the Leaf Economics Spectrum (LES), the SLA-LDMC plane, and the CSR triangle. The LA was positively correlated with both CGR and CNR, while LDMC was only negatively correlated with CNR. Other leaf traits did not correlate with CGR or CNR. Despite a wide range of growth strategies measured, all cover crops were classified as resource-acquisition species by the SLA-LDMC plane and the LES. The CSR triangle positioned most cover crops along the Competition/Ruderality axis. Their positioning along the competition axis showed that competitive species have the highest CGR and CNR and thus appear particularly efficient in producing ecosystem services as catching nitrate and N green manuring. Leaf functional traits, especially LA and LDMC, and associated methods allowed differentiating some species strategies related to their CGR and CNR and thus the ability of cover crops to provide ecosystem services. However they also showed a lack of sensitivity to allow a precise characterisation. We hypothesised this may be due to the effect of domestication, which would have reduced the range of species strategies and modified the trait syndrome in comparison to wild species.

## **1.1 Introduction**

Cover crops are defined as plant covers in agrosystems whose ecosystem services reduce negative environmental impacts of agriculture, i.e. protects soils against erosion and improve its fertility. Currently, cover crops are sown to i) reduce nitrate leaching by maximising nutrient uptake, particularly nitrogen (N) before leaching becomes significant during the drainage period (i.e. the “catch crop effect”) (Justes et al., 2012b; Kristensen and Thorup-Kristensen, 2004); ii) produce “N green manure” by N recycling for later release to the next cash crop (Tonitto et al., 2006); iii) produce biomass, which is important for soil carbon sequestration; and iv) reduce weed growth and soil seed-bank formation during the fallow period of “bare soil” between two main cash crops (Justes et al., 2012b; Thorup-Kristensen et al., 2003). The amount of N available for the next cash crop is influenced by the amount of N acquired by the cover crop, and the cover crop residue quality and decomposition (Thorup-Kristensen et al., 2003). Biomass production and acquired N determine the C:N ratio of cover crop residues, which controls the dynamics of N release from residues incorporated into the soil and then its green manure effect (Jensen, 1991; Justes et al., 2009). Regardless of the current strong interest in cover crops in intensive cropping systems, only little scientific-based information is available to choose cover crop species on a sound basis. To the best of our knowledge, the only study proposing a method to select cover crops according to the targeted ecosystem services deals with tropical cover crops in banana agrosystem (Damour et al., 2014). In the present study, we apply the functional trait-based screening approach widely used in plant ecology (Garnier and Navas, 2011; Grime, 1979; Violle et al., 2007) to characterise a large diversity of species’ potential to be used as cover crops.

Ecological theories (Grime, 1977; Westoby, 1998) and empirical works (Grime et al., 1997; Reich et al., 1992) showed that, in wild species, relative growth rate is related to various leaf functional traits (Poorter and Garnier, 2007; Reich et al., 1998) and that it is one of the components underlying plant strategies (Grime, 1977; Westoby, 1998). Ecologists have established the existence of a fundamental trade-off between traits allowing rapid resource capture (fast-growing species) and those allowing efficient resource conservation (slow-growing species). Specific leaf area (SLA) and leaf N concentration (LNC) appear to be effective predictors of a species’ position along the “Leaf Economic Spectrum” (LES) that captures the gradient from “acquisitive” (high SLA and LNC) to “conservative” (low SLA and LNC) species (Wright et al., 2004). As proposed by Wilson et al., (1999), leaf dry matter content (LDMC, the ratio of leaf dry mass to water-saturated fresh mass) is complementary to SLA for assessing plant functioning and resource acquisition/conservation strategies. Indeed

acquisitive strategies are associated with high SLA and low LDMC whereas conservative strategies are associated with low SLA and high LDMC (Cruz et al., 2010; Garnier et al., 2007; Poorter and Bergkotte, 1992). Moreover LDMC is more strongly related to litter decomposition than SLA: litters with high LDMC tend to decompose more slowly than litters with low LDMC, leading to litter accumulation (Fortunel et al., 2009), thereby potentially delaying N release and then the green manure effect from cover crop residues. As emphasised above, another important potential service attributed to cover crops is limiting weed proliferation by a rapid growth, soil coverage and N acquisition, inducing a strong competition for resources (light, water, nutrients), which relates to their competitive abilities. Grime (1977) identified three main avenues for species' adaptive specialisations that reflect trade-offs between their capacities to be competitive (C), stress tolerant (S) and ruderal (R). Locating species within the CSR space, and thereby assessing their relative competitive ability, normally requires measuring a large number of traits (Grime et al., 1997; Hodgson et al., 1999). Moreover, Pierce et al. (2013) recently developed a new method that uses only three leaf traits: SLA, LDMC and leaf area (LA), which allows one to assess relatively quickly species' ability to compete for light with neighbouring plants.

A key criterion to evaluate ecosystem services provided by cover crops is the quantity of biomass produced during the growing period, which strongly depends on the crop growth rate (CGR) (Damour et al., 2014; Poorter and Garnier, 2007; Wilson, 1981). Fast-growing species are more suitable for short fallow periods, i.e. 2 to 4 months, because they can produce ecosystem services quickly, but they usually cannot resist cold temperatures and frost because of their advanced stage of development in winter or because of the frost sensitivity. Conversely, slow-growing species are more suitable when temperatures are low; although they may have lower autumn biomass production, they may maintain growth and live biomass over the winter, and are better adapted to longer fallow periods, i.e. 6 to 8 months. Another relevant indicator for cover crops is the amount of N acquired by the plants over a given period, which depends on crop N acquisition rate (CNR): the faster a species acquired N, the higher nitrate catch crop (especially for non-legume species) and N green manure effects (true for legume and non-legume species) (Damour et al., 2014).

In this study we used a functional-trait approach to characterise and classify the resource-use and ecological strategies of the different cover crop species to assess their ability to act as a nitrate catch crop and to provide N green manure for short fallow periods. Species from various botanical families were analysed all together as recommend by Diaz et al. (2004). We make the hypothesis that these ecological strategies are applicable to evaluate strategy of

species in sole crop and at the stand level. We hypothesised that, over cover crop species, a gradient of resource acquisition/ conservation strategies corresponding to growth and N acquisition rates gradients could be investigated by functional traits. It can be assumed that species with acquisitive strategies having high SLA and low LDMC would ensure rapid development of plant growth and soil covering and short-term nutrient capture, while conservative species having low SLA and high LDMC would ensure the storage of C and nutrients during and after the fallow period (since their litter decomposes slowly, increasing soil organic matter content).

The aim of our study was to perform a functional characterisation of 34 species and 2 species varieties (36 taxa) using leaf traits and CGRs and CNRs measured in plants grown in monocrops at two locations. To better understand where these cover crop species lie in previously identified axes of resource-use strategy variation (Westoby et al., 2002), these experimental data were compared to those from two large published data sets measured among a wide range of wild species (Annexe A2): (i) one based on the identification along the LES (Wright et al., 2004) and (ii) the other that describes the relationship between SLA and LDMC corresponding to the trade-off between acquisitive and conservative resource strategies (E Garnier et al., 2001). Finally, we used the methodology of Pierce et al. (2013) to position the 36 taxa tested on the CSR space (Grime, 1977). We focused our study on leaf functional traits because they are easily measurable, making the approach potentially repeatable for a wide range of species used as cover crops, and because of the existence of large databases of these traits for characterising species' strategies.

## **1.2 Materials and Methods**

### **1.2.1 Growth conditions**

Two field experiments were conducted from August to October 2012 on research public areas at the INRA site in Auzeville-Tolosane (43°31' N, 1°30' E), southwest France and the Agroscope site in Nyon (46°22' N, 6°14' E), Switzerland. This study was permitted by the both institutions of INRA and Agroscope and field studies did not involve endangered or protected species. The French and Swiss sites had mean temperatures of 18.4°C and 16.7°C during this period, respectively, and both have deep clay loam soils. Species were sown, at their recommended plant density for each cover crop use and constituted homogeneous dense plant covers, in plots of 10 and 11 m<sup>2</sup> on 3 August 2012 in Switzerland and 16 August 2012 in France, respectively. Species were grown in non-water- and non-N-limiting conditions as

proposed by Grime and Hunt (1975) so as to minimize genotype x environment interactions and compare the potential abilities of species independently of environmental conditions. Irrigation was performed regularly and 100 kg ha<sup>-1</sup> of N from fertilizer was provided to non-N fixing species at sowing. It was assumed that the symbiotic fixation of N<sub>2</sub> of Fabaceae was effective thanks to the presence in soils of efficient *Rhizobium* and observed good nodulation in roots for all legumes which assured optimal growth conditions and N acquisition.

### 1.2.2 Species characteristics, crop growth and N acquisition rates

Thirty-six taxa (34 species and two varieties of *Vicia faba* and *Pisum sativum*) were selected as cover crop species based on their supposed ability to provide ecosystem services during fallow period (Justes et al., 2012a, 2012b; Thorup-Kristensen et al., 2003). These species are adapted to grow in French and European soils and climates and match the range of species commonly used as cover crops by farmers. Most of these species are not used as main crop in current arable cropping systems and are non-host species for pests and diseases of the main cash crops. The species were also chosen to represent a diversity of botanical families: Fabaceae, Brassicaceae, Poaceae, Asteraceae, Polygonaceae and Hydrophyllaceae. Species from the Fabaceae family are commonly used to provide green manure effect whereas Brassicaceae or Poaceae are more often used to catch nitrate from soil (Thorup-Kristensen et al., 2003; Tonitto et al., 2006). These taxa were also chosen to ensure a wide range of growth and N acquisition rates, phenology, shoot architecture and taxonomic diversity. All species had an annual life cycle except four biannual or triennial grassland species: *Secale multicaule*, *Medicago lupulina*, *Melilotus officinalis* and *Onobrychis viciifolia*. The Poaceae family was separated into two groups according to photosynthetic pathway (C<sub>3</sub> versus C<sub>4</sub>).

We used crop growth rate (CGR) and crop N acquisition rate (CNR) measured at the cover level as two indicators of the efficiency of cover crop species in providing targeted ecosystem services of nitrate catching and green manuring by N recycling. Degree-days (DD) using a base temperature of 0°C were calculated from the date of sowing (T<sub>0</sub>) until the date of the harvest (T<sub>1</sub>) (12 October and 28 September 2012 for the French and Swiss sites, respectively). At T<sub>1</sub>, DD equalled 1160 DD and 1118 DD for French and Swiss sites, respectively. CGR (in kg DM ha<sup>-1</sup> DD<sup>-1</sup>) and CNR (in g N ha<sup>-1</sup> DD<sup>-1</sup>) were calculated for each species at both sites using the following equations:

$$CGR = \frac{DMI - DM0}{T1 - T0} \quad \text{and} \quad CNR = \frac{Nacq1 - Nacq0}{T1 - T0}$$

where DM1 and DM0 are shoot dry matters (DM) ( $\text{kg ha}^{-1}$ ) and Nacq1 and Nacq0 are the amounts of N acquired by plants in shoots ( $\text{g ha}^{-1}$ ) at T1 and T0, respectively. DM0 and Nacq0 are equal to 0 because they correspond to DM and Nacq at the sowing date. N acquired was estimated by shoot biomass (DM) and total-N concentration which was measured by elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany).

### **1.2.3 Leaf trait measurements**

At each experimental site, leaf traits were measured at the vegetative stage (Cornelissen et al., 2003) on 20 individual plants per species. Immediately after harvest, the 20 plant samples were placed in demineralised water and stored in the dark at  $4^{\circ}\text{C}$  for at least seven hours water was regularly sprayed on leaves to ensure that they were water-saturated (Cornelissen et al., 2003; Garnier et al., 2001). Traits were measured on the youngest mature leaf (lamina and petiole) free from herbivore or pathogen damage. Leaves were weighed to obtain the water-saturated leaf mass and scanned with a manual scanner (Scanjet 3770, Hewlett-Packard) to measure their surface area. Leaves were then oven-dried at  $60^{\circ}\text{C}$  for 48h and weighed to measure their dry mass. Finally, leaves were ground and total-N content was measured.

LDMC was calculated as the ratio of dry mass (mg) of a leaf to its water-saturated fresh mass (g) and is expressed in  $\text{mg g}^{-1}$ . SLA was calculated as LA ( $\text{m}^2$ ) divided by its dry mass (kg) and is expressed in  $\text{m}^2 \text{kg}^{-1}$ . LNC was calculated as the total amount of N (mg) in leaf dry mass (g) and is expressed in  $\text{mg g}^{-1}$ .

### **1.2.4 Data analysis**

Co-variations among traits were detected using principal component analysis (PCA). We assessed the position of cover crop species along the LES axis of variation using the relationship between SLA and LNC established by Wright et al. (2004). We did the same for the SLA-LDMC relationship established for 1392 wild herbaceous species based on a compilation of published data (Annexe A2). The position of species on the CSR triangle was assessed with ternary coordinates calculated from LA, LDMC and SLA values, as described by Pierce et al. (2013). Predictive regressions of this method translated PCA coordinates in multidimensional space into coordinates in a “trade-off” triangle.

As a first analysis of the possible effect of domestication and breeding on species traits, we compared the traits measured on cover crop species at the experimental sites to those for annual wild species of the same genus in the TRY database (Kattge et al., 2011 and Annexe A3). Leaf traits were extracted from the database for all native species of the same genera as

those used in our experiment. For each trait, the mean value for all species within the wild genus was calculated and compared to the values obtained for the corresponding genus in our experiment.

Statistical analyses were performed with STATGRAPHICS Centurion XV software (version 15.2.06).

### 1.3 Results

#### 1.3.1 Trait values and rankings are maintained between the two sites

There were no significant differences between the mean values of the four leaf traits measured in France and Switzerland (Student's t-tests  $P$ -values: 0.10 for LNC, 0.42 for LDMC, 0.89 for LA and 0.90 for SLA). Consequently, species ranking based on these traits did not differ between the two sites: Spearman correlation coefficients between values obtained in France and Switzerland were 0.93, 0.87, 0.77 and 0.91 ( $P < 0.0001$ ) for SLA, LDMC, LNC and LA, respectively. Similar results were observed for CGR and CNR: no significant differences in mean values ( $P=0.23$  and  $0.21$  respectively), and species ranking seemed conserved between both sites, with Spearman correlation coefficients of 0.67 ( $P=0.0001$ ) and 0.62 ( $P=0.0003$ ).

#### 1.3.2 Species' functional characterisation and growth and N acquisition rates

CGR and CNR varied respectively from  $0.50 \text{ kg DM ha}^{-1} \text{ DD}^{-1}$  and  $12.4 \text{ g N ha}^{-1} \text{ DD}^{-1}$  for *Medicago lupulina* to  $5.66 \text{ kg DM ha}^{-1} \text{ DD}^{-1}$  and  $155.8 \text{ g N ha}^{-1} \text{ DD}^{-1}$  for *Helianthus annuus*. Globally, all Brassicaceae and Asteraceae had high CGR and CNR values, except *Camelina sativa* which had lower values (Table 1). CGR were significantly positively correlated ( $R^2=0.73$  and  $P<0.0001$ ) with CNR using a logarithm regression model ( $\text{CNR}=60.\ln(\text{CGR})+43$ ).

Leaf traits also spanned a wide range of values: SLA varied from  $14.3 \text{ m}^2 \text{ kg}^{-1}$  for *Brassica rapa* to  $52.2 \text{ m}^2 \text{ kg}^{-1}$  for *Vicia villosa*, with a mean of  $31.9 \text{ m}^2 \text{ kg}^{-1}$ . LDMC varied from  $78.9 \text{ mg g}^{-1}$  for *Brassica rapa* to  $209.6 \text{ mg g}^{-1}$  for *Sorghum bicolor var. sudanense*, with a mean of  $127.7 \text{ mg g}^{-1}$ . Although all species were grown under non-limiting N conditions, LNC also varied over a wide range, from  $25.2 \text{ mg g}^{-1}$  for *Trigonella foenum-graecum* to  $63.4 \text{ mg g}^{-1}$  for *Vicia villosa*. Finally, LA varied from  $6.1 \text{ cm}^2$  for *Lupinus angustifolius* to  $222.8 \text{ cm}^2$  for *Helianthus annuus* (Table 1). All families with more than two species (i.e. Brassicaceae, Fabaceae and Poaceae) had large differences in traits values.

**Table 1** Botanical family, taxonomic status, leaf functional traits values, crop growth rate (CGR) and crop N acquisition rate (CNR) of the 36 taxa studied. Values are means  $\pm$  SDs of both experimental sites. DM is dry matter and DD is degree day. SLA is specific leaf area, LDMC is leaf dry matter content, LNC is leaf nitrogen content and LA is leaf area.

Botanical family	Species	Id.	SLA (m <sup>2</sup> kg <sup>-1</sup> )	LDMC (mg g <sup>-1</sup> )	LNC (mg g <sup>-1</sup> )	LA (cm <sup>2</sup> )	CGR (kg DM ha <sup>-1</sup> DD <sup>-1</sup> )	CNR (g N ha <sup>-1</sup> DD <sup>-1</sup> )
Asteraceae	<i>Guizotia abyssinica</i>	GA	38.2 $\pm$ 6.5	121.6 $\pm$ 12.9	41.4 $\pm$ 1.3	58.1 $\pm$ 20.4	3.43 $\pm$ 0.96	128.6 $\pm$ 32.8
	<i>Helianthus annuus</i>	HA	25.4 $\pm$ 2.4	115.9 $\pm$ 6.8	45.9 $\pm$ 3.5	222.8 $\pm$ 47.3	5.66 $\pm$ 0.52	152.8 $\pm$ 10.2
Brassicaceae	<i>Brassica carinata</i>	BC	20.1 $\pm$ 2.6	105.8 $\pm$ 8.5	39.2 $\pm$ 5.8	132.1 $\pm$ 38.6	3.71 $\pm$ 0.47	127.4 $\pm$ 12.7
	<i>Brassica juncea</i>	BJ	24.2 $\pm$ 2.8	116.6 $\pm$ 8.9	47.7 $\pm$ 6.9	79.4 $\pm$ 31.6	4.48 $\pm$ 0.28	115.6 $\pm$ 4.3
	<i>Brassica napus</i>	BN	19.1 $\pm$ 3.1	106.8 $\pm$ 11.6	38.2 $\pm$ 2.0	169.4 $\pm$ 35.9	3.21 $\pm$ 1.07	130.2 $\pm$ 40.1
	<i>Brassica rapa</i>	BR	14.3 $\pm$ 2.6	78.9 $\pm$ 9.5	38.8 $\pm$ 3.5	222.4 $\pm$ 76.9	3.07 $\pm$ 0.39	102.9 $\pm$ 15.6
	<i>Camelina sativa</i>	CS	44.9 $\pm$ 4.7	99.7 $\pm$ 11.0	61.9 $\pm$ 3.1	10.1 $\pm$ 2.8	1.96 $\pm$ 1.26	89.7 $\pm$ 55.9
	<i>Eruca sativa</i>	ES	22.6 $\pm$ 4.3	93.2 $\pm$ 19.5	51.9 $\pm$ 14.1	48.6 $\pm$ 13.4	1.97 $\pm$ 0.48	117.6 $\pm$ 25.4
	<i>Raphanus sativus</i>	RS	17.1 $\pm$ 3.0	82.7 $\pm$ 9.2	40.5 $\pm$ 5.0	222.6 $\pm$ 83.0	3.32 $\pm$ 0.29	140.8 $\pm$ 8.7
	<i>Sinapis alba</i>	SA	26.6 $\pm$ 3.4	146.6 $\pm$ 11.0	48.1 $\pm$ 6.5	80.4 $\pm$ 24.4	4.56 $\pm$ 0.48	134.2 $\pm$ 10.8
Fabaceae	<i>Lupinus angustifolius</i>	LA	19.2 $\pm$ 4.8	131.4 $\pm$ 14.6	32.7 $\pm$ 9.1	6.1 $\pm$ 1.7	1.2 $\pm$ 0.36	33.6 $\pm$ 10.8
	<i>Lens nigricans</i>	LN	47.4 $\pm$ 6.1	141.5 $\pm$ 20.4	52.5 $\pm$ 0.5	9.4 $\pm$ 1.7	1.77 $\pm$ 0.06	65.5 $\pm$ 3.8
	<i>Lathyrus sativus</i>	LS	40.3 $\pm$ 3.4	105.5 $\pm$ 6.1	60.2 $\pm$ 2.1	19.5 $\pm$ 6.4	2.62 $\pm$ 0.16	108.8 $\pm$ 3.8
	<i>Medicago lupulina</i>	ML	26.4 $\pm$ 4.5	165.0 $\pm$ 17.9	42.0 $\pm$ 5.7	9.9 $\pm$ 2.5	0.50 $\pm$ 0.02	12.2 $\pm$ 0.2
	<i>Melilotus officinalis</i>	MO	29.2 $\pm$ 3.6	156.5 $\pm$ 10.3	58.3 $\pm$ 1.9	14.9 $\pm$ 4.7	1.41 $\pm$ 0.31	71.6 $\pm$ 14.1
	<i>Onobrychis viciifolia</i>	OV	21.2 $\pm$ 2.1	160.5 $\pm$ 13.9	38.1 $\pm$ 1.6	15.9 $\pm$ 3.6	1.36 $\pm$ 0.54	51.4 $\pm$ 19.1
	<i>Pisum sativum</i> cv ASSAS	PSA	40.4 $\pm$ 4.3	111.0 $\pm$ 12.7	58.5 $\pm$ 3.1	52.8 $\pm$ 12.3	2.60 $\pm$ 0.91	124.0 $\pm$ 40.3
	<i>Pisum sativum</i> cv PFX	PSP	32.0 $\pm$ 3.3	106.5 $\pm$ 13.5	56.3 $\pm$ 2.7	34.9 $\pm$ 14.5	1.61 $\pm$ 0.75	72.5 $\pm$ 32
	<i>Trifolium alexandrinum</i>	TA	32.9 $\pm$ 5.3	169.4 $\pm$ 16.1	49.8 $\pm$ 1.1	13.9 $\pm$ 3.6	2.93 $\pm$ 0.47	81.9 $\pm$ 11
	<i>Trifolium incarnatum</i>	TI	30.1 $\pm$ 3.7	101.0 $\pm$ 7.9	42.2 $\pm$ 4.6	19.3 $\pm$ 4.2	1.99 $\pm$ 0.36	83.4 $\pm$ 13.1
	<i>Trigonella foenum-graecum</i>	TF	26.3 $\pm$ 4.3	143.2 $\pm$ 17.6	25.2 $\pm$ 3.5	9.0 $\pm$ 2.8	0.90 $\pm$ 0.40	20.0 $\pm$ 8.4
	<i>Vicia benghalensis</i>	VB	42.7 $\pm$ 5.8	132.9 $\pm$ 18.8	60.7 $\pm$ 0.3	34.2 $\pm$ 7.6	2.41 $\pm$ 0.06	111.1 $\pm$ 0.3
	<i>Vicia faba</i> cv LAURA	VFL	35.1 $\pm$ 3.7	105.4 $\pm$ 10.0	59.9 $\pm$ 3.3	85.6 $\pm$ 18.5	2.49 $\pm$ 0.17	94.5 $\pm$ 3.9
	<i>Vicia faba</i> cv SSNS	VFS	38.2 $\pm$ 3.9	104.0 $\pm$ 6.8	58.1 $\pm$ 1.7	126.1 $\pm$ 35.7	2.74 $\pm$ 0.32	96.2 $\pm$ 8.9
	<i>Vicia sativa</i>	VS	40.5 $\pm$ 3.9	122.4 $\pm$ 13.4	58.1 $\pm$ 4.5	26.8 $\pm$ 5.1	2.03 $\pm$ 0.58	59.5 $\pm$ 15.4
<i>Vicia villosa</i>	VV	52.2 $\pm$ 6.2	120.4 $\pm$ 15.1	63.4 $\pm$ 2.4	21.3 $\pm$ 5.7	2.63 $\pm$ 0.51	124.2 $\pm$ 21.1	
Hydrophyllaceae	<i>Phacelia tanacetifolia</i>	PT	35.6 $\pm$ 5.6	91.7 $\pm$ 12.2	55.5 $\pm$ 6.7	68.5 $\pm$ 25	2.56 $\pm$ 1.26	137.5 $\pm$ 64.6
Poaceae C3	<i>Avena sativa</i>	ASa	28.2 $\pm$ 3.9	146.3 $\pm$ 16.8	45.2 $\pm$ 3.4	46.1 $\pm$ 9.7	2.18 $\pm$ 0.86	72.7 $\pm$ 30.4
	<i>Avena strigosa</i>	ASt	35.2 $\pm$ 7.0	143.9 $\pm$ 21.8	47.7 $\pm$ 5.2	31.9 $\pm$ 7.2	1.60 $\pm$ 0.85	65.4 $\pm$ 36.2
	<i>Lolium hybridum</i>	LH	36.3 $\pm$ 4.0	118.5 $\pm$ 10.0	40.5 $\pm$ 5.0	22.8 $\pm$ 5.2	2.21 $\pm$ 0.51	83.6 $\pm$ 21.2
	<i>Lolium multiflorum</i>	LM	41.6 $\pm$ 5.6	132.3 $\pm$ 11.2	44.3 $\pm$ 1.7	18.5 $\pm$ 4.3	1.93 $\pm$ 0.36	77.5 $\pm$ 12.3
	<i>Secale cereale</i>	SC	32 $\pm$ 4.0	140.9 $\pm$ 14.7	51.4 $\pm$ 4.3	21.1 $\pm$ 12.2	0.91 $\pm$ 1.09	47.5 $\pm$ 57.5
	<i>Secale multicaule</i>	SM	37.5 $\pm$ 5.4	139.4 $\pm$ 15.8	48.1 $\pm$ 5.0	25.0 $\pm$ 5.7	2.01 $\pm$ 0.53	98.2 $\pm$ 23.5
Poaceae C4	<i>Setaria italica</i>	SI	28.2 $\pm$ 3.2	195.3 $\pm$ 19.1	57.4 $\pm$ 3.1	43.5 $\pm$ 22.5	1.74 $\pm$ 0.59	58.6 $\pm$ 18.3
	<i>Sorghum bicolor</i> var. <i>sudanense</i>	SS	27.8 $\pm$ 2.4	209.6 $\pm$ 28.2	35.7 $\pm$ 1.6	145.5 $\pm$ 29	3.50 $\pm$ 1.40	85.8 $\pm$ 36.3
Polygonaceae	<i>Polygonum fagopyrum</i> / <i>Fagopyrum esculentum</i>	PF	37.8 $\pm$ 7.4	131.7 $\pm$ 18.5	39.8 $\pm$ 6.3	27.0 $\pm$ 6.3	4.32 $\pm$ 0.10	98.9 $\pm$ 0.2

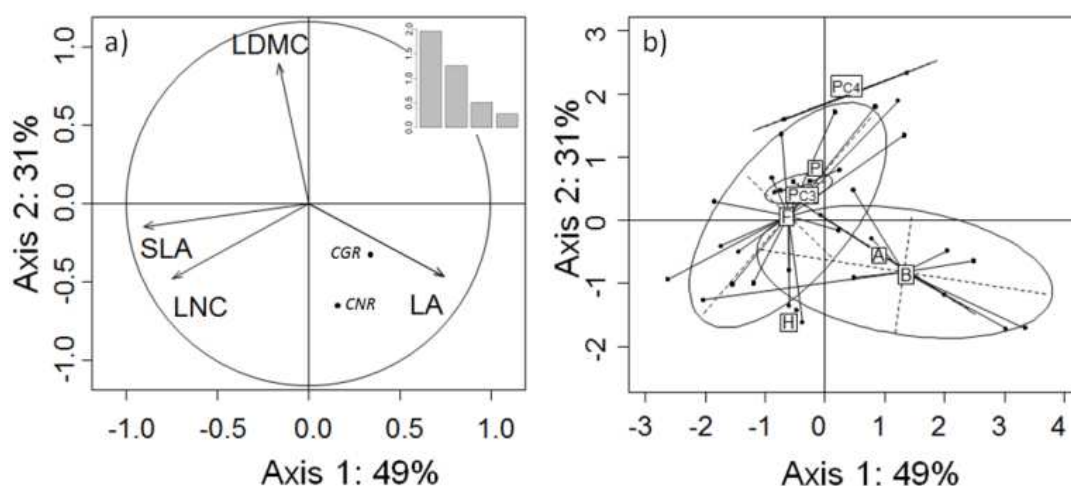
### 1.3.3 Co-variations among plant traits

No significant correlation was detected between CGR and SLA, LDMC or LNC (Table 2 and Annexe A4). The only significant positive relationship was found between LA and CGR, but the correlation was relatively weak ( $R^2=0.37$ ;  $P=0.001$ ). For CNR, significant positive relationships were found but also relatively weak with LA ( $R^2=0.35$ ;  $P=0.0001$ ) and also with LDMC ( $R^2=0.27$ ;  $P=0.001$ ) (Table 2 and Annexe A5).



**Table 2** Pearson's correlations between crop growth rate (CGR) and crop N acquisition (CNR) of cover crop species (n=36) and leaf functional traits: specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) and leaf area (LA). DM is dry matter and DD is degree day.

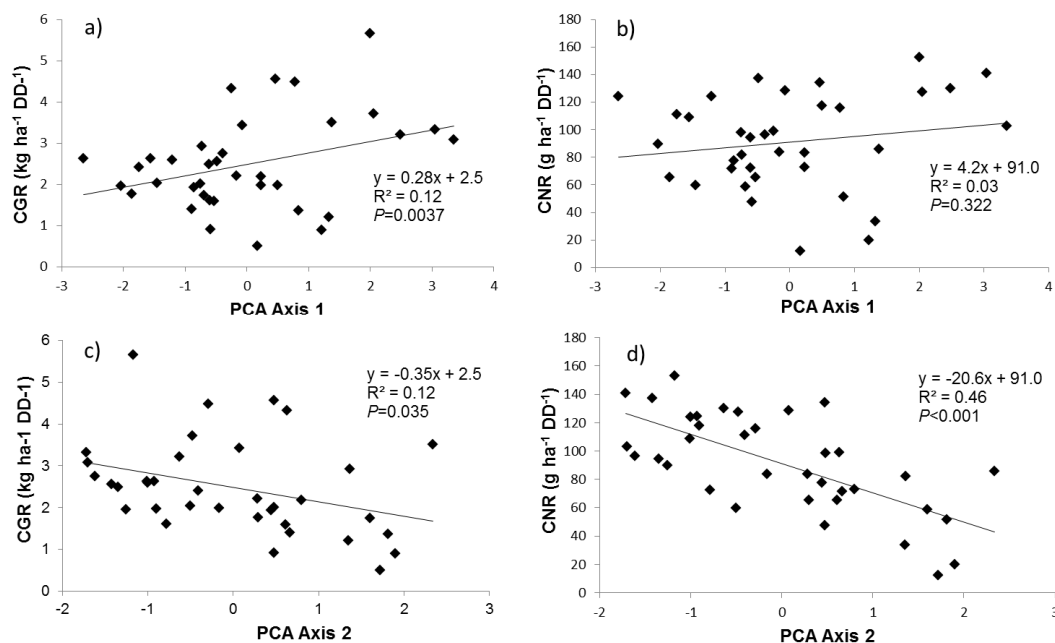
Leaf functional traits	CGR (kg DM ha <sup>-1</sup> DD <sup>-1</sup> )		CNR (g N ha <sup>-1</sup> DD <sup>-1</sup> )	
	Corr.	P-value	Corr.	P-value
SLA	-0.13	0.44	0.02	0.90
LDMC	-0.22	0.20	-0.52	0.001
LNC	-0.01	0.72	0.23	0.18
LA	0.65	<0.0001	0.59	0.0001



**Figure 9** Principal component analysis (PCA) based on four functional traits measured on 36 cover crop species. (a) Correlation circle between SLA (specific leaf area), LDMC (leaf dry matter content), LNC (leaf nitrogen content) and LA (leaf area) and loadings; coordinates position of CGR (crop growth rate) and CNR (crop N acquisition rate); (b) Botanical families and cover crop species position along the first two axes of the PCA; A: Asteraceae; B: Brassicaceae; F: Fabaceae; H: Hydrophyllaceae; P: Polygonaceae; PC3: C3 Poaceae; PC4: C4 Poaceae.

The first two axes of the PCA of functional traits accounted for 80% of the variability in the data (49% and 31%, respectively). Axis 1 opposed LA to SLA and LNC (Figure 9a). These traits strongly correlated with Axis 1 (Loading coefficients were -0.91 for SLA, -0.75 for LNC, and 0.74 for LA; all  $P < 0.001$ ). As a result, species with negative coordinates on this axis, such as *Vicia villosa*, *V. benghalensis*, *V. sativa*, *Len nigrican*, *Camelina sativa*, had high SLA and LNC but low LA. In contrast, species with positive coordinates, such as Brassicaceae (e.g. *Brassica rapa*, *Raphanus sativus*, *B. napus*) had the opposite syndrome (Figure 9b). Axis 2 was strongly and significantly positively correlated with LDMC (Pearson's correlation coefficient was 0.89;  $P < 0.001$ ). *Sorghum bicolor var. sudanense*, *Trigonella foenum-graecum* and *Onobrychis viciifolia* had the highest positive coordinates on this axis, and *Brassica rapa*, *Raphanus sativus* and *Vicia faba cv SSNS* the lowest. All C<sub>3</sub> Poaceae were located around the PCA centre and inside the Fabaceae ellipse, and the scatter

of C<sub>3</sub> Poaceae species in the former group was low. Both C<sub>4</sub> Poaceae were located at a high positive position on Axis 2. Correlations between PCA axes and cover crops growth and N acquisition rates (CGR and CNR not active in PCA) were assessed (Figure 10): Axis 1 was significantly positively correlated with CGR but not with CNR, whereas Axis 2 was weakly negatively correlated with CGR and was more strongly correlated with CNR.



**Figure 10** Relationships between crop growth rate (CGR) and crop N acquisition rate (CNR) and strategy axes from principal component analysis (PCA): axis 1 and 2. The significance of differences was assessed by Student's *t*-tests. Values of traits and CNRs and CGRs are means of both experimental sites.

### 1.3.4 Functional strategies of cover crop species

Grass and herbs wild species in the LES axis had SLA values ranging from 1.5-69.4 m<sup>2</sup> kg<sup>-1</sup> (mean: 19.7 m<sup>2</sup> kg<sup>-1</sup>) and LNC values ranging from 4.5-63.6 mg g<sup>-1</sup> (mean: 26.4 mg g<sup>-1</sup>) (Wright et al., 2004). Cover crop species spanned 55% of the SLA range for herbaceous species and 63% of the LNC range and were located in the high SLA - high LNC zone of the LES relationship (Figure 11). A significant positive correlation was found for cover crops species between log SLA and log LNC (R<sup>2</sup>=0.36 and P=0.0001), however species from different cover crop families were not distinguishable from each other. Brassicaceae showed the lowest SLA and the highest LNC, except for one species that was located outside of the Brassicaceae point cloud, corresponding to *Camelina sativa* (Figure 11).

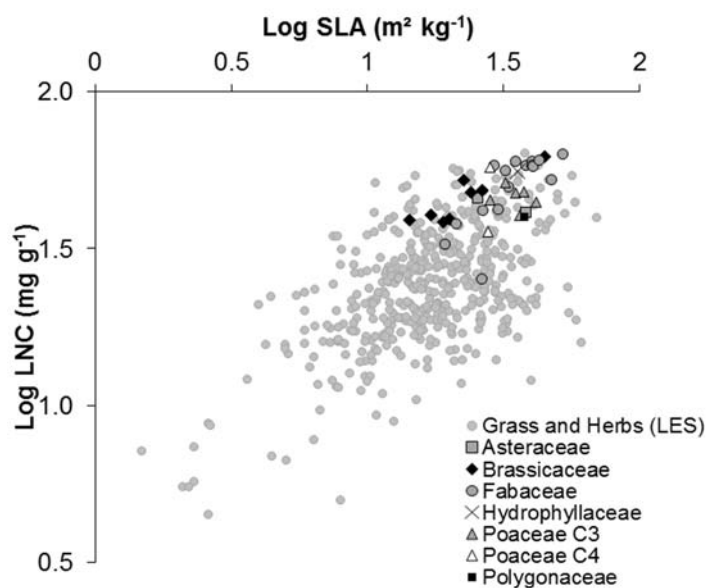


Figure 11 Position of cover crop species along grass and herbs of the Leaf Economic Spectrum (LES) (Wright et al. 2004) based on specific leaf area (SLA) and leaf nitrogen content (LNC) axes (the latter logarithmic).

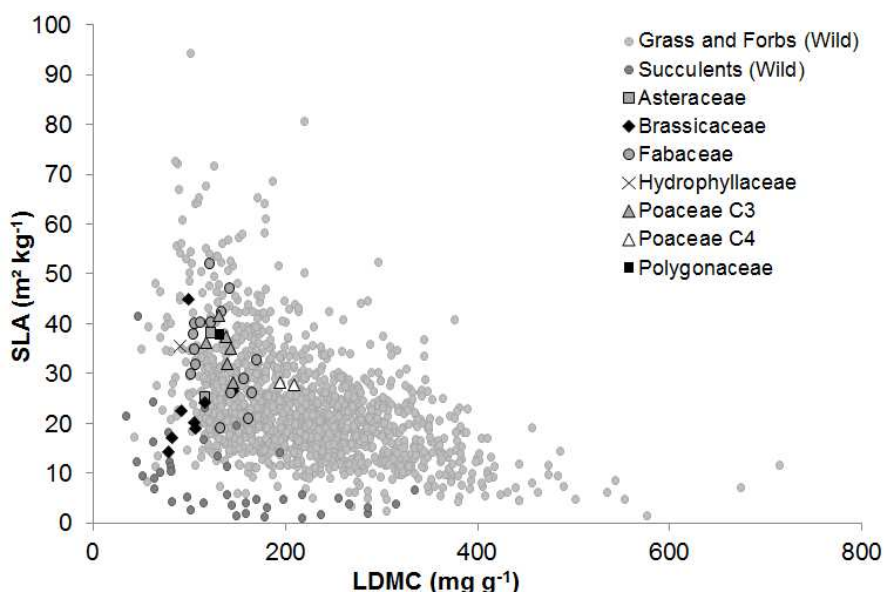


Figure 12 Cover crop species position compared to the relationship between leaf dry matter content (LDMC) and specific leaf area (SLA) established for 1392 grass, forbs and succulents wild species (Annexe A2).

Cover crop species were positioned on the SLA-LDMC plane based on a data set composed of herbaceous wild species (Figure 12). LDMC of wild species composed of grasses and forbs varied from 42-714 mg g<sup>-1</sup> (mean: 221 mg g<sup>-1</sup>), while their SLA varied from 2-94 m<sup>2</sup> kg<sup>-1</sup> (mean: 23 m<sup>2</sup> kg<sup>-1</sup>). Cover crops are mostly located in the high SLA - low LDMC zone of the plane (Figure 12) but no relationship was found between SLA and LDMC in our data set ( $R^2=0.0006$  and  $P=0.88$ ). Fabaceae, C<sub>3</sub> Poaceae, Asteraceae, Polygonaceae and Hydrophyllaceae tended to be mixed in this space. In contrast, most Brassicaceae species had relatively low SLA and LDMC and were surprisingly close to succulent species in the plane. The two C<sub>4</sub> Poaceae species clearly had higher LDMC than the other cover crop species.

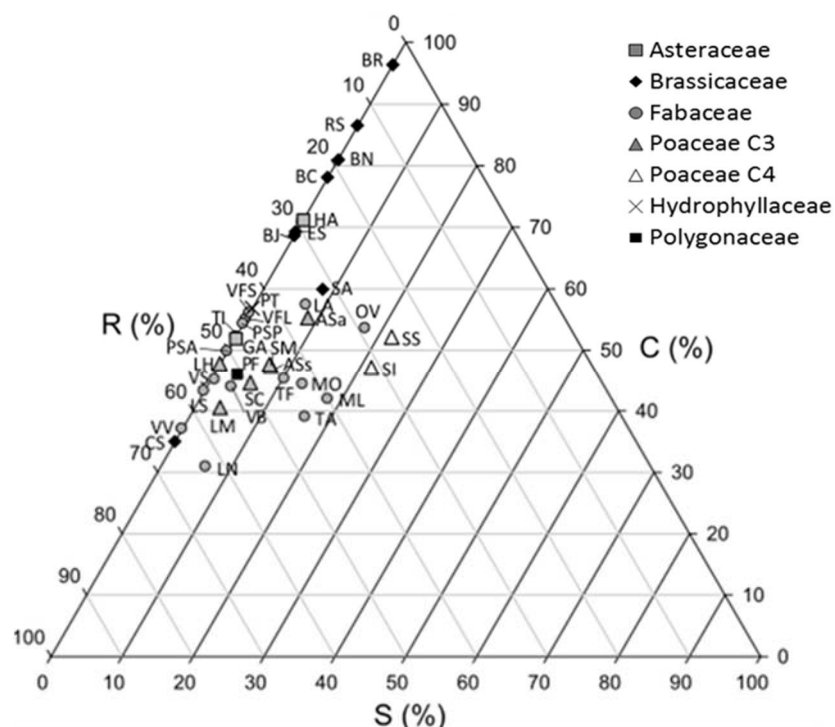


Figure 13 Cover crop species position on Grime's CSR triangle (Pierce et al. 2013). C: competition, S: stress-tolerance, R: ruderality. Letters correspond to species identification (Table 1).

Cover crop strategies corresponded to a restricted region of the CSR triangle: 21 taxa were located in the C-R range in a gradient from C to R strategies (Figure 13). All Brassicaceae were classified as competitive, with C-axis coordinates from 60-96%, except *Camelina sativa* (35% on this axis). *Helianthus annuus* (Asteraceae) was also classified as competitive, at 71% on the C-axis, as expected due to its high CGR and CNR. Species from other families were mostly positioned between competitive and ruderal (47% and 46%, on average, along the C- and R-axes, respectively). Some species were positioned as ruderal, scoring up to 65% on the R-axis (e.g. *Camelina sativa*, *Lens nigricans* and *Vicia villosa*). Species generally scored low on the stress tolerance axis, with the highest values reached by the two C<sub>4</sub> Poaceae (*Sorghum bicolor* var. *sudanense* and *Setaria italica*), but with a maximum value of only 22%. Correlations with Grime's triangle axes showed that CGR and CNR were positively and significantly related to the C axis ( $R^2=0.22$ ;  $P=0.004$  for both) but negatively and significantly related to the S axis, especially for CNR ( $R^2=0.18$ ;  $P=0.050$  for CGR and  $R^2=0.35$ ;  $P<0.001$  for CNR). Concerning the R axis both CGR and CNR were negatively related to the axis ( $R^2=0.11$  and  $R^2=0.04$  respectively) however only the relationship with CGR was barely significant ( $P=0.049$  and  $P=0.223$  respectively).

## 1.4 Discussion

### 1.4.1 The functional-trait approach is robust for cover crop species

Results showed that leaf functional-trait values and rankings were closely maintained among cover crop species grown under non-limiting water and N availabilities at two experimental sites with different soil and climate characteristics. These results are consistent with those of Al Haj Khaled et al. (2005) and Kazakou et al. (2014). However, in our study, in which N fertiliser was added to both sites and legumes showed good nodulation allowing efficient N<sub>2</sub> fixation, LNC ranking was maintained. This stability in rankings and values confirms the robustness of classifications based on leaf traits for characterising functional strategies of domesticated species.

### 1.4.2 Leaf traits related to cover crop growth and N acquisition rates

Axis 1 of the PCA of leaf traits showed a gradient ranging from species with small leaves, high LNC and high SLA to those with large leaves, low LNC and low SLA. This axis was positively correlated with CGR, suggesting that species with high LA, low SLA and low N content are actually growing more quickly than those with low LA, high SLA and high LNC. However, we demonstrated that leaf area was the only leaf functional trait significantly correlated with CGR, showing that species with large leaves are growing more quickly than those with small leaves which is consistent with the study of Hajek et al (2013) based on species used in forestry system. Contrary to our initial hypothesis, we did not find any correlation between CGR and SLA or LNC. This result suggests that there is no direct functional link between net photosynthesis expressed *per* unit leaf mass, captured by a combination of SLA and LNC (Wright et al., 2004), and the absolute growth rate of the cover crop (CGR). Leaf traits that influence growth rates of the aboveground cover ( $\text{g ha}^{-1} \text{DD}^{-1}$ ) therefore appear to differ from those that influence relative growth rate expressed on a plant biomass basis (RGR:  $\text{g g}^{-1} \text{DD}^{-1}$ ).

Axis 2 distinguished species according to their LDMC and was negatively correlated with CGR and especially with CNR. This axis indicates a gradient of species ability to acquire N (fast N uptake or high N<sub>2</sub> fixation) and thus to provide green manure effect. As a result, the LDMC appeared to be the most efficient traits to estimate species ability to acquire N. Finally, the cover crop species positioned positively with axis 1 and negatively with axis 2 would be the species the most efficient as cover crops. This position corresponds to Brassicaceae and Asteraceae which is consistent with their highest CGR and CNR.

### 1.4.3 Cover crop species position along functional and ecological strategies

With their high SLA and LNC, cover crop species are located in the “acquisitive” part of the LES, which is generally associated with a high photosynthetic rate, high respiration rate and rapid leaf turnover (Wright et al., 2004). Although the cover crop species follow the relationship between log SLA and log LNC, none of these traits alone were correlated with CGR and CNR. For the cover crops tested, the LES-plane did not allow to classify them into a gradient of resource acquisition strategies. Moreover, even if leaf traits of cover crops were measured on non-limiting conditions, we found a relatively large range of SLA-LDMC distribution all positioned on the SLA-LDMC wild species plane. However, our data set of cover crop species does not present any SLA-LDMC relationship conversely to species coming from natural ecosystems (Garnier et al., 2001). Especially the Brassicaceae being located close to succulents, which was not expected because of the large difference in growth rates (high for Brassicaceae and low for succulents). Despite their low SLA, Brassicaceae species had high CGR and CNR and are therefore very efficient acquisitive species that produce much biomass and are specifically sought after as cover crops adapted to short fallow periods. That was consistent to their low LDMC values, showing that the LDMC seems more informative than the SLA to discriminate cover crop species strategies.

We showed that positioning species on the Grime’s triangle (Grime et al., 1997) was consistent for exploring differences between cover crop species; in fact, CGR and CNR were positively correlated with the C axis. As a consequence the most competitive cover crop species are those having the highest CGR and CNR. All Brassicaceae (except *Camelina sativa*) and *Helianthus annuus* were identified as highly competitive and thus corresponds to their ability to rapidly uptake soil nitrate and occupy the space needed to acquire light. Again according to the CSR triangle, most of the cover crop species seem not to tolerate stress, except for C<sub>4</sub> Poaceae. This is in agreement with the fact that C<sub>4</sub> plants, especially sorghum, are often resistant to water stress (Lopes et al., 2011; Ludlow and Muchow, 1990). Cover crop species with low CGR seem more ruderal and those with low CNR more stress tolerant, based on the negative correlations with R and S axes. However, we found that *Sorghum bicolor var. sudanense*, which should be the most stress-tolerant, had one of the highest CGRs and CNRs pointed out some lack of precision of the Grime’s triangle to accurately predict the strategies of cover crop species.

#### 1.4.4 A first hypothesis explaining a cover crop domestication syndrome

The hypothesis tested here is that domestication or breeding modified the cover crop leaf traits syndrome and could explain why ecological methods have been found insufficiently accurate for characterising and ranking cover crop species. In a first approach, leaf traits values of cover crops were compared to their homologous wild annual species from the same genus found in the TRY database (Table 3).

**Table 3** Leaf functional traits measured in cover crop botanical families in experimental fields and leaf functional traits of corresponding wild species from the TRY plant-trait database (Kattge et al. 2011 and Annexe A2). Traits of wild species are means of annual species of same genus. Values are means  $\pm$  SDs of species of each family (na: data not available in TRY database). Only cover crop species with a corresponding wild species in TRY were included in the mean. The significance of differences was assessed by Student's t-tests, testing the effect of the species by family all together. SLA is specific leaf area, LDMC is leaf dry matter content and LNC is leaf nitrogen content.

Botanical family	SLA (m <sup>2</sup> kg <sup>-1</sup> )			LDMC (mg g <sup>-1</sup> )			LNC (mg g <sup>-1</sup> )		
	Cover crops	Wild species	P-value	Cover crops	Wild species	P-value	Cover crops	Wild species	P-value
Brassicaceae	23.6 $\pm$ 9.4	17.6 $\pm$ 7.5	0.145	103.8 $\pm$ 21.4	173.7 $\pm$ 35.2	0.003	45.8 $\pm$ 8.3	na	na/
Poaceae	33.9 $\pm$ 5.7	27.3 $\pm$ 0.7	0.070	147.3 $\pm$ 29.0	221.0 $\pm$ 9.5	0.001	45.2 $\pm$ 3.1	24.7 $\pm$ 3.9	0.000
Fabaceae	36.8 $\pm$ 7.7	25.3 $\pm$ 2.0	0.002	125.1 $\pm$ 26.1	224.3 $\pm$ 19.5	0.000	52.5 $\pm$ 10.1	43.6 $\pm$ 11.4	0.001
Polygonaceae	37.8	32.4	/	131.7	213.7	/	39.8	20.4	/

The analysis was done at the genus level because no data was available for the wild ancestor of each cover crop species. The comparison indicated that cover crop species presented systematically higher SLA and LNC and lower LDMC than wild species. Moreover, Fabaceae which did not receive any fertilizer in our experiment also presented a significant higher LNC than wild species; this fact constitutes a first argument indicating that the comparison of leaf trait values of TRY database to our experimental data could make sense to test the hypothesis that domestication would have modified the leaf traits. This is also consistent with Kazakou et al (2014) who showed that SLA, LDMC and LA are not very sensitive to N fertilisation. Then the comparisons of cover crops and wild species traits suggest that domestication and breeding would have induced a decrease of LDMC and an increase of SLA and LNC. This could be related to their adaptation to different environments and reflect fundamental differences in the strategies they use to acquire, invest and use resources (Aerts and Chapin, 2000; Lambers and Poorter, 1992). Similar results were illustrated for certain species by Gambino and Vilela (2011), who found that domesticated primroses (*Primula vulgaris*) accumulated more biomass than wild ones and had higher SLA. In another study, domesticated cassava (*Manihot esculenta*) had a higher SLA and CO<sub>2</sub> exchange rate and

lower LDMC than wild ones (Pujol et al., 2008). However, this is a first argumentation of a potential domestication effect on leaf traits syndrome and further specific studies are needed for a full demonstration. Especially, this analysis have been made on the average of traits for species of the genus of corresponding wild species, it would be worth to investigate the wild ancestor at the species level and in the same growth conditions on a larger scale.

#### **1.4.5 Cover crop traits and the N green-manure service from residues**

Considering the decomposition of crop residues, García-Palacios et al. (2013), who worked with some of the species tested in our experiments (*Avena sativa*, *Helianthus annuus*, *Secale cereale*, *Sorghum sudanense*), highlighted that domesticated species produced high quality litter, with higher nitrate availability and lower C:N ratio than wild species of the same genus. These decomposition characteristics may promote rapid mineralisation and increase N availability for following crops, increasing the green-manure service. In a modelling study, Justes et al. (2009) demonstrated that the lower the C:N ratio, the more rapidly N is released from cover crop residues after their incorporation into the soil. Moreover, litters with high LDMC have low decomposition rates (Fortunel et al., 2009; Kazakou et al., 2009). Our results suggest that cover crop species have lower LDMC values than wild species. If so, cover crop species would tend to produce higher-quality residue, increasing the rate of N release after residue incorporation into the soil and then produce N green manure effect. Our results indicate that cover crop species are better adapted than wild species for increasing N availability and nutrition of the succeeding cash crop. Among the cover crop species considered in this study, *Brassicaceae* and *Fabaceae* are better able to provide this N recycling service, which highlights their particular interest as a cover crop species.

### **1.5 Conclusion**

Leaf functional traits allow us to explore differences in the ability of cover crop species to produce ecosystem services in agrosystems for N recycling. All studied species, despite the wide range of CGR and CNR, belong to the acquisition-use strategy as indicated by converging results from the three ecological methods tested. We demonstrated that LA and LMDC were correlated to crop growth and N acquisition rates as an interesting result, but other traits did not provide any relevant information. Furthermore, this study pointed out the lack of precision to characterise and rank cover crops for producing ecosystem services. We hypothesised that domestication and breeding would have modify the trait syndrome of cover



crops and would have maximised their ability to have high growth rate and, as a consequence, a fast resource acquisition. Further studies are necessary to better understand links between functional traits and cover crop services to improve the use of functional traits as an accurate method for screening and adapting cover crop species to the targeted ecosystem services.

## **1.6 Acknowledgements**

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**Références indiquées à la fin du manuscrit.**



## **2 Determination of cardinal temperatures for germination and related functional groups for a wide range of cover crop species.**

*In preparation for submitting to European Journal of Agronomy.*

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### **2.1 Introduction**

A diversity of cover crops are increasingly used during fallow periods. First as it became compulsory to sow them for catching nitrate and limit water pollution, and also as they provide at the same time different other services such as producing “green manure” effect (increasing nitrogen available for next main crop), protecting soils against erosion (Justes et al., 2012b; Thorup-Kristensen et al., 2003; Wilke and Snapp, 2008) and help to control pests and diseases. Many species belonging to different families (Brassicaceae, Poaceae, Fabaceae or other families) are available and some have been specifically selected to be efficient as cover crops with a large and fast biomass production, while others are tested for different potential interests, which are not always clearly established. In temperate climates like in Europe, cover crops are commonly sown in late summer for two to several months according to the fallow period duration determined by the cash crop succession. The challenge is to successfully establish cover crops stand in late summer in order to achieve all the expected ecosystem services in a few months. Seed germination and seedling emergence are crucial processes, which depend on climate conditions and, particularly, temperature which is one of the most important factor regulating germination (Baskin and Baskin, 1988) in interaction with water availability (Gummerson, 1986). When cover crops are sown in late summer, high temperatures over 30 or 35°C can be frequent, which influence the ability of species to germinate in the field. In France and in Europe, the variety of climates and sowing dates requires adapting the choice of cover crop species and to better know the germination response to temperatures of a large number of species. The effects of temperatures on seed germination are widely examined through the germination maximum percentage and the germination speed, defined as 1/time to germination of a defined seed percentage (Roberts, 1988). The range of temperatures favorable to seed germination can be described by cardinal temperatures: minimum, optimum and maximum temperature values for germination (Bewley and Black, 1994). The minimum temperature ( $T_0$ ) is the lowest temperature at which a seed can germinate. The optimum temperature ( $T_{opt}$ ) corresponds to the temperature at which the

fastest germination speed occurs, and the maximum temperature ( $T_{\max}$ ) is the highest temperature at which seeds can germinate. The response of germination speed to temperature can be fitted to the Yin model, for which three parameters are the cardinal temperatures. The base temperature value is a concept close to  $T_0$  value, used for calculating cumulative thermal time. It is the intercept with the x axis of the linear part of the positive relation between germination speed and increasing temperatures until  $T_{\text{opt}}$  (Gummerson, 1986). Calculating base temperature and cardinal temperatures allow comparing species and also the parameterization of models simulating the time of various species for germination and emergence under contrasting climatic conditions. The effects of temperature on germination have been studied for many species but only few studies investigated germination of cover crop species such as white mustard, Italian ryegrass or hairy vetch (Dorsainvil, 2002; Jacobsen et al., 2010; Lee et al., 2009; Schopfer et al., 1979; Wilke and Snapp, 2008) and no comparative study over a large range of temperatures on seed germination was carried out for a wide range of cover crop species. There is thus a lack of knowledge for choosing the most adapted species of cover crops according to pedoclimatic conditions.

The aim of this study was to determine the response of germination to temperature (final percentage and speed) and to calculate the cardinal temperatures and  $T_b$  for a diversity of cover crops species. These characteristics were also used to propose functional groups of cover species having the same temperature response.

## **2.2 Materials and Methods**

### **2.2.1 Cover crop species and seeds**

Thirty-six taxa (34 species and two varieties of *Vicia faba* and *Pisum sativum*) from six botanical families were selected to ensure a wide range of species more or less used as cover crops (Table 4). The Poaceae family was separated into two groups according to photosynthetic pathway ( $C_3$  vs.  $C_4$ ). Seeds were produced and commercialized by French seed companies and no seed treatment was applied. For each taxon, the same seed lot was used for all modalities tested in order to avoid a potential “lot effect” which allow evaluating only temperature effect on germination.

### **2.2.2 Germination experiments**

Germination was measured in laboratory at 8 temperatures from 4.5°C to 39.5°C and also at 43°C for taxa obtaining good germination at 39.5°C. The germinations at temperatures from

11.5°C to 36°C were measured on an automatic digital imaging system. It was composed of a Jacobsen table made of grids on which flat blotter paper with seeds were placed and a hydraulic power in continuous flow of demineralized water assured optimal moisture for seed germination. The table is covered by a hood opening every two hours, for picture acquisition under white light thanks to four cameras. Four replicates of 25 seeds were sown for each taxon and were split under each camera. Image analysis was performed with Fiji merging the pixels from the color images into two classes (seeds or background) and then measuring each seed thanks to a labeling module (Demilly et al., 2014; Ducournau et al., 2005, 2004). A seed was considered as germinated when the radicle pointed out of the integuments. This method allows obtaining a very precise dynamic of germination. For the extreme temperatures (4.5°C, 39.5°C and 43°C) the seeds were sown on flat germination paper in plastic Petri boxes, moistened with 5 ml deionized water placed in the dark in incubators with controlled temperature. Deionized water was added after each measurement. Germinated seeds (radicle pointing out of the teguments) were counted two or three times per day depending on temperature. Temperatures were recorded every half hour in order to measure the precise temperature of each experiment.

### 2.2.3 Data analysis

The cumulative germination dynamic over time was fitted for each species and temperature to the Gompertz function expressing cumulative germination,  $G(t)$ , in percentage (%) at  $t$  time after sowing (h):  $G(t) = G_{max} \cdot EXP \left[ \left( -\frac{b}{c} \right) \cdot EXP(-c \cdot t) \right]$

where  $G_{max}$  was the final germination percentage (maximum cumulative germination) and  $b$  and  $c$  were shape parameters. The germination speed was calculated for  $G = 20^{th}$ ,  $30^{th}$ ,  $40^{th}$  and  $50^{th}$  percentiles as  $1/t(G)$  and fitted to Yin model (Yin, 1995) for each taxon, over all temperatures:  $\frac{1}{t(G)} = EXP(\mu) \cdot (T - T_0)^\alpha \cdot (T_{max} - T)^\beta$

where  $T$  was the measured temperature,  $T_0$  and  $T_{max}$  were the minimal and maximal temperatures at which germination stopped,  $\mu$ ,  $\alpha$  and  $\beta$  were shape parameters. The optimal temperature for germination ( $T_{opt}$ ) was calculated for each taxon based on the parameters of previous equation as:  $T_{opt} = (\beta \cdot T_{max} + \alpha \cdot T_0) / (\alpha + \beta)$

The base temperature ( $T_b$ ) was calculated for each percentile from  $20^{th}$  to  $50^{th}$  based on data for the  $1/t(G)$  which increased linearly ( $1/t(G) = a \cdot T + b$ ) with temperature (Dahal and Bradford, 1994; Gummerson, 1986),  $T_b$  was estimated as the values for which germination was null when fitting the speed of germination to the temperature for the linear response

phase only. All cardinal temperatures of germination ( $T_{opt}$ ,  $T_0$ ,  $T_{max}$ ) and  $T_b$  were calculated for each percentile and averaged to obtain a better estimation. All fittings were adjusted by minimizing the sum of square error. Statistical analysis and fittings were performed using R software (version 2.14.0). ANOVA and a posteriori Student-Newman-Keuls tests were used with level of significant  $P < 0.05$ . Hierarchical classification was done using Ward method based on the three cardinal temperatures  $T_0$ ,  $T_{opt}$  and  $T_{max}$ ; we have chosen to investigate the use of  $T_0$  instead of  $T_b$  to evaluate the minimal temperature at which the species can germinate because  $T_0$  is generally a better indicator thanks to the more precise method to estimate it, compared to  $T_b$ . Distances between taxa were estimated by Euclidean square.

## 2.3 Results

### 2.3.1 Large variations among species in germination response to temperatures

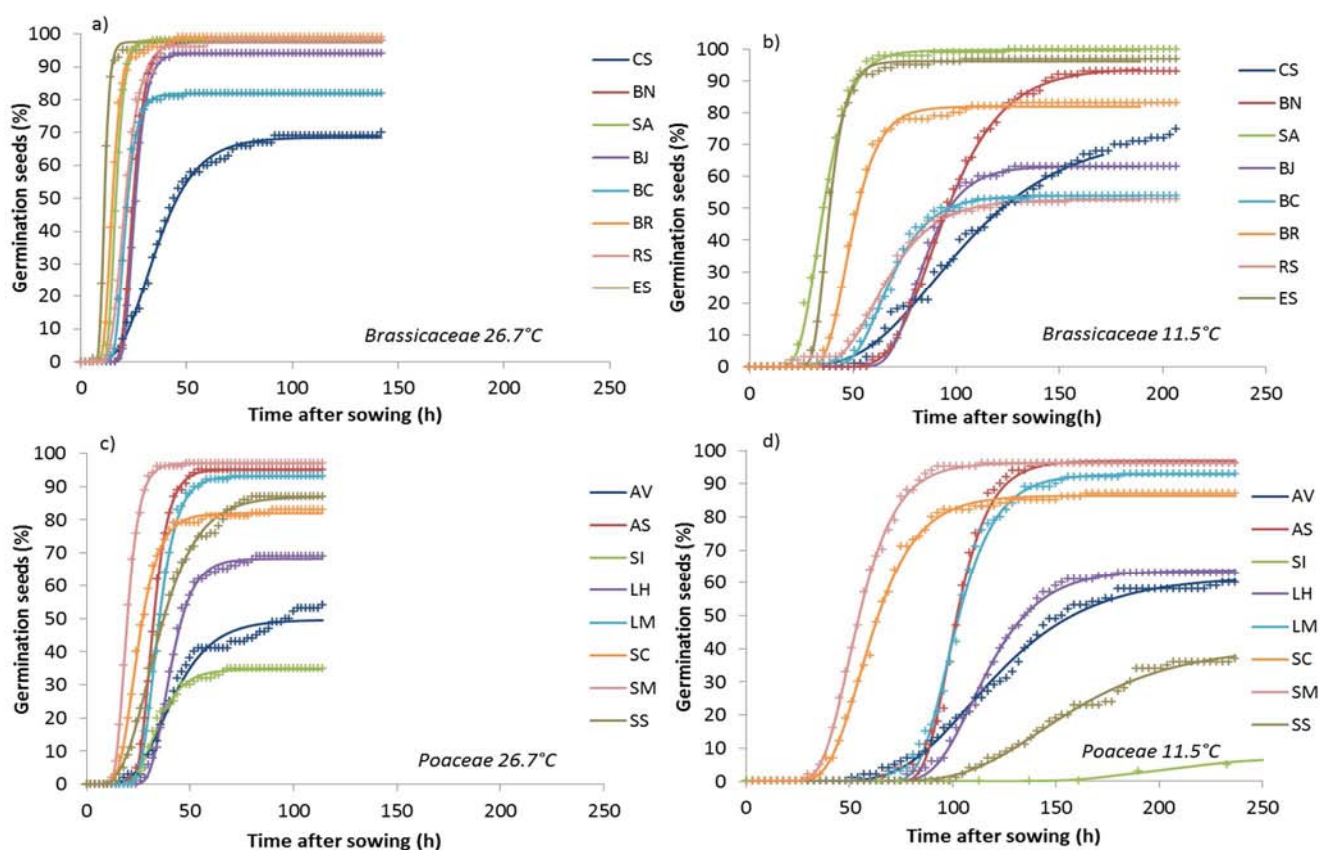


Figure 14 Variation in germination dynamic for a) Brassicaceae cover crops at 26.7°C and b) at 11.5°C and for c) Poaceae cover crops at 26.7°C and d) at 11.5°C. The crosses represent the observed data and the corresponding line represents the fittings to a Gompertz function. CS is *Camelina sativa*, BN is *Brassica napus*, SA is *Sinapis alba*, BJ is *Brassicca juncea*, BC is *Brassica carinata*, BR is *Brassica rapa*, RS is *Raphanus sativus*, ES is *Eruca sativa*, AV is *Avena sativa*, AS is *Avena strigosa*, SI is *Setaria italica*, LH is *Lolium hybridum*, LM is *Lolium multiflorum*, SC is *Secale cereale*, SM is *Secale multicaule* and SS is *Sorghum bicolor var. sudanense*.

The cumulative germination percentage varied widely among taxon. For Brassicaceae at 26.7°C (Figure 14a) all species began to germinate very early (from 8 to 18h after sowing)

and reached fastly a high final germination percentage (from 22 to 42h after sowing), except *Camelina sativa*. For Poaceae, at the same temperature, germination began later (from 12 to 28h after sowing) and final germination percentages were much more variable among species (35% to 98%). At 11.5°C, germination results were more variable for all species but still more variable for Poaceae than for Brassicaceae.

In Poaceae (Figure 14c-d), the strongest differences between the two temperatures were observed for the two  $C_4$ , *Sorghum bicolor var. sudanense* and *Setaria italica*, for which the final germination percentage was strongly affected (56% and 80% of reduction respectively).

Another illustration of contrasted germination dynamics according to a range of temperatures is given in Figure 15a-b for two species: *Guizotia abyssinica* and *Phacelia tanacetifolia*. For *Guizotia abyssinica*, the optimal germination dynamic occurred for temperatures from 26.7 to 36.0°C with an imbibition time very short (8-12h) and thus a fast germination. In contrast, for *Phacelia tanacetifolia* the optimal germination dynamic allowing fast germination occurred at lower temperatures, from 15.4 to 20.6°C.

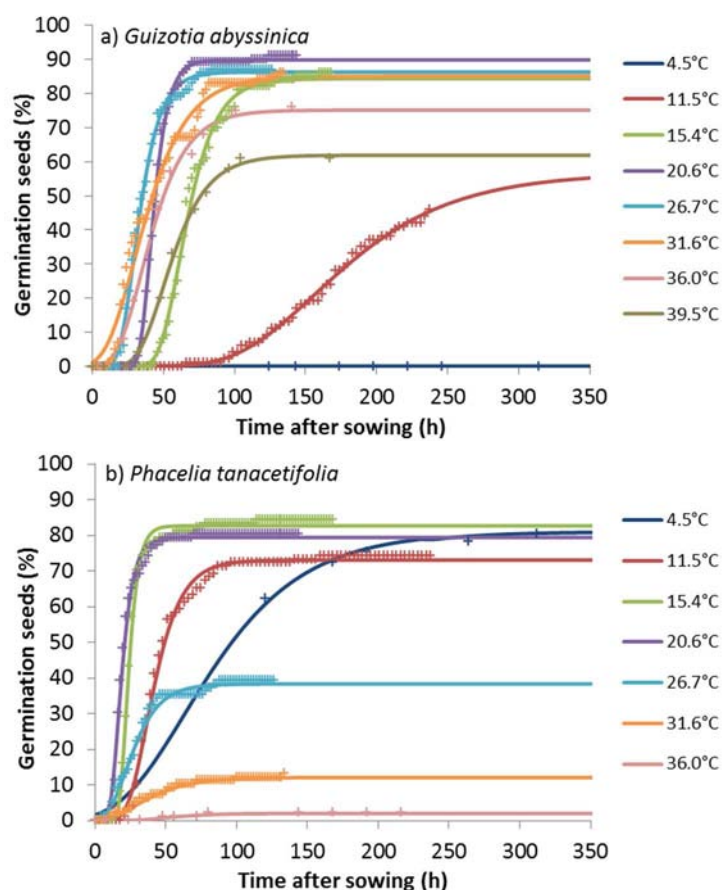


Figure 15 Examples of variation in germination dynamic for a) *Guizotia abyssinica* and b) *Phacelia tanacetifolia* according to temperatures. The crosses represent the observed data and the corresponding line represents the fittings to a Gompertz function.

The final germination percentage and/or the time to reach it decreased in both cases with higher and lower temperatures in comparison to the optimal ones. However the two species respond differently to extremes temperatures. The germination dynamic of *Guizotia abyssinica* was strongly affected only by very low temperatures (low final germination percentage, late germination at 11.5°C and no germination at 4.5°C) compared to *Phacelia tanacetifolia* dynamics which was strongly affected only by high temperatures since the final germination percentage significantly decrease at 26.7, 31.6 and then 36.0°C.

### 2.3.2 Temperature effect on final germination percentage and germination speed

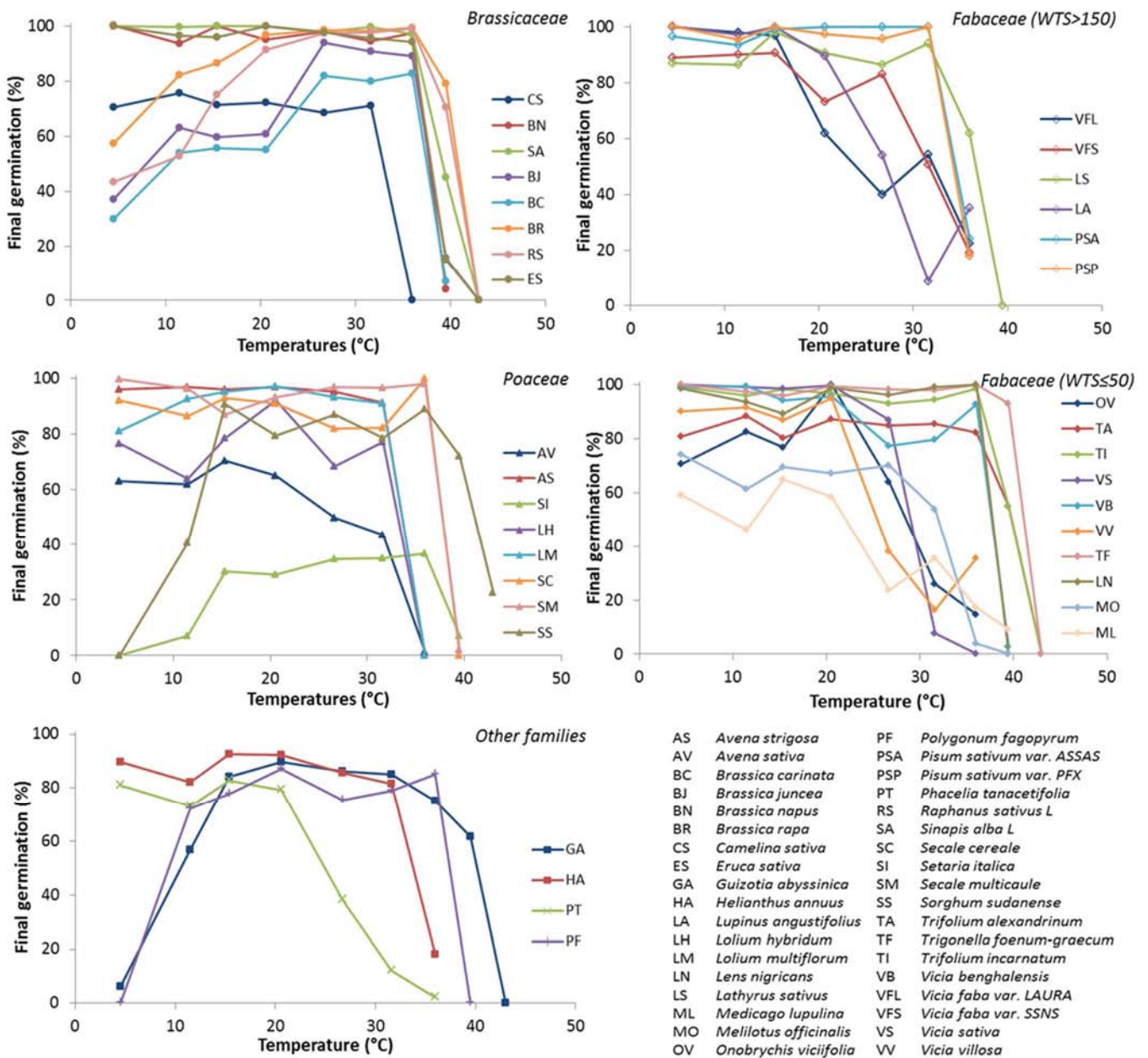


Figure 16 Variation in the final germination percentage for cover crops taxa of various families according to temperatures.



In Figure 16, globally all taxa reached a high maximal final germination percentage, ranging from 80 to 100%, except *Setaria italica* (37%), *Medicago lupulina* (62%), *Avena sativa* (66%), *Melilotus officinalis* (74%) and *Camelina sativa* (75%), maybe due to a seed lot effect. Two types of response to temperature of final germination percentage can be observed: (i) the taxa maintained a final germination percentage constant whatever temperature in the range 11 to 35 °C, and it decreased sharply only at the highest one and particularly 39.5 and 43°C; (ii) the final germination percentage of the other taxa was the lowest at both minimal and maximal temperatures. The first case was particularly applicable to all Fabaceae taxa, all C<sub>3</sub> Poaceae, only a part of Brassicaceae (*Brassica napus*, *Sinapis alba* and *Eruca sativa*), *Phacelia tanacetifolia* and *Helianthus annuus*. For example, *Phacelia tanacetifolia* maintained a final germination percentage close to 80% from 4.5 at 20.6°C then it decreased gradually with higher temperatures, some other species such as *Eruca sativa* or *Pisum sativum* for which the final germination percentage severely decreased. The second case concerned the majority of Brassicaceae, the two C<sub>4</sub> Poaceae and also *Guizotia abyssinica* and *Polygonum Fagopyrum*. For example, *Brassica carinata* final percentage increased with temperature from 30% at 4.5°C to more than 80% from 26.7 to 36°C before to decrease to only 7% at 39.5°C. Curves of germination percentages of 30<sup>th</sup> percentile (Figure 17) fitted well the data with R<sup>2</sup> from 0.81 for *Vicia villosa* to 0.99 for *Secale cereale* and *Medicago lupulina*; and 0.95 in average of all taxa. The germination speed increased gradually from minimal temperature until the optimal temperature then it severely decreased to a very low germination speed. Globally, the Brassicaceae obtained the highest germination speed (0.030 h<sup>-1</sup>) and particularly *Eruca sativa*, *Brassica rapa*, *Raphanus sativus* and *Sinapis alba* (0.040 h<sup>-1</sup> in average). The Poaceae had average germination speed of 0.016 h<sup>-1</sup>, with *Secale* particularly fast to germinate (0.025 h<sup>-1</sup>) unlike *Avena* which had low germination speed (0.011 h<sup>-1</sup>). The Fabaceae with small seeds had a higher germination speed (0.018 h<sup>-1</sup>) than those with large size seeds (0.008 h<sup>-1</sup>). Both *Trifolium* and *Trigonella foenum-graecum* were the fastest Fabaceae to germinate (0.033 h<sup>-1</sup> in average).

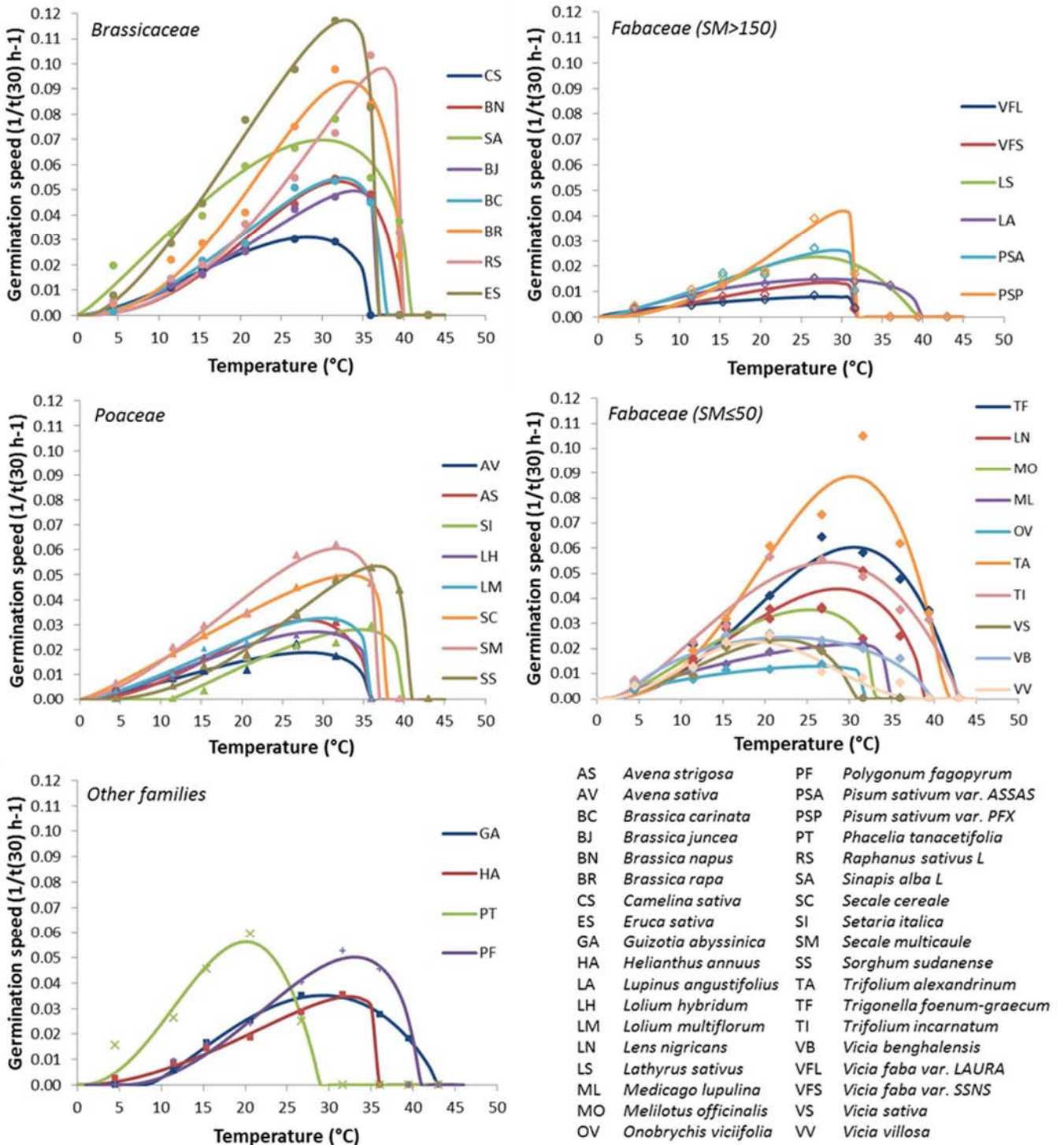


Figure 17 Variation in germination speed (1/t(30)) for cover crops taxa of various families according to temperature. SM is seed mass (mg). The points represent the observed data and the corresponding line represents the fittings.

### 2.3.3 Cardinal temperatures and $T_b$

The fittings of the 50th germination percentile were close to the observed data (examples in Figure 18).

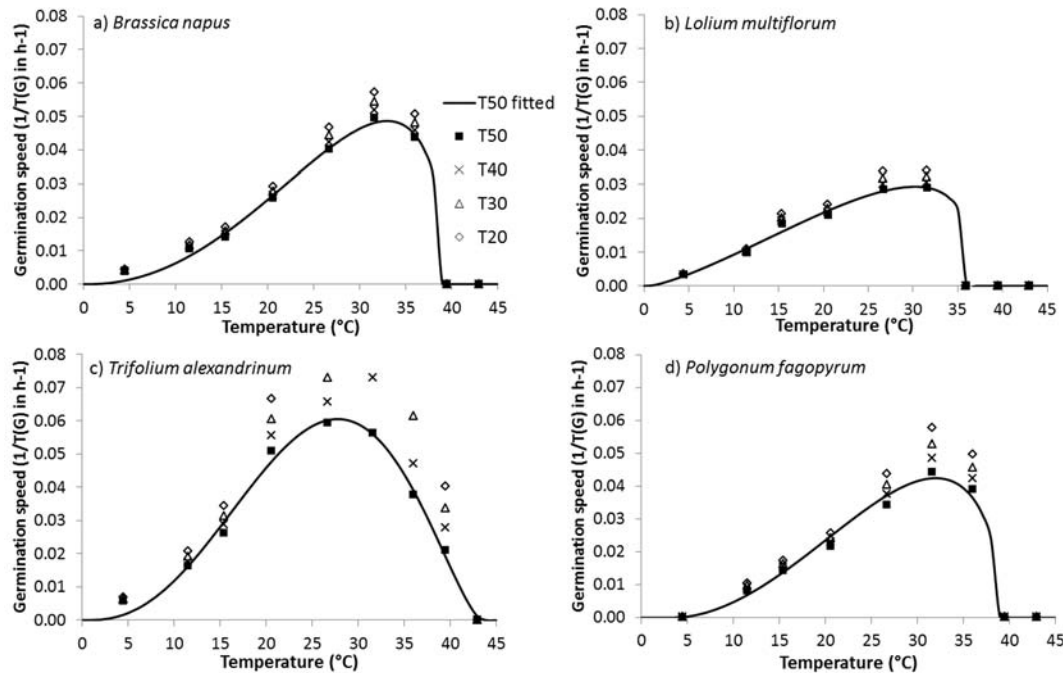


Figure 18 Examples of germination speeds for four fractions of the seed population 20<sup>th</sup> (◇), 30<sup>th</sup> (△), 40<sup>th</sup> (×) and 50<sup>th</sup> (■) according to temperature, in the case of *Brassica napus* (Brassicaceae), *Lolium multiflorum* (Poaceae), *Trifolium alexandrinum* (Fabaceae) and *Polygonum fagopyrum* (Polygonaceae).

The different percentiles (20<sup>th</sup>, 30<sup>th</sup>, 40<sup>th</sup> and 50<sup>th</sup>) presented the same germination speed curve according to temperature leading to globally close estimation of  $T_{opt}$ ,  $T_0$  and  $T_{max}$ . The mean  $T_{opt}$  of all cover crops taxa was estimated at 29.1°C and varied from 20.0°C for *Vicia villosa* to 37.2°C for *Raphanus sativus* (Table 4).

The Brassicaceae had an optimal germination speed at high temperature (32.4°C in average) which was in average the highest in comparison with other botanical families, it was significantly ( $P=0.002$ ) higher than the Fabaceae (26.4°C in average). For the Brassicaceae, *Camelina sativa* and *Sinapis alba* had  $T_{opt}$  at significantly ( $P<0.0001$ ) lower temperatures than others taxa. For the Fabaceae,  $T_{opt}$  of *Lens nigricans*, *Trigonella foenum-graecum* and *Trifolium alexandrinum* were significantly the highest whereas  $T_{opt}$  of *Vicia villosa*, *V. sativa*, *V. benghalensis* and *V. faba* var. LAURA were the lowest. The Poaceae were in average close to the Brassicaceae (31.3°C) but some Poaceae species such as *Avena strigosa* and *A. sativa* had  $T_{opt}$  (27.8 and 25.5°C) were significantly ( $P<0.0001$ ) lower than species with the highest  $T_{opt}$  such as the two C<sub>4</sub> Poaceae (35.8°C in average). Globally,  $T_0$  was estimated at 1.4°C as average of all cover crops taxa, from about 0.0°C for many taxa, especially Brassicaceae, to

11.3°C for *Setaria italica*. The average  $T_{max}$  of all taxa was 36.5°C, varying from 27.7°C for *Phacelia tanacetifolia* to 43.4°C for *Trifolium incarnatum*.

**Table 4 Seed mass, germination cardinal temperatures and  $T_b$  values of the 36 cover crops taxa. Mean and standard error of four percentiles.**

Family	Taxa	Id.	Seed mass (mg)	$T_0$	$T_{opt}$	$T_{max}$	$T_b$
Asteraceae	<i>Guizotia abyssinica</i>	GA	3.3	8.7 ± 2.8	28.7 ± 1.3	42.9 ± 0.3	8.1 ± 0.9
	<i>Helianthus annuus</i>	HA	48.0	2.3 ± 1.1	32.5 ± 0.6	36.0 ± 0.0	4.4 ± 0.5
Brassicaceae	<i>Camelina sativa</i>	CS	1.3	0.0 ± 0.0	28.3 ± 0.1	35.8 ± 0.4	2.1 ± 0.1
	<i>Brassica napus</i>	BN	2.7	0.0 ± 0.0	32.7 ± 0.4	38.9 ± 0.5	7.2 ± 0.2
	<i>Sinapis alba</i>	SA	8.0	0.0 ± 0.0	29.6 ± 0.7	40.4 ± 0.3	1.2 ± 0.1
	<i>Brassica juncea</i>	BJ	3.0	0.3 ± 0.6	33.7 ± 0.6	37.8 ± 1.4	6.8 ± 0.5
	<i>Brassica carinata</i>	BC	5.0	0.0 ± 0.0	32.3 ± 0.3	37.1 ± 0.2	6.7 ± 0.9
	<i>Brassica rapa</i>	BR	3.7	0.0 ± 0.0	33.1 ± 0.5	39.7 ± 0.0	6.6 ± 0.0
	<i>Raphanus sativus</i>	RS	13.0	1.2 ± 0.9	37.2 ± 0.2	39.5 ± 0.0	7.3 ± 0.6
	<i>Eruca sativa</i>	ES	1.3	0.8 ± 0.6	32.5 ± 0.4	36.2 ± 0.1	5.4 ± 0.1
	Poaceae	<i>Avena sativa</i>	AV	39.4	0.5 ± 0.8	25.5 ± 4.5	32.7 ± 3.5
<i>Avena strigosa</i>		AS	16.1	0.0 ± 0.0	27.8 ± 0.4	35.8 ± 0.2	4.8 ± 0.1
<i>Setaria italica</i>		SI	2.2	11.3 ± 0.3	36.1 ± 1.5	39.5 ± 0.0	10.6 ± 0.1
<i>Lolium hybridum</i>		LH	3.4	0.9 ± 0.7	29 ± 0.3	36.0 ± 0.0	1.1 ± 0.3
<i>Lolium multiflorum</i>		LM	2.7	0.4 ± 0.3	30.1 ± 0.1	34.6 ± 1.3	1.9 ± 0.3
<i>Secale cereale</i>		SC	32.3	0.5 ± 0.7	34.5 ± 1.5	38.1 ± 1.5	0.6 ± 0.2
<i>Secale multicaule</i>		SM	18.8	0.0 ± 0.0	32.1 ± 1.1	37.1 ± 0.9	3.1 ± 0.8
<i>Sorghum bicolor var. sudanense</i>		SS	13.8	5.3 ± 3.0	35.6 ± 3.0	40.6 ± 1.5	9.4 ± 0.6
Hydrophyllaceae		<i>Phacelia tanacetifolia</i>	PT	1.8	0.3 ± 0.6	21.3 ± 1.0	27.7 ± 1.0
Fabaceae	<i>Trigonella foenum-graecum</i>	TFG	16.0	0.0 ± 0.0	30.1 ± 0.3	43.0 ± 0.0	4.2 ± 1.0
	<i>Vicia faba LAURA</i>	VFL	442.8	0.2 ± 0.4	23.8 ± 2.6	33.9 ± 2.7	0.0 ± 0.0
	<i>Vicia faba SSNS</i>	VFS	359.6	0.5 ± 0.5	28.1 ± 0.3	31.6 ± 0.0	1.2 ± 2.0
	<i>Lathyrus sativus</i>	LS	176.0	0.3 ± 0.6	26.8 ± 0.7	39.1 ± 0.6	3.5 ± 0.2
	<i>Lens nigricans</i>	LN	21.5	0.3 ± 0.5	31.8 ± 2.2	37.4 ± 1.4	0.8 ± 1.4
	<i>Lupinus angustifolius</i>	LA	179.4	1.3 ± 0.8	25.7 ± 3.7	35.4 ± 4.2	0.0 ± 0.0
	<i>Melilotus officinalis</i>	MO	2.5	1.1 ± 1.1	24.9 ± 2.3	33.5 ± 1.3	0.8 ± 1.3
	<i>Medicago lupulina</i>	ML	1.5	2.1 ± 0.5	26.2 ± 4.1	30.3 ± 4.8	0.1 ± 0.9
	<i>Pisum sativum ASSAS</i>	PSA	168.8	0.7 ± 0.7	28.5 ± 0.9	33.5 ± 3.2	1.1 ± 1.5
	<i>Pisum sativum PFX</i>	PSP	214.5	0.0 ± 0.0	29.3 ± 1.3	32.0 ± 0.6	3.9 ± 0.7
	<i>Onobrychis viciifolia</i>	OV	23.0	1.8 ± 1.1	24.2 ± 1.2	31.7 ± 0.2	0.0 ± 0.0
	<i>Trifolium alexandrinum</i>	TA	3.0	1.1 ± 1.9	30.0 ± 1.4	41.6 ± 1.2	6.1 ± 0.3
	<i>Trifolium incarnatum</i>	TI	4.7	1.5 ± 1.2	26.5 ± 1.9	43.4 ± 0.5	6.4 ± 0.3
	<i>Vicia sativa</i>	VS	53.8	0.6 ± 1.0	22.0 ± 0.8	30.0 ± 1.7	4.1 ± 0.0
	<i>Vicia benghalensis</i>	VB	41.4	2.6 ± 0.5	23.6 ± 0.8	39.5 ± 0.0	0.6 ± 0.3
<i>Vicia villosa</i>	VV	26.7	0.5 ± 0.8	20.2 ± 1.1	33.1 ± 6.8	1.4 ± 0.9	
Polygonaceae	<i>Polygonum Fagopyrum</i>	PF	25.0	3.8 ± 0.4	32.3 ± 0.4	39.4 ± 0.2	7.8 ± 0.9

In general, the germination speeds increased linearly from 10 to 25°C, even if the minimal and maximal temperatures depended on the taxon (examples in Figure 19). These fittings based on 20<sup>th</sup>, 30<sup>th</sup>, 40<sup>th</sup> and 50<sup>th</sup> allowed determining very closely  $T_b$ , the differences between  $T_b$  of each percentile were not significant ( $P=0.72$ ). For all cover crops taxa,  $T_b$  in average was 3.8°C, varying from about 0.0°C for three Fabaceae species to 10.6°C for *Setaria italica*. Globally, Brassicaceae had higher  $T_b$  (5.4°C) than Fabaceae (2.1°C), the difference being

significant ( $P=0.05$ ). For Fabaceae family, the two *Trifolium* obtained the highest  $T_b$  (6.3°C as mean), which was significantly different than the other taxa ( $P<0.0001$ ). Inside the Poaceae family, two contrasted behaviors occurred: the  $C_4$  species reached high  $T_b$  (10.0 as mean) instead the Poaceae  $C_3$  obtained an average  $T_b$  (2.3°C as mean) close to those of Fabaceae.

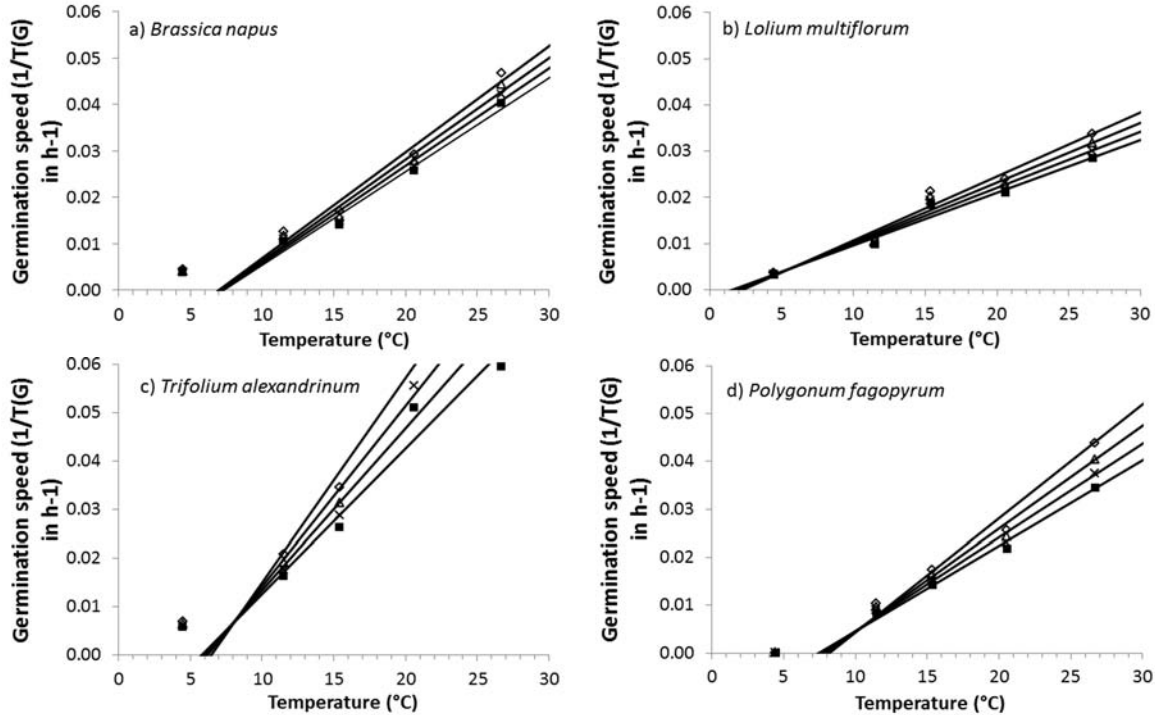


Figure 19 Examples of relationships between germination speed and temperature for four fractions of the seed population 20% ( $\diamond$ ), 30% ( $\Delta$ ), 40% ( $\times$ ) and 50% ( $\blacksquare$ ) in the case of *Brassica napus* (Brassicaceae), *Lolium multiflorum* (Poaceae), *Vicia sativa* (Fabaceae) and *Polygonum fagopyrum* (Polygonaceae). Lines are linear regression fitted for each percentile.

### 2.3.4 Imbibition times vary with seed mass and species families

Cover crop taxa presented a large range of seed mass, varying from 1.3 mg for *Eruca sativa* to 442.8 mg for *Vicia faba* var. LAURA and a mean at 55.0 mg (Table 4). At 20°C, which was the temperature for which globally all cover crop taxa germinated with the highest maximum percentage, the time of seed imbibition, defined as the time required for starting the germination (estimated as the time between sowing and the first seed germination), was positively correlated with the seed mass ( $R^2=0.79$ ) (Figure 20a). Taxa with the biggest seeds needed more time to begin the germination than those with the smallest seeds.

The time of seed imbibition was also influenced by temperature with a response curve as power function ( $R^2=0.87$ ) (Figure 20b). The time of seed imbibition was the highest for minimal temperatures and it was the lowest for temperatures the range 20 to 35°C. A linear response was observed from 10 to 25°C for all taxa indicating that the imbibition time was negatively correlated to non-extreme temperatures ( $R^2=0.94$ ). The relationship was found

significant for all families with  $R^2$  from 0.82 to 0.99 (Figure 20c). The Brassicaceae had the lowest imbibition times compared to the Fabaceae and the Poaceae.

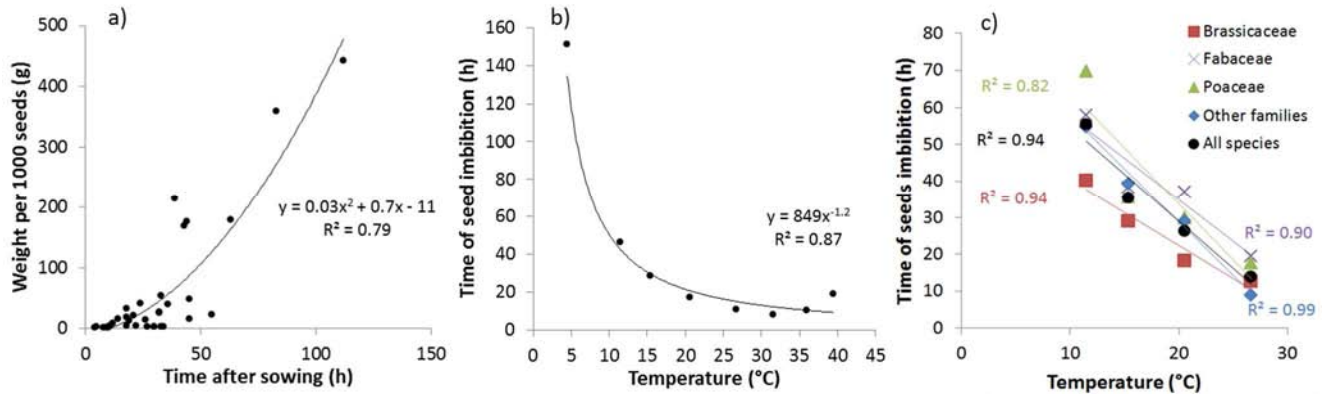


Figure 20 Relationships between the seed mass and the time of seed imbibition for all cover crop species (a), the time of seed imbibition and temperature for taxa germinating at more than 39.5°C (b) and for all cover crops averaged by family and all confounded (c).

### 2.3.5 Species functional groups for germination

The hierarchical classification allowed classifying the 36 cover crop taxa in five functional groups corresponding to different germination profiles (Figure 21). All differences between groups were highly significant with  $P < 0.0001$ .

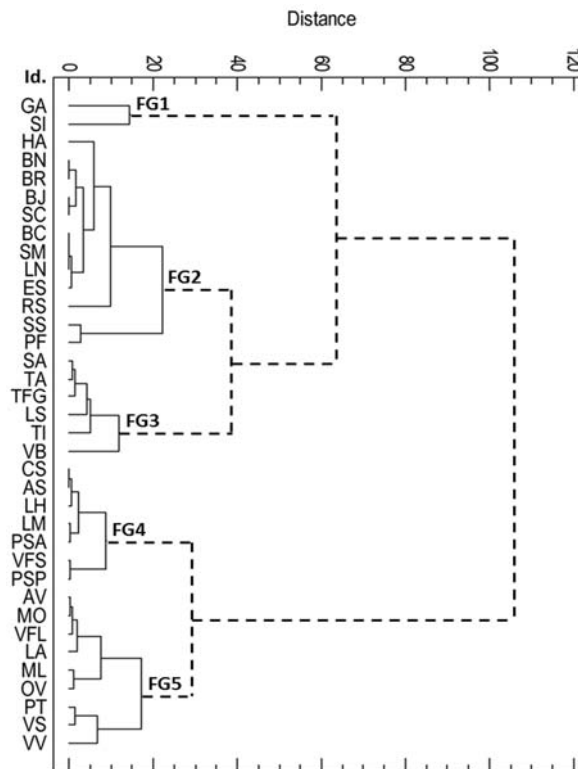


Figure 21 Hierarchical classification based on cardinal temperatures ( $T_0$ ,  $T_{max}$  and  $T_{opt}$ ) estimated on 36 cover crop taxa using Ward method ( $P < 0.05$ ). Distance between species for five functional groups (FG) is represented in solid lines and distance between all groups is in dotted lines. See Table 1 for species names (Id.).

**Tableau 5 Cardinal temperatures for germination for each functional group issued from hierarchical classification. Mean and standard deviation of all species of the functional group. Letters 'a', 'b', 'c' correspond to functional groups significantly different at  $P < 0.05$  for each cardinal temperature.**

Functional group	$T_0$		$T_{opt}$		$T_{max}$	
1	10 ± 1.8	b	32.4 ± 5.2	c	41.2 ± 2.4	c
2	1.2 ± 1.7	a	33.4 ± 1.6	c	38.2 ± 1.5	b
3	0.9 ± 1	a	27.8 ± 2.6	b	41.2 ± 1.8	c
4	0.4 ± 0.4	a	28.7 ± 0.8	b	34.2 ± 1.9	a
5	0.9 ± 0.7	a	23.8 ± 2.1	a	32 ± 2.4	a

The largest group was the group 2 with 12 taxa (33% of total taxa) composed of six Brassicaceae, three Poaceae which were the two *Secale* and *Sorghum bicolor var. sudanense*, and *Helianthus annuus*, *Polygonum Fagopyrum* and the Fabaceae *Lens nigrican*. This group showed abilities to germinate between 1.2°C ( $T_0$ ) to 38.2°C ( $T_{max}$ ) with  $T_{opt}$  around 33.2°C (Tableau 5). This group 2 presented the significantly highest  $T_{opt}$  (33.4°C), as it was the case for the group 1 (32.4°C). However the functional group 1 was composed of two species (*Guizotia abyssinica* and *Setaria italica*) with higher  $T_{max}$  but not able to germinate at low temperatures. At the opposite, the group 5, composed of nine taxa, which were mainly from Fabaceae family and also *Avena sativa* and *Phacelia tanacetifolia*, presented the significant lowest  $T_{opt}$  (23.8°C) than other functional groups and with a  $T_{max}$  fairly low (32.0°C). As intermediate abilities to germinate at medium  $T_{opt}$  (28.7°C) and  $T_{max}$  (34.2°C), the functional group 4 was composed of three Poaceae, three Fabaceae and *Camelina sativa*. Finally, the group 3 presented the largest range of germination from 0.9°C to 41.1°C, even if the  $T_{opt}$  was also intermediate compared to other groups. This group 3 was mainly composed of Fabaceae such as the two *Trifolium* or *Trigonella foenum-graecum* but also of *Sinapis alba*.

## **2.4 Discussion and conclusion**

This study provides results on the effect of one major environmental factor, the temperature, which affects the establishment of cover crops during fallow period for a sowing in late summer where soil temperature could greatly vary according to the location and date of sowing. The cardinal temperatures calculated can be used in a crop emergence model in order to simulate the emergence of cover crop in different pedoclimatic conditions which can help to choose the most adequate species to sow in function of site, date of sowing or different sowing conditions. It was shown by Dorsainvil et al. (2005) and Constantin et al. (Submitted) using the SIMPLE emergence model (Dürr et al., 2001) that the duration from sowing to emergence can vary a lot for cover crops. Moreover, the cardinal temperatures and  $T_b$  of germination can also be used in crop model as a estimation of the cardinal temperatures and

$T_b$  of the plant growth and development (Parent and Tardieu, 2012) and could thus be used for new species parameterization. The determination and comparison of cardinal temperatures for germination such as  $T_0$ ,  $T_{opt}$ ,  $T_{max}$  and  $T_b$  of 36 cover crop taxa from various botanical families was not available for all these taxa and presented a large variability according to species. For the species which have already been studied, the cardinal temperatures for germination we calculated, especially  $T_b$ , were globally consistent with those found in literature, even if the majority of the references were estimated for emergence ( $T_{b-emerg}$ ) measured in field situation.

**Table 6 Cardinal temperatures for germination and emergence in field ( $T_{b-emerg}$ ) issued from literature for the same species studied as cover crops.**

Species	$T_b$ (°C)	$T_{opt}$ (°C)	$T_{max}$ (°C)	$T_{b-emerg}$ (°C)	References
<i>Helianthus annuus</i>	1.0 to 5.1	34.0 to 36.7	45.5 to 50.9	7.9	Mwale, Hamusimbi and Mwansa 2003 Angus et al. 1981
<i>Camelina sativa</i>				-0.7	Allen, Vigil and Jabro 2014
<i>Brassica napus</i>	2.6 to 3.5	22 to 24		2.6	Marshall and Squire 1996 Angus et al. 1981
<i>Sinapis alba</i>	3.3	27	39		Dorsainvil 2002
<i>Avena sativa</i>				1.6	Yusoff et al. 2012
				2.2	Angus et al. 1981
<i>Setaria italica</i>				10.9	Angus et al. 1981
	9.3	37.0	46.0		Kamkar et al. 2006
<i>Lolium multiflorum</i>	4.6	27	38		Dorsainvil 2002
	1.8				Yusoff et al. 2012
<i>Secale cereale</i>				2.6	Angus et al. 1981
<i>Faba bean</i>	0.40	25.4	37.08		Dumur, Pilbeam and Craigon 1990
				1.2	Yusoff et al. 2012
<i>Lupinus angustifolius</i>	-0.8 to 0.7			1.7	Iannucci, Terribile and Martiniello 2008
<i>Pisum sativum</i>				1.4	Dracup, Davies and Tapscott 1993 Angus et al. 1981
	-0.4 to 9.6				Olivier and Annandale 1998 Raveneau et al. 2011
<i>Onobrychis viciifolia</i>				2.4	Iannucci, Terribile and Martiniello 2008
<i>Trifolium alexandrinum</i>				5.5	Iannucci, Terribile and Martiniello 2008
<i>Vicia sativa</i>				3.5	Iannucci, Terribile and Martiniello 2008
<i>Vicia villosa</i>				0.0	Iannucci, Terribile and Martiniello 2008
<i>Polygonum fagopyrum</i>				1.9	Iannucci, Terribile and Martiniello 2008
				11.1	Angus et al. 1981

In our data, *Setaria italica* and *Polygonum fagopyrum* were distinguished from other taxa for their high  $T_b$  measured (from 7.8 to 10.6°C), which were consistent with literature ( $T_{b-emerg} = 10.9°C$  and 11.1°C respectively) (Angus et al., 1981; Kamkar et al., 2006). However, some different values in base temperature were also reported from literature for certain taxa when available such as *Onobrychis viciifolia* (0.0°C vs 5.5°C). For some species in literature the range of values calculated by various authors was large (Table 6) probably because of various



methodologies and calculations used but also because of different species varieties. Moreover,  $T_b$  for the germination is not exactly similar than the one for the emergence, generally higher than for the germination, as shown on Fabaceae by Brunel et al. (2009) and Raveneau et al. (2011), the comparison to them is thus not perfectly correct.

In addition, the determination of the base temperature for germination and other cardinal temperatures is useful for choosing cover crop species according to climatic conditions. In summer sowing situations where the soil temperature in the seedbed can be high ( $>30^{\circ}\text{C}$ ) it is crucial to know the optimum and maximum temperature values for germination of species. Most cover crop taxa tested showed to be well adapted to late summer conditions because the average optimal temperature were  $29^{\circ}\text{C}$  in average. However some species -such as *Phacelia tanacetifolia* or *Vicia sativa*- did not germinate at temperatures higher than  $30^{\circ}\text{C}$ , these species should be better adapted to summer sowing in sites with relatively mild temperatures. Moreover, most cover crop taxa were affected by higher temperatures which abruptly reduced final germination percentage.

A way to describe the diversity of temperature response on a wide range of species could be to define functional groups with the same range of cardinal temperatures for germination. The species were regrouped independently of their botanical family which is a feature not sufficient to characterize species. Cover crop species ranking allowed to regroup species which would be adapt to same climatic conditions. For example, in intercropping (species mixtures) for which two or more species are grown and sown in same time and thus in same conditions, it could be useful to know the species characteristics for making optimal mixtures. In fact, a species with an early germination could lead to early growth and nutrients acquisition inducing that the species could be advantaged in mixture and thus apply competition on the associated species, as supported for barley in mixture by Hauggaard-Nielsen et al. (2006). In order to balance interspecific interactions it could be interesting to adapt species choice to germination ability. For example to give a chance to both species to assure early growth without too much dominance of one species on the other, and then choosing species having the same abilities to germinate (species in the same group). Or if a species presents a low growth rate it could be interesting to sow earlier for favoring its establishment.

Another interesting way to know and valorize the germination abilities would be to avoid climatic risk, especially during fallow period, by intercropping several species with contrasted germination abilities (from different functional groups) in order to spread the risk of non-emergence. For example, associating in mixture *Guizotia abyssinica* (Group 1) well adapted

to high temperatures with *Vicia sativa* (Group 5) which prefers lower temperatures and *Avena sativa* (Group 3) with an intermediate position, could be a way to increase the chance to obtain an homogenous cover whatever the seedbed temperature. Moreover all species tested here had good final germination percentage in the range of optimal temperature except a few of them. For the taxa which did not reach a satisfactory level of seed germination percentage, it can be hypothesized that there was a presence of hard seeds in the lot of seeds. This property seems specific to the species, as it is known for *Medicago* which have low germination percentage explaining by very hard seed coats breaking seed dormancy with difficulty (Uzun and Aydin, 2004).

Finally, the Brassicaceae germinated globally faster than other families. That could give to these species an advantage in emergence and crop establishment and also in competition for resources acquisition. This is particularly true at high temperatures which can be often recorded in soil seedbeds in summer. It seems consistent with their competitive strategy to rapidly grow and have access to resource as supported by Tribouillois et al. (submitted to PlosOne) for the same cover crops species. Moreover, the cover crop species which are able to have a fast germination, especially at high temperatures, would tend to have an early emergence in field. The taxa presenting the ability to germinate early at high temperature (30-40°C) and with high final germination percentage were, for example, *Sorghum bicolor var. sudanense*, *Raphanus sativus* or *Trigonella foenum-graecum*. An early emergence may allow a early cover rate leading to a fast soil protection but also an advanced root and shoot growth leading to early catch nitrate and thus prevent nitrogen losses.

However, another important factor influencing seed germination, especially in summer sowing of cover crops, is the water availability in the soil and particularly in the upper 0-10 cm layers (Constantin et al. Submitted; Dorsainvil et al. 2005). Of course the germination could occur only if the soil hydric potential is sufficient for inducing seed imbibition. Our results showed that the time of seed imbibition generally increased with the mass of the seeds, probably because the seeds with the larger size, such as *Vicia faba* or *Lupinus angustifolius*, needed more water to initiate germination. These results are consistent with the study of Håkansson et al. (2011) who showed that the fastest emergence was obtained from small seeds with a shallow sowing. In order to better understand the effect of soil hydric potential on all cover crops germination, a complementary work is needed in order to know the water potential and then the range of variation of these 36 cover crop taxa.

**Références indiquées à la fin du manuscrit.**

## **Chapitre 2 : Analyse des performances de mélanges bispécifiques de cultures intermédiaires dans le cas d'une destruction automnale sur trois sites contrastés**

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## **Cover crop mixtures including legumes could be efficient to simultaneously produce ecosystem services of nitrate catching and green N manuring.**

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### **1 Introduction**

Cover crops (CC) are commonly sown during fallow period, between two main crops, to provide various ecosystem services within agroecosystems. Indeed, CC increase organic matter, reduce erosion, improve the soil physical characteristics, reduce weeds and diseases and especially, they provide ecosystem services concerning nitrogen (N) management in annual crop rotations (Snapp et al., 2005). They are efficient to acquire soil mineral N (SMN) and thus reduce N leaching avoiding water pollution. They also increase N availability for next main crop (green manure effect) allowing to reduce N fertilization (Justes et al., 2012b; Thorup-Kristensen et al., 2003). However, providing ecosystem services with CC will depend on appropriate species choice. A wide range of plant species can be used as CC, including various botanical families such as Fabaceae, Poaceae, Brassicaceae or Hydrophyllaceae. Even if species from the different families can provide both ecosystem services related to N management, legume species (Fabaceae) would be mostly attempted to produce green N manure effect. With these species, it will be maximized thanks to their ability to fix atmospheric N<sub>2</sub> through symbioses with N-fixing rhizobacteria, accumulate large amount of N acquired and thus increase soil N availability during decomposition (e.g. Touchton et al. 1984; Peoples, Herridge and Ladha 1995; Tonitto, David and Drinkwater 2006). Legumes in mixtures may thus be used to reduce the dependence of conventional agriculture from synthetic-N (Jensen and Hauggaard-Nielsen, 2003). In addition, species from non-legume families would be mostly attempted to be performant to acquire soil mineral N, especially NO<sub>3</sub><sup>-</sup>, leading to reduce N leaching (e.g. Meisinger et al. 1991; Constantin et al. 2011; Thomsen and Hansen 2014). Growing species in mixtures as intercrop (IC) CC composed of a legume and a non-legume species, may maximize the benefits of each species thanks to the principle of niche complementarity (Jensen, 1996b), the N<sub>2</sub> fixation and the nutrient cycling leading to provide additional ecosystem services compared to sole crops (SC) (Kuo and Sainju, 1998; Ranells and Waggoner, 1997; Wortman et al., 2012a). Moreover by mixing species with a good complementarity it can be expected to balance the carbon (C) to N ratio in

order to favour N release for next main crop and avoid the negative pre-emptive effect for next main crop (Quemada and Cabrera, 1995; Tosti et al., 2012a). Most studies concerning intercropping have focused on legume-cereal mixtures as main crops. In this type of mixtures, the cereal species was mostly the stronger competitor and acquired N from soil reserve (Hauggaard-nielsen et al., 2001) while legume was the dominated species and enhances the use of atmospheric N<sub>2</sub> thanks to an improvement of its biological N-fixation ability (Jensen, 1996b). These systems have demonstrated higher yield productivity, community stability and increasing resource-use efficiency (light and nutrients resources) compared to SC (e.g. Corre-Hellou et al., 2006; Bedoussac and Justes, 2010). Further studies have investigated legume and non-legume mixtures as CC and have illustrated that CC mixtures tend to enhance productivity or the production of ecosystem services (Kramberger et al., 2013; Kuo and Sainju, 1998; Möller et al., 2008; Ranells and Waggoner, 1997; Summers et al., 2014; Tosti et al., 2012b). The Land Equivalent Ratio (LER) has been developed long time ago to describe interactions in mixture (Willey and Osiru, 1972). This indicator has been mostly used to evaluate mixtures as main crops, as for examples, durum wheat and winter pea (Bedoussac and Justes, 2011), barley and pea (Hauggaard-Nielsen et al., 2001) or mustard and legumes (Banik et al., 2000). Only a few studies used it to evaluate interactions in CC mixtures (Schipanski and Drinkwater, 2012; Smith et al., 2014; Wortman et al., 2012b). These studies provided a first evaluation that CC mixtures are able to over-yielding compared to SC. It also has been discussed how the proportion of the associated species could interfere the interspecific interactions (Hauggaard-Nielsen et al., 2006; Ofori and Stern, 1987). However, to our knowledge, no accessible study was found in the scientific literature concerning the evaluation of the performances using the LER for a large number of bispecific CC mixtures.

We tested the hypothesis that some well associated bispecific legume and non-legume CC mixtures could simultaneously produce two ecosystem services for N recycling in agrosystems, such as being: i) as efficient as non-legume SC species to acquire nitrate and the to avoid nitrate leaching, and ii) increasing the N release for the next main cash crop by allowing to balance the C:N ratio and the amount of N acquired in comparison to a sole legume CC. We evaluated the performance of CC mixtures growing from late summer to late autumn. The objectives of this study were to: i) evaluate the bispecific CC mixtures performances to provide ecosystem services related to N management compared to SC and bare soil (without CC neither volunteers); ii) analyse the interspecific interactions between the associated species occurring in bispecific mixtures.

## 2 Material and methods

### 2.1 Experimental sites and design

Three experiments were conducted from August to November 2012 in France on three experimental sites: at Institut National de la Recherche Agronomique (INRA) site in Auzeville-Tolosane, southwest France (43°31' N, 1°30' E); at Arvalis-Institut du végétal- site in Bignan, west France (47°52' N, 2°46' W) and at Arvalis site in Lyon, east France (45°43' N, 5°4' E). Sites description and growth conditions are detailed in Table 7. Bignan site presented a high level of soil organic matter and of initial SMN available at the sowing date compared to the two other sites. The main crop before CC sowing was faba bean for Auzeville, barley for Bignan and wheat for Lyon. The experimental site of Lyon presented the characteristic to be very pebbly with approximately 30% of pebbles in 0-30cm; 50% in 30-60cm and until 70% in 60-90cm (deep alluvial gravel soil type).

**Table 7 Experimental sites, soil and climate description. Climatic variables are presented as cumulative or average of the growing season from sowing to cover crop destruction.**

Site description and climate	Auzeville	Bignan	Lyon
Sowing date	16/08/2012	17/08/2012	01/08/2012
Biomass harvest date	24/10/2012	13/11/2012	21/11/2012
Soil texture	Clay loam	Silt loam	Loam
Soil mineral nitrogen at sowing (kg N ha <sup>-1</sup> )	53	112	31
Soil organic matter (0-30cm) (g kg <sup>-1</sup> )	14	34	16
Irrigation (mm)	60	0	0
Rainfall (mm)	80	323	331
ETP (mm)	237	159	343
Daily average of temperature (°C)	19.1	13.5	18.4
Daily average of global radiation (J cm <sup>2</sup> )	1430	1031	1187

Soil mineral nitrogen (SMN) measured on 0-90 cm for Bignan and Lyon and 0-120 cm for Auzeville

The experimental design was a complete randomized plan replicated with three blocks for INRA site and two blocks for Arvalis sites. The elementary plot area was at least 11m<sup>2</sup> for each treatment. The CC treatments were 10 SC (five legumes and five non-legumes), 25 different bispecific mixtures (composed of one legume and one non-legume), corresponding to an orthogonal bispecific crossing of the five legumes x five non-legumes species, and a bare soil as control without any CC. The ten different species were composed of i) five legumes: crimson clover (*Trifolium incarnatum*), purple vetch (*Vicia benghalensis*), wild lentil (*Lens nigrican*), forage pea (*Pisum sativum*) and faba bean (*Vicia faba*) and ii) five non-legumes: turnip rape (*Brassica rapa*), foxtail millet (*Setaria sativa*), bristle oat (*Avena strigosa*), Italian ryegrass (*Lolium multiflorum*) and phacelia (*Phacelia tanacetifolia*). The

species were chosen to represent families' diversity (Fabaceae, Poaceae, Brassicaceae, Asteraceae, Polygonaceae and Hydrophyllaceae), architectures and morphologies and, *ex ante*, to present a sufficient early growth speed compatible to be used as CC. For non-legumes, we have chosen to study species with reasonable height in order to limit light competition on legume. Species were sown at their recommended plant density according to the expertise known for CC use (Annexe A1), in order to constitute a homogeneous dense plant covers with rapid grow and soil covering. For the three experiments, the seeds came from the same seed lot commercialized by seed companies. In mixture, sowing density was the half of corresponding SC for both species. Seeds of both species were mixed on the row. No fertilizer was added during the experiment. In Auzeville site, irrigations were provided during the first two weeks to ensure plant emergence because the soil was dry at sowing and no rain occurred. No irrigation was done after this stage on this site and none was performed on Bignan and Lyon sites.

## **2.2 Measurements and calculations**

The quantification of the ecosystem services provided by CC was assessed by harvesting CC shoots biomass at the soil surface from 1m<sup>2</sup> area for each replicate. The sampling harvest occurred in autumn 2012 on 24/10 for Auzeville, 13/11 for Bignan and 21/11 for Lyon (Table 7). The biomass of each species was separated, dried at 80°C for 48h and weighted. The sum of DD was 1380 in Auzeville site, 1200 in Bignan site and 1870 in Lyon site; corresponding to respectively 72, 89 and 114 days after sowing. In order to take into account the difference in the duration of the growing period, we estimated the crop growth rate (CGR) in kg ha<sup>-1</sup> DD<sup>-1</sup>, where DD is degree-days using a base temperature of 0°C calculated from the date of sowing (T0) until the date of the harvest (T1), using the following equation :

$$CGR = \frac{DM1 - DM0}{T1 - T0}$$

where DM1 and DM0 are shoot dry matters (DM) (kg ha<sup>-1</sup>) at T1 and T0, respectively. DM0 is equal to 0 because it corresponds to DM at the sowing date. After shoot dry matter weighted, a sub-sampling of each treatment was grinded to measured total-N and C concentrations by elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany) and the <sup>15</sup>N concentration for legumes with stable isotope ratio mass spectrometer in continuous flow (Isoprime 100, England). In order to evaluate the total (shoots + roots) amount of N acquired by CC, we also harvested the root biomass on 0-25 cm for SC treatments and the same N analysis methods that for shoots were assessed. The ratio



root and shoot was assumed constant for a species in SC and in mixture in order to estimate the total amount of N acquired for all treatments.

For legumes, we calculated the amount of N<sub>2</sub> fixed by estimating the percentage of plant N derived from the atmosphere (%Ndfa) using the <sup>15</sup>N natural abundance (δ<sup>15</sup>N) method (Amarger et al., 1979; Unkovich et al., 2008). It was calculated using the following equation:

$$\%Ndfa = 100 * \frac{\delta^{15}N_{ref} - \delta^{15}N_{legume}}{\delta^{15}N_{ref} - \beta_{fix}}$$

where β<sub>fix</sub> is the isotopic fractionation factor for N<sub>2</sub> fixation of legume. The β<sub>fix</sub> was considered as equal to -0.78 for faba bean (Peoples et al., 2002), -1.55 for forage pea (Schipanski and Drinkwater, 2012), -1.18 for wild lentil, -0.67 for crimson clover (Gebhard, 2012) and -0.79 for purple vetch (Unkovich et al., 2008). The value of δ<sup>15</sup>N<sub>ref</sub> was for mixtures those of the associated non-legume species and for legume SC the average of the non-legume species at proximity. Finally, in order to evaluate the amount of N acquired from soil (i.e; mineral-N acquired) by the legume, we subtracted the amount of N coming from N<sub>2</sub> fixation to the total amount of N accumulated in the total cover.

Soil samples were taken on 90 cm (both Arvalis sites) or on 120 cm depth (INRA site) with a hydraulic coring shortly after biomass harvest. Six soil cores were sampled on each plot to take into account soil variability. They were then pooled together for determining water content and mineral-N (nitrate and ammonium) analysis. Soil mineral content was evaluated by KCl extraction by colorimetric reactions in a continuous flow autoanalyser (Skylar 51000, Skalar analytic, Erkelenz, Germany).

Finally, as an evaluation of the allelopathic of Brassicaceae species produces by glucosinolates (Dam et al., 2008; Kirkegaard and Sarwar, 1998) that can limit the development of the associated species in mixture, we harvested (on 11/10/2012) 10 roots per sample of five Brassicaceae species (see Figure 25). They were grown in bispecific mixtures associated independently to three legumes (faba bean, purple vetch and wild lentil) during the same period and in the same experiment field at Auzeville site but in an additional plot. The glucosinolate concentration in roots of each sample was measured in laboratory by liquid chromatography.

### **2.3 Estimation of ecosystem services with the STICS model**

We used the STICS soil-crop model to estimate the green manure and catch crop effect of the different treatments. The STICS model is a dynamic model which simulates C, N and water cycles with a daily time according to soil and climatic characteristics and agricultural

practices (Brisson et al., 2008). This model has already been parameterized and evaluated as satisfactory for simulating CC residues decomposition by Justes et al. (2009), it is also efficient for simulating  $\text{NO}_3^-$  leaching under CC (Constantin et al., 2012).

We made simulations for the three experimental sites with the specific soil initialization (water,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  contents in the different 30 cm soil layers) and climatic parameters. We made the simulations in two steps. Firstly, we run the model from sowing to the date of CC harvest and destruction to simulate the amount of N leached under legume and non-legume SC and the bare soil. For doing this, we previously made an optimization of the different CC species parameters based on the method proposed by Guillaume et al. (2011) using the JavaStics optimizer (simplex algorithm). We were not able to perform these simulations for mixtures. However in the second step, we simulated the CC destruction and incorporation of all treatments, SC and mixtures, and we run the model from the date of CC destruction and incorporation (corresponding to the day of biomass harvest) until the 31<sup>st</sup> of March of the next year to simulate the amount of  $\text{NO}_3^-$  leached (corresponding to the end of drainage period). We run the model until the 31<sup>st</sup> of May of the next year to estimate the amount of N mineralized from CC residues (corresponding to the period of N needs of the next cash crop). We simulated the amount of N mineralized from CC residues and the quantity of  $\text{NO}_3^-$  leached based on the biomass, the C:N ratio and the amount of SMN at the destruction for each CC treatment and bare soil.

On Bignan and Lyon sites, few weeds were imperfectly controlled on bare soils, however their biomass and the amount of N acquired were estimated and took into account in the N balance, the analysis and the STICS simulations.

## **2.4 Evaluation of interactions in mixture**

The efficiency of bispecific mixture to grow together and to produce targeted ecosystem services was evaluated by the Land Equivalent Ratio (LER) which is commonly used to evaluate the productivity of crop mixtures relative to corresponding SC (Bedoussac and Justes, 2011; Malézieux et al., 2009; Vandermeer, 1989). LER is defined as the land area that would require to grow SC to achieve the same level of productivity as attained by a mixture (Willey, 1979). We calculated the LER for shoot biomass dry matter of a mixture as the sum of the partial LER (LER<sub>p</sub>) values for both legume and non-legume species as:

$$LER_p = \frac{DM_{IC}}{DM_{SC}}$$

$$LER = LER_{p_{legume}} + LER_{p_{non-legume}}$$

where  $DM_{IC}$  is the shoot biomass dry matter of a species (legume or non-legume) in the considered mixture and  $DM_{SC}$  is the same species shoot biomass dry matter achieved in SC. When a mixture reaches a total  $LER > 1$ , it indicates that resources are used more efficiently in mixture than in SC and thus that more land area would be required to grow SC than growing an equivalent biomass of a mixture. On the contrary when  $LER < 1$  the mixture is disadvantaged for sharing resources (competition > complementarity or facilitation) compared to SC and thus less land area would be necessary for SC to achieve an equivalent level of biomass mixture. Moreover, partial LER indicates how each species is advantaged or not in a given mixture. In our case,  $LER_p$  would be compared to 0.5 because each species in mixture was sown at the half of the SC densities. It means that in the absence of any interspecific interactions, the  $LER_p$  of each species would be 0.5. As a consequence, the  $LER_p$  indicates whether each species was positively ( $LER_p > 0.5$ ) or negatively ( $LER_p < 0.5$ ) affected by the interactions occurring in mixture relatively to the SC.  $LER_p$  and LER were calculated for each replicates.

## **2.5 Statistical analyses**

Means and standard deviations measured on different species were calculated and reported on the figures and tables. Data used to evaluate CC performances were evaluated with analysis of variance ANOVA considering the treatment as factor to explain each variable. If significant treatment differences were detected we performed *a posteriori* a Newman-Keuls tests with level of significant  $P < 0.05$  (or  $P < 0.10$  for some specific cases). Statistical tests were performed for each site separately in order to detect site-specific effects. Statistical analyses were performed with STATGRAPHICS Centurion XV software (version 15.2.06).

## **3 Results**

### **3.1 Cover crop performances**

Analyses of CC performances were expressed by nine different variables presented in Table 8. These data indicated significant differences according to the experimental site ( $P < 0.0001$  for all variables except 'Mineralized N from CC residues' for which  $P = 0.79$ ); therefore, the data from each experimental site were analyzed separately. Concerning the CGR, no significant difference was found between mixtures and SC for Auzeville and Bignan sites but in both cases legume SC tended to produce lower biomass. However in Lyon site, mixtures and legume SC produced significantly faster than non-legume SC ( $2.3$  and  $2.4 \text{ kg ha}^{-1} \text{ DD}^{-1}$

for legume SC and mixtures; 1.9 kg ha<sup>-1</sup> DD<sup>-1</sup> for non-legume SC). For the green manure effect, in terms of total N acquisition by CC, in all sites the total N acquired by mixtures were higher than those of non-legume SC, and even significantly the same than legume SC in Lyon (Table 8). The amount of this total N acquisition coming from N<sub>2</sub> fixation was significantly lower, about approximately the half, in mixtures than in legume SC for all sites. Moreover the C:N ratio of mixtures was significantly lower than for non-legume SC but significantly higher than for legume SC (in all sites; Table 8). As a consequence, the simulation of the amount of N mineralized from CC residues was also significantly higher for mixtures than for non-legume SC but was significantly lower than for legume SC.

**Table 8** The performances of cover crop treatments and bare soil measured at the date of destruction for ‘Crop growth rate’, ‘Total N acquired’, ‘N<sub>2</sub> fixed’, ‘N acquired from soil’, ‘C:N ratio’ and ‘Amount of mineral N in soil 0-90cm’ and simulated with the STICS model for ‘NO<sub>3</sub><sup>-</sup> leached from sowing to CC destruction’, ‘NO<sub>3</sub><sup>-</sup> leached after CC destruction’ and ‘Mineralized N from CC residues’.

		Crop growth rate (CGR) (kg ha <sup>-1</sup> DD <sup>-1</sup> )	Total N acquired (kg ha <sup>-1</sup> )	N <sub>2</sub> fixed (kg ha <sup>-1</sup> )	Mineral N caught from soil (kg ha <sup>-1</sup> )	C:N ratio	Mineral N at CC destruction in soil 0-90 cm (kg ha <sup>-1</sup> )	Simulated NO <sub>3</sub> <sup>-</sup> leached from sowing to CC destruction (kg ha <sup>-1</sup> )	Simulated NO <sub>3</sub> <sup>-</sup> leached after CC destruction (kg ha <sup>-1</sup> )	Simulated mineralized N from CC residues (kg ha <sup>-1</sup> )
Auzeville	SC NL	1.5 ± 0.4	47 ± 19 c	0 c	47 ± 19 a	20 ± 6 a	28 ± 9 c	0	15 ± 6 c	11 ± 13 c
	SC Leg	1.4 ± 0.4	78 ± 21 a	46 ± 15 a	31 ± 11 b	11 ± 1 c	46 ± 12 b	0	28 ± 4 b	34 ± 7 a
	IC	1.6 ± 0.4	67 ± 15 b	20 ± 13 b	48 ± 12 a	15 ± 4 b	31 ± 12 c	0	18 ± 9 c	22 ± 8 b
	Bare soil	/	/	/	/	/	89 ± 21 a	0	48 ± 11 a	/
Bignan	SC NL	3.1 ± 1	79 ± 20	0 c	79 ± 20 a	19 ± 3 a	66 ± 17 b	32 ± 16	56 ± 8 c	13 ± 5 c
	SC Leg	2.2 ± 1	87 ± 48	45 ± 35 a	42 ± 16 b	12 ± 2 c	68 ± 21 b	39 ± 10	72 ± 16 b	36 ± 14 a
	IC	2.8 ± 1	87 ± 30	18 ± 19 b	70 ± 25 a	16 ± 3 b	66 ± 18 b	ND	63 ± 10 bc	22 ± 9 b
	Bare soil	/	/	/	/	/	96 ± 2 a	48	85 ± 6 a	/
Lyon	SC NL	1.9 ± 0.6 b*	69 ± 28 b	0 c	69 ± 28	24 ± 6 a	22 ± 8 b	3 ± 5 b	15 ± 5 c	2 ± 7 c
	SC Leg	2.3 ± 0.5 a	139 ± 47 a	83 ± 57 a	64 ± 33	14 ± 2 c	37 ± 16 b	8 ± 2 b	43 ± 16 ab	46 ± 19 a
	IC	2.4 ± 0.5 a	118 ± 35 a	48 ± 29 b	71 ± 24	19 ± 6 b	31 ± 16 b	ND	30 ± 15 bc	24 ± 21 b
	Bare soil	/	/	/	/	/	65 ± 18 a	32 a	57 ± 15 a	/

‘CC’ is cover crop; ‘SC NL’ is the average of the non-legume sole crops; ‘SC Leg’ is the average of the legume sole crops; ‘IC’ is the average of the mixtures (intercrops); ‘/’ means no corresponding data. Letters ‘a’, ‘b’, ‘c’ significantly different at  $P < 0.05$  and ‘\*’ at  $P < 0.1$ .

ND: not determined (STICS model not parameterized for these species in intercrop version).

Concerning the evaluation of the effect of mixtures to mitigate SMN, results indicated that mixtures acquired the same amount than non-legume SC which were significantly higher than for legume SC, except in Lyon site where differences were not significant probably because of the presence of weeds in legume SC which also acquired N from soil. Concerning the estimations of NO<sub>3</sub><sup>-</sup> leaching from sowing to the date of CC destruction, simulations indicated that no leaching would occur whatever the treatment in Auzeville site. At the opposite, NO<sub>3</sub><sup>-</sup> leaching would occur under bare soil and CC treatments in Bignan and Lyon sites; the

differences were found significant in Lyon between both legume and non-legume SC and the bare soil. For all sites (Table 8), the simulated amount of  $\text{NO}_3^-$  leached after mixtures was not significantly different of the amount after SC NL and stayed significantly lower than with bare soil.

The highest efficiency for reducing  $\text{NO}_3^-$  leaching was obtained in Auzeville site, even if no leaching occurred during CC growing, the CC mixtures composed with turnip rape reduced  $\text{NO}_3^-$  leaching after CC destruction by 85% in average in comparison to bare soil (Figure 22). The mixtures composed with turnip rape had significantly higher reduction rates than mixtures with bristle oat or foxtail millet (average of 62%) which produced higher reduction rates than mixtures with phacelia or Italian ryegrass (average of 52%). However, the species of legume associated in mixture had no significant impact, and then no negative effect on the potential of CC to reduce  $\text{NO}_3^-$  leaching in this site where drainage was moderated. It can also be noted that legume SC would reduce of 42% in average  $\text{NO}_3^-$  leaching in comparison to the bare soil, indicating that legumes acquired a great proportion of their N accumulated by SMN uptakes in the soil and then can produce a nitrate catching service.

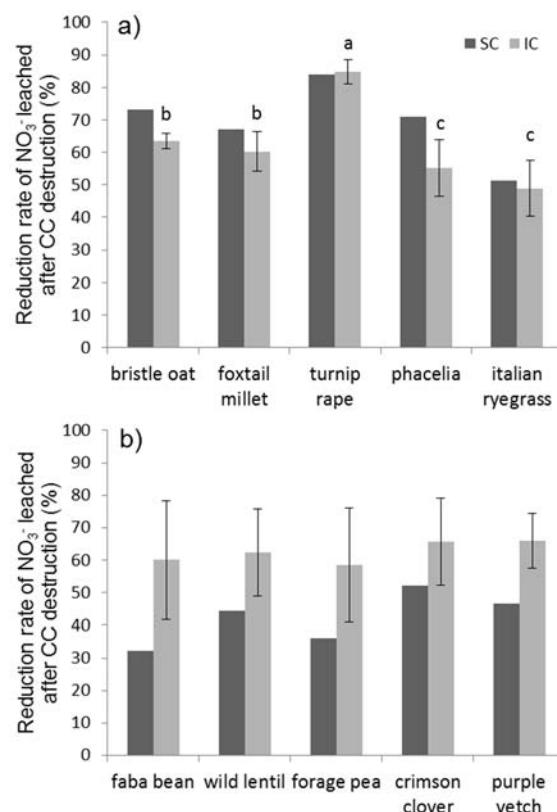
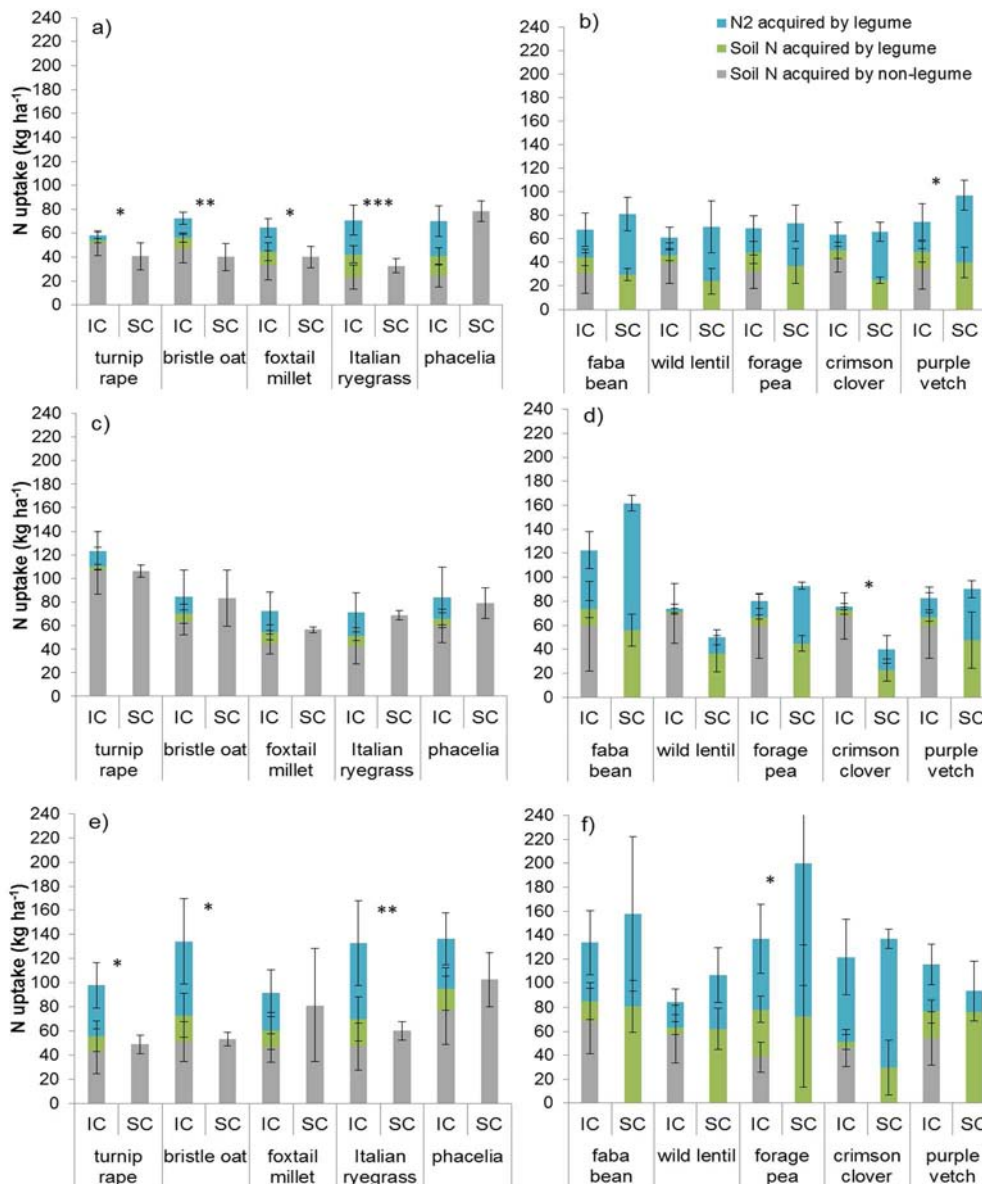


Figure 22 Reduction rates of estimated  $\text{NO}_3^-$  leached after cover crop destruction compare to bare soil (corresponding to 100%) in Auzeville site for a) non-legume treatments and b) legume treatments. Reduction rates under mixtures (IC for intercrops) are in pale grey and under the corresponding sole crop (SC) in dark grey. Standard deviation corresponds at the difference between the five mixtures in which the considered species was associated to the five different species. Letters 'a', 'b', 'c' significantly different at  $P < 0.05$ .



**Figure 23** Amount of soil mineral N acquired by legume and non-legume species and amount of N<sub>2</sub> acquired from atmosphere by legume in mixtures (IC) and in sole crops (SC) for three experimental sites: a) Auzeville – non-legume treatments and b) Auzeville – legume treatments; c) Bignan – non-legume treatments and d) Bignan – legume treatments; e) Lyon – non-legume treatments and f) Lyon – legume treatments. Standard deviation corresponds for ‘IC’ at the difference between the five mixtures in which the considered species was associated and for ‘SC’ at the difference between the five legume or non-legume species in sole crops. ‘\*\*\*’ significantly different at  $P < 0.001$ ; ‘\*\*’ significantly different at  $P < 0.01$  and ‘\*’ significantly different at  $P < 0.05$ .

Moreover, the different species associated in mixture obtained various performances according to the experimental sites (Figure 23). In both Auzeville and Lyon sites, mixtures composed with turnip rape, bristle oat, foxtail millet and Italian ryegrass acquired significantly more N than their corresponding SC due to N<sub>2</sub> fixed by legumes. Moreover in Auzeville and Lyon sites, mixtures composed with turnip rape acquired significantly less N than mixtures with other non-legume species (except with foxtail millet), indicating a possible problem of compatibility of Brassicaceae with legume species. At the opposite in Bignan site, mixtures with turnip rape acquired significantly the highest N amount (Figure 23c-d).

Concerning the behavior of legumes, wild lentil acquired significantly less N than other legume species in particular in Lyon site, and faba bean acquired significantly more N than others in particular in Bignan site. However, in Auzeville site, the species of legume in mixtures had no significant effect for N total acquisition. In Bignan site, crimson clover and wild lentil in SC did not grow well and acquired significantly less N than the corresponding mixtures.

Concerning the amount of N acquired coming from N<sub>2</sub> fixation by legume, same results that for the total N acquisition were found in Auzeville according to the non-legume species associated. However in Bignan and Lyon sites, no significant differences were found for the non-legume species associated. Whereas for legume species, mixtures with faba bean fixed significantly more N<sub>2</sub> than mixtures with forage pea or purple vetch which, them, fixed significantly more than mixtures with crimson clover or wild lentil; this result is particularly true in Bignan site (Figure 23c-d). On the contrary, in Lyon site, mixtures with crimson clover acquired significantly the most N<sub>2</sub>. Finally, for all sites no significant difference for the fraction of N acquired from soil was found according to the legume species associated. However in Auzeville, mixtures with turnip rape or bristle oat acquired significantly the most SMN in comparison to mixtures with other non-legume species (Figure 23a). In Bignan mixtures with turnip rape acquired significantly more SMN than mixtures composed with bristle oat or phacelia which, them, acquired significantly more SMN than mixtures with foxtail millet or ryegrass (Figure 23c). In Lyon, only mixtures with phacelia significantly acquired SMN than others mixtures (Figure 23e). All these results indicated that species behaviour in SC and in mixtures depends on the pedoclimatic conditions and the initial state of SMN and water content.

## **3.2 Interspecific interactions in mixtures**

### **3.2.1 Species advantaged and disadvantaged in mixtures**

In Figure 24, mixtures positioned above the diagonal line, corresponding to total mixture LER=1, indicate a better resource valorization than corresponding sole crops. For Auzeville and Lyon sites, almost all mixtures were above the diagonal line and thus obtained total LER values >1 whereas in Bignan only 9 mixtures out of the 25 mixtures had total LER values >1. Mixtures in Auzeville and Lyon sites reached total LER (averages of 1.18 and 1.25 respectively) significantly higher than those obtained in Bignan site (average of 0.90). In all sites, mixtures with turnip rape showed total LER values  $\geq 1$ , they were 1.30, 1.11 and 1.45 in

average respectively in Auzeville, Bignan and Lyon. Moreover, partial LER (LERp) was used to analyze the behavior of the two species in mixture. The theoretical value of LERp if no interaction occurred between the two species is 0.5 because we used a substitutive design of mixtures by sowing half density of those sown in SC.

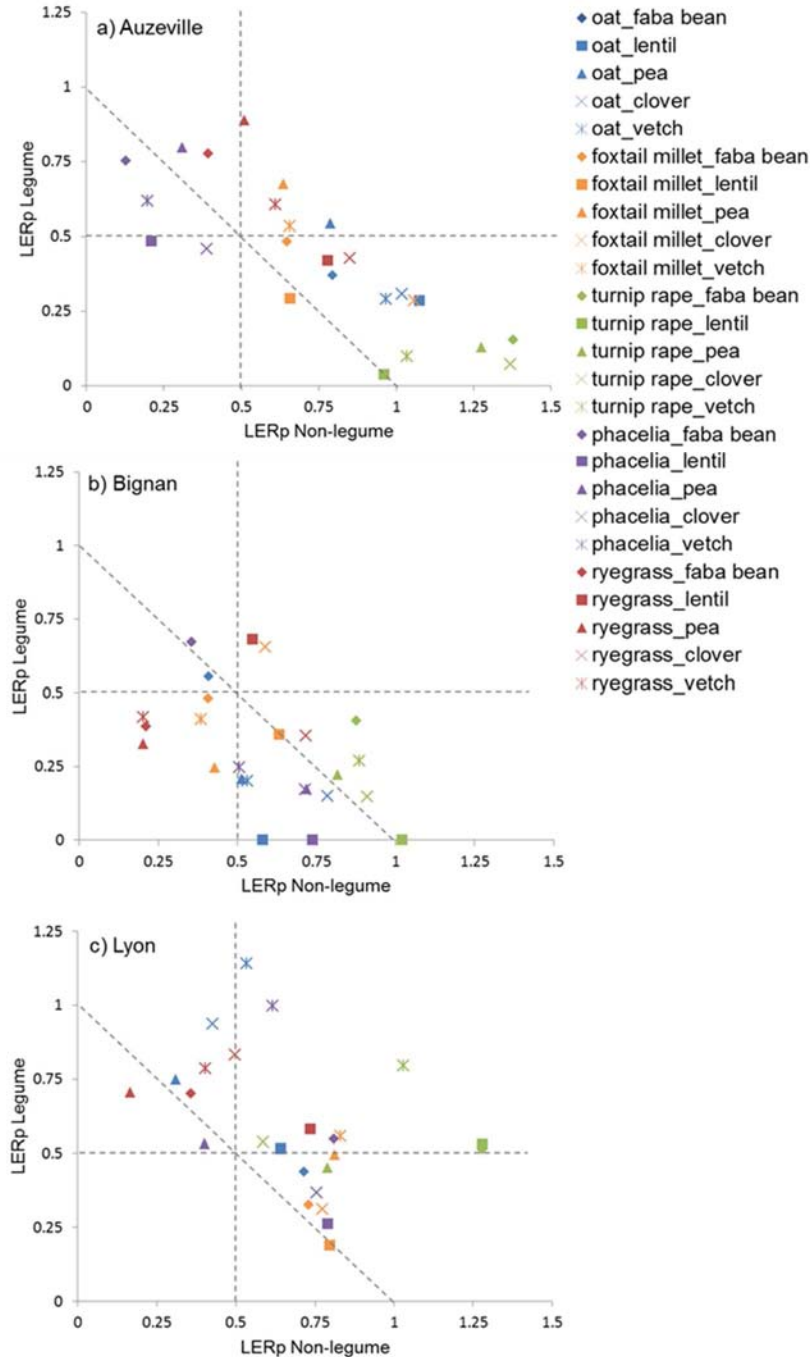


Figure 24 Partial Land Equivalent Ratios (LERp) for biomass of Legumes versus LERp of non-legumes in mixtures in the three experimental sites. In the legend, the color of the markers corresponds to a considered non-legume species in mixture and the symbol corresponds to a considered legume species in mixture. Vertical and horizontal dotted lines indicate limit values for LERp (0.5) in the case of neutral interaction between the species. And the diagonal dotted line indicates the limit value of the total LER for which a mixture is equal to sole crops.



In Figure 24, when a mixture is positioned below the horizontal line, corresponding to legume  $LER_p=0.5$ , means that the legume is disadvantaged in mixture. At contrary, if the mixture is positioned above the horizontal line, the legume is advantaged and may apply competition over non-legume. The same representation occurs for the non-legume species with the vertical line, corresponding to non-legume  $LER_p=0.5$ . On the left part of this line, the non-legume species is disadvantaged in mixture, and on the contrary, on the right part, the non-legume is advantaged.

In our experiments, the variability of species behavior in mixture produced a wide variability in the  $LER_p$  of legumes and non-legumes, in the three sites, indicating a great variability of species response according to interspecific interactions. Globally, in Auzeville and Bignan sites, respectively 17 and 18 mixtures advantaged the non-legume species ( $LER_p>0.5$ ) whereas in Lyon, the legume species were more advantaged (legume  $LER_p$  of 16 mixtures  $>0.5$ ) (Figure 24). Finally 2, 5 and 9 mixtures respectively in Bignan, Auzeville and Lyon allowed increasing biomass in mixture for both species ( $LER_p$  of legume and non-legume  $>0.5$ ).

In the three sites, the turnip rape was the most advantaged species in mixtures with  $LER_p>0.5$  but also, and surprisingly sometimes higher than 1 indicating a very better behavior in mixture than in SC according to the pedoclimatic conditions. In Auzeville site, bristle oat also obtained  $LER_p$  close to 1. At the opposite, phacelia was strongly disadvantaged in mixture with a very low  $LER_p$  of 0.25. In Bignan, only  $LER_p$  of turnip rape (0.90) was significantly higher than other non-legume species (average of 0.51). The  $LER_p$  of phacelia and bristle oat were  $>0.5$  expect when associated with faba bean. In Auzeville and Bignan, the  $LER_p$  of legumes were not significantly different according to the non-legume associated. However in Lyon, differences between  $LER_p$  legume species were significant for legume species:  $LER_p$  of purple vetch were significantly the highest (0.86) rather than  $LER_p$  of faba bean and lentil were the lowest (0.50 and 0.42).

Finally, concerning the effect of the associated species influencing the  $LER_p$  of the given species: turnip rape and bristle oat reduced the most the  $LER_p$  of associated legume for Auzeville and Bignan sites, whereas foxtail millet and phacelia impacted the most negatively the  $LER_p$  of associated legume in Lyon. In all sites, Italian ryegrass was the non-legume species inducing the least competition since it induced the highest  $LER_p$  of legume associated. The species of legume associated did not significantly influence the  $LER_p$  of non-legume for Auzeville, whereas faba bean in Bignan and forage pea in Lyon induced competition on non-legume associated.

In all sites, the turnip rape reached LERp >0.5 but LERp of associated legume <0.5, except in Lyon where three mixtures reached for both species LERp >0.5. We tested the hypothesis of an allelopathic effect produce by high level of glucosinolates concentration in roots of the turnip rape inhibiting the development of the associated legume in mixtures. Results showed that turnip rape presented high level of glucosinolates in its roots significantly higher than other species of crucifers (Brassicaceae) (Figure 25a). Moreover, in Figure 25b, we found a negative correlation between the glucosinolate concentration and the LERp of the legume concentration. The faba bean had a lower LERp decrease according to the glucosinolate concentration than LERp of other legumes associated with Brassicaceae species.

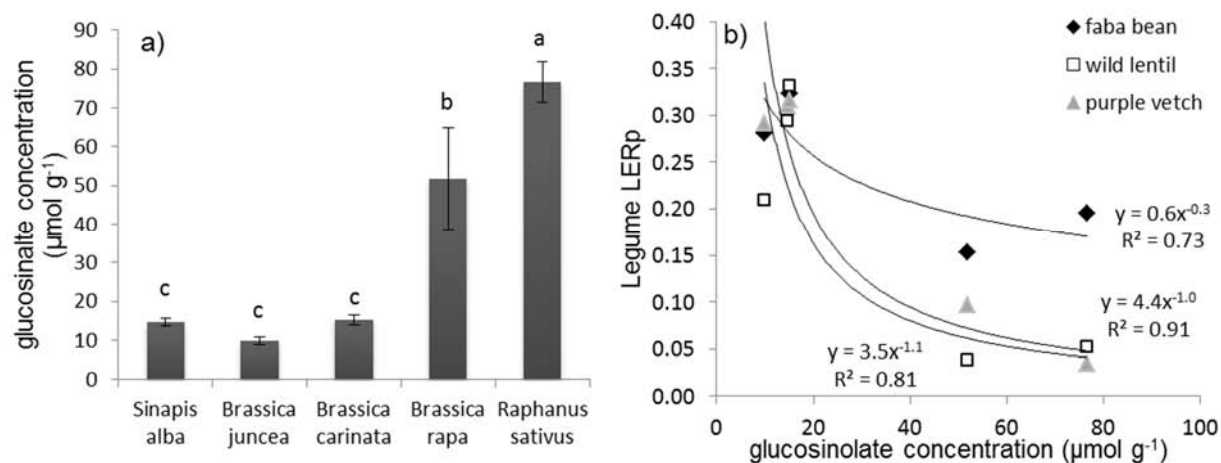
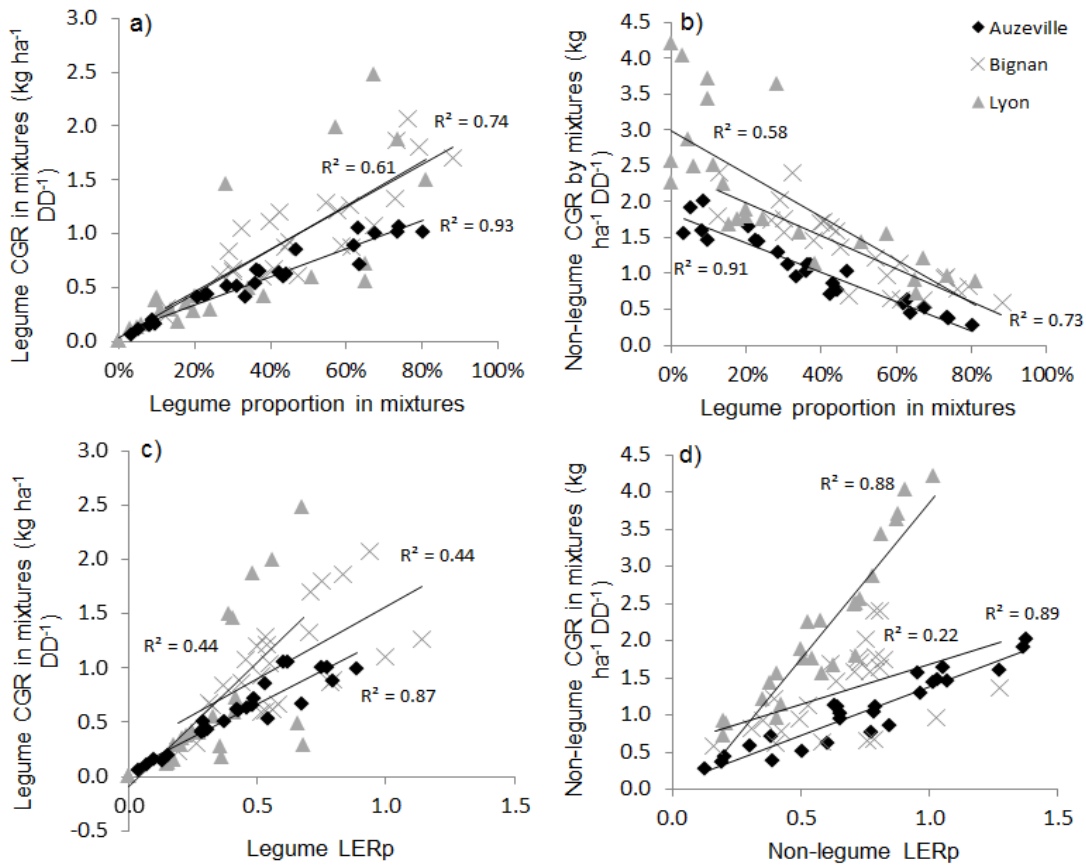


Figure 25 Glucosinolate concentration (µmol g<sup>-1</sup>) in roots of five Brassicaceae (a) and relationship with the associate legume LERp in mixture (b). Letters 'a', 'b', 'c' significantly different at P<0.05.

### 3.2.2 Species behaviour in mixture influences the production of ecosystem services

In Figure 26, we tested the hypothesis that a fast growing species would induce a high species proportion in mixture and would be more advantaged than a slower species. Indeed, on each experimental site, a wide range of legume proportions were observed over the different mixtures: from 3% to 80% in Auzeville, from 0 to 81% in Bignan and from 12 to 88% in Lyon. The results showed a significant positive correlation between the legume biomass proportion in mixture and the CGR of the considered legume species ( $P<0.0001$  for all sites) (Figure 26a). As a consequence, the results also showed a significant negative correlation between the legume proportion and the CGR of the non-legume species associated ( $P<0.0001$  for all sites) (Figure 26b). Moreover, in Figure 26c-d, the results pointed out a significant positive correlation between legume LERp and the CGR of the considered legume ( $P<0.0001$  for Auzeville and  $P=0.0003$  for Bignan and Lyon) and a significant positive correlation

between non-legume LERp and the CGR of the considered non-legume ( $P < 0.0001$  for Auzeville and Lyon;  $P = 0.017$  for Bignan).

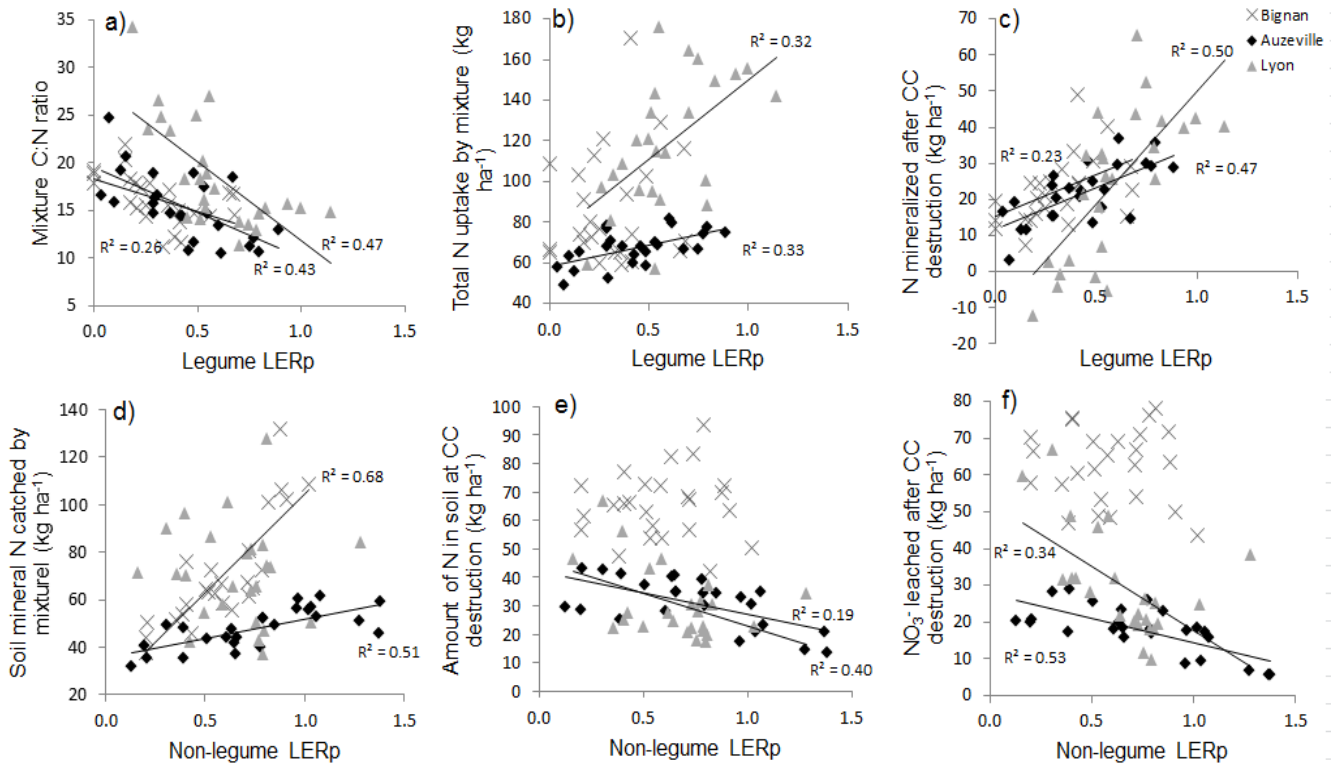


**Figure 26** Relationships between Legume CGR in mixtures and a) Legume proportion in total biomass mixture and c) Legume LERp; and between Non-Legume CGR in mixtures and b) Legume proportion in total biomass mixture and d) Non-legume LERp for the three experimental sites. Lines correspond to the linear regressions.

In Figure 27 we tested the hypothesis that a legume advantaged in mixture ( $LERp > 0.5$ ) would enhance the production of green manure effect of the whole mixture. This green manure effect would be expressed by a low C:N ratio, a high amount of N acquired, and a large amount of N mineralized from CC residues. At contrary, we tested if a non-legume species advantaged in mixture ( $LERp > 0.5$ ) would lead to maximize the nitrate catching effect of the whole mixtures by increasing the amount of N acquired from soil, decreasing the residual amount of SMN and thus reducing the amount of nitrate leaching after CC destruction.

As expected, LERp of legume species was significantly and negatively correlated with C:N ratio obtained in total mixture ( $P = 0.0004$ ,  $P = 0.009$  and  $P = 0.0001$  respectively for Auzeville, Bignan and Lyon), indicating that the better is the behavior of legume in mixture, the lower is the C:N ratio of the mixture (Figure 27a) The LERp of legume species was also significantly and positively correlated ( $P = 0.003$  in both Auzeville and Lyon) with the total amount of N acquired by the total mixtures, except in Bignan ( $P = 0.45$ ) (Figure 27b). Again for the three

sites, LERp of legume was significantly and positively related to the amount of simulated N mineralized from CC residues ( $P=0.0002$ ,  $P=0.015$  and  $P=0.0001$ ) (Figure 27c). In summary, the higher was the legume LERp, i) the higher was the total N uptake by mixture, ii) the lower was the C:N ratio and iii) the higher was the amount of simulated N mineralized after CC destruction, which then indicating that the green N manure function was reinforced by an advantaged legume in mixture.



**Figure 27** Relationships between legume LERp and a) C:N ratio of mixtures, b) Total N acquired by mixtures and c) Amount of N mineralized after cover crop destruction; and between non-legume LERp and d) Amount of soil mineral N acquired by mixtures, e) Amount of residual mineral N in soil at the day of CC destruction and f) Amount of  $\text{NO}_3^-$  leached after cover crop destruction for the three experimental sites. Lines correspond to the linear regressions.

Concerning nitrate catching function, LERp of non-legume species was found an interesting variable. In fact, a positive and significant correlation was found between LERp and the amount of SMN acquired by mixtures ( $P=0.0001$  and  $P<0.0001$  in Auzeville and Bignan), except in Lyon site ( $P=0.77$ ) (Figure 27d). Moreover non-legume LERp was also correlated negatively with the amount of residual SMN at the day of CC destruction ( $P=0.03$  and  $P=0.0007$  for Auzeville and Lyon), except in Bignan ( $P=0.48$ ) (Figure 27e). Finally, the non-legume LERp was also negatively correlated with the amount of  $\text{NO}_3^-$  leached after CC destruction ( $P<0.0001$  and  $P=0.002$  for Auzeville and Lyon), except in Bignan ( $P=0.68$ )

(Figure 27f). In summary, the higher was the non-legume LERp, i) the higher was the amount of SMN acquired by mixtures, ii) the lower was the residual SMN at CC destruction, and iii) the lower was the amount of  $\text{NO}_3^-$  leached, and then the higher was the nitrate catching function of the mixture.

## 4 Discussion

### 4.1 Green N manure effect of cover crop mixtures

The objective of this study was to evaluate CC mixture performances in order to produce ecosystem services related to N management during autumnal fallow period. The green manure effect was assessed by the total N acquired by plants, the amount of N fixed, the C:N ratio and the simulation of the amount of N mineralized from CC residues after destruction. As expected, our results confirm that legume and non-legume CC mixtures are globally more efficient to provide a green manure effect than non-legume SC and, as a consequence, would improve growing conditions for the next cash crop (Snapp et al., 2005). This conclusion is supported by an enhancement of N accumulated in plant, especially by an addition of a substantial amount of atmospheric  $\text{N}_2$  by the legume and a reduction of C:N ratio leading to an improvement of the amount of mineralized N from CC residues for the next main crop. These results are consistent with those found by Tosti et al. (2012a) for the barley-hairy vetch mixture used as CC to provide green manure effect. Moreover according to Möller et al. (2008) a high legume proportion within CC mixtures increase total N content in biomass and decrease the C:N ratio. In our study we showed that legume species with a fast biomass production (high CGR) will enhance its proportion in the mixture and will then be more advantaged to be intercropped with non-legumes species by in improvement of its LERp. Moreover we found that a high legume LERp was associated with a decrease in the C:N ratio and an increase in the amount of N accumulated and finally produced a higher amount of N mineralized from CC mixture residues. These results indicate that if the legume species is fast growing, it will be more advantaged than a slower species and will provide a best green N manure effect.

Globally, legume species tended to be the dominated species in mixture, except in Lyon site where legumes in mixture were fairly advantaged. The fact that legumes seemed more competitive within mixture, in Lyon site, can be explained by the influence of environmental factors and especially N availability (Möller et al., 2008), since Lyon site was the experimental site having the least amount of SMN at sowing. Moreover, for the three sites,

the fact that legumes in mixtures can provide approximately the half amount of  $N_2$  fixed than legume SC can be explained by an efficient biological symbiosis of legume in such mixtures despite the interspecific competition and a global lower CGR of legumes in comparison to non-legumes species (Schipanski and Drinkwater, 2010).

#### **4.2 Cover crop mixtures could be efficient as catch crops**

In order to evaluate CC mixtures to perform nitrate mitigation as catch crops, we analyzed the amount of SMN acquired, the residual SMN at the destruction and we simulated the amount of  $NO_3^-$  leached under CC and after their destruction. We were not able to simulate the amount of  $NO_3^-$  leached during growing period under mixtures because the STICS model was not parameterized to simulate the growth of CC species used in our experiments in bispecific mixture and thus their N acquisition in intercropping. However during the growing period, results of the simulation comparing legume and non-legume SC with bare soil pointed out that legume SC are also able to acquire substantial amount of SMN and then reduce  $NO_3^-$  leaching of 42% in average compared to bare soil. This conclusion supports the results found in literature indicating that legume SC were able to acquire about 40% of N compared to bare soil (Meisinger et al., 1991; Tonitto et al., 2006). At Auzeville site, no  $NO_3^-$  leaching occurred during fallow period and then under the CC and bare soil until the date of CC destruction, it was thus possible to evaluate precisely the differences in terms of residual SMN at the destruction and then to evaluate the CC mixtures effect for reducing  $NO_3^-$  leaching. We found that some CC mixtures, especially those composed with turnip rape, were as efficient as non-legume SC for decreasing the amount of SMN at the date of CC destruction. In that case, some mixtures reduced leaching up to 88%, as for example the turnip rape\_faba bean mixture. As demonstrated in literature, both species in mixtures tend to have deeper and faster root growth (Li et al., 2006; Tosti and Thorup-Kristensen, 2010) which can allow a more efficient SMN uptake than bare soil (Kristensen and Thorup-Kristensen, 2004). In Bignan and Lyon sites, all CC treatments decreased the amount of SMN at CC destruction in comparison with bare soil. However, we did not found any difference between CC treatments which could be explained by large diversity of species behavior inside mixtures which do not allow generalizable conclusions. In Bignan site it can also be explained by an early nitrate leaching which occurred at the beginning of the growing period. Indeed, the STICS simulation estimated that leaching would have started on the 1<sup>st</sup> of October, only six weeks after sowing for Bignan. The CC and their roots would not have been developed enough to avoid nitrate leaching under any treatment, except under those with turnip rape which was found to be a

very fast growing species as usually known for Brassicaceae species (Thorup-Kristensen, 2001).

However, for the three sites, CC mixtures saved approximately the same amount of nitrate from leaching than non-legume SC. Bispecific mixtures, even if composed with legume species, can be efficient tools to reduce  $\text{NO}_3^-$  leaching. This conclusion is consistent with results found in other studies for CC mixtures containing legume (Hauggaard-Nielsen et al., 2003; Möller et al., 2008; Tosti et al., 2012b). In our study, mixtures composed with turnip rape but also bristle oat or phacelia were found efficient to acquire SMN but their behavior varied in function of the site. The faster is the non-legume species to grow (high CGR) the lower will be the proportion of legume in mixture and the more advantaged the non-legume will be in mixture, as indicated by its LERp. The enhancement of the non-legume LERp, due to better resource-use efficiency in mixture compared to SC, globally led to increase the amount of SMN acquired, decrease the residual amount of SMN at the date of CC destruction and thus saved nitrate leaching.

The nitrate leaching simulations were performed from CC destruction, around November, until end of March corresponding to a few days or weeks before the next cash crop sowing. This four months period after CC destruction and incorporation would induce substantial nitrate leaching, for example until more than  $70\text{kg NO}_3^- \text{ ha}^{-1}$  for some mixtures in Bignan (e.g. bristle oat\_faba bean or turnip rape\_forage pea) because of CC residues mineralization and rainfall. Moreover, in Lyon site, the fact that legume species were advantaged and acquired large amount of N may have induced large and rapid N mineralization after CC destruction in agreement with a low C:N ratio (Justes et al., 2009; Kumar and Goh, 2002; Quemada and Cabrera, 1995). These conditions could lead, in case of heavy rainfall and associated high drainage, to large amount of  $\text{NO}_3^-$  leached including a proportion of  $\text{NO}_3^-$  coming from CC residues N mineralization. In climatic sites with substantial rainfall, as for example in Bignan site, or filtering soil as Lyon site (very pebbly), the CC destruction date must be adapted and, in that case, delayed to the end of winter in order to avoid that a fraction of the mineralized-N from CC residues be leached. To maintain the cover until the end of winter may thus maximize both green manure effect and nitrate leaching (Kumar and Goh, 2002; Quemada and Cabrera, 1995; Tejada et al., 2008a).

### 4.3 Examples of mixtures to produce both ecosystem services

Table 9 gives an illustration of the two best mixtures to provide i) catch crop effect, ii) green manure effect and iii) simultaneously both green manure and catch crop effects significantly higher than 50%-50%. For an evaluation of the 25 mixtures on the three sites, see Annexe A6. It can be noted that mixtures with turnip rape allowed a higher efficiency for producing a catch crop effect in comparison to the average of non-legume SC, which was particularly noteworthy in Auzeville site (> 200%). This very high level of achievement is explained by a better growth of the turnip rape in mixture than in SC where a limited development and yellow leaves were observed. It can be hypothesized that a strong intraspecific competition occurred in SC perhaps due to a too high plant density, even if the seed rate recommended by experts was used. It cannot be excluded that another limiting factor not identified occurred in SC. It can be also noted that in Lyon site, the best catch crop mixtures induced a negative or neutral green manure effect due to strong competition from the non-legume on the legume species, especially for the foxtail millet\_lentil mixture, leading to high C:N of total mixture and strong N immobilization and thus pre-emptive competition for the next cash crop.

**Table 9 Examples of mixture performances to provide catch crop and green manure effects for the three experimental sites (the complete list for all mixtures is available in Annexe A6). The catch crop effect was presented as the achievement level by mixtures in reference to the average of non-legume sole crops based on the amount of NO<sub>3</sub><sup>-</sup> leached after cover crop destruction. The achievement level of the green manure effect was estimated for the mixture in reference to the average of the legume sole crops based on the amount of N mineralized from cover crop residues.**

Auzeville			Bignan			Lyon		
Mixture	catch crop effect	green manure effect	Mixture	catch crop effect	green manure effect	Mixture	catch crop effect	green manure effect
<b>Best catch crop</b>			<b>Best catch crop</b>			<b>Best catch crop</b>		
turnip rape_faba bean	260%	33%	foxtail millet_purple vetch	118%	66%	foxtail millet_lentil	155%	-27%
turnip rape_crimson clover	258%	9%	turnip rape_lentil	128%	54%	phacelia_crimson clover	130%	7%
<b>Best green manure</b>			<b>Best green manure</b>			<b>Best green manure</b>		
phacelia_pea	52%	104%	bristle oat_faba bean	74%	111%	bristle oat_pea	23%	115%
phacelia_purple vetch	74%	108%	turnip rape_faba bean	78%	135%	ryegrass_pea	26%	143%
<b>Mutualization both services</b>			<b>Mutualization both services</b>			<b>Mutualization both services</b>		
bristle oat_lentil	93%	70%	phacelia_faba bean	97%	80%	turnip rape_crimson clover	62%	68%
ryegrass_purple vetch	81%	87%	ryegrass_faba bean	84%	92%	phacelia_faba bean	85%	58%

The mixtures allowing the best achievement of services may have benefited from facilitation processes or niche complementarity for water and nutrients. For example, in Bignan site, the turnip rape associated with faba bean presented a high N concentration in its shoots, even higher than the legume, which could be induced by indirect N transfers from the faba bean to the turnip rape because of N rhizodeposition, as demonstrated for other species in literature (Jensen, 1996a; Stern, 1993; Xiao et al., 2004). This would induced a better N nutrition status



of the cruciferous and then led to a low C:N ratio of the total mixture; consequently this would allow producing a high green manure effect, reaching almost the same calculated effect than those permitted by the legume SC.

#### **4.4 Competition and complementarity in cover crop mixtures**

In our study the LERp were calculated on the basis of biomass produced in order to analyze growth behavior of each species in bispecific mixtures tested. As discussed previously, the LERp was found correlated with the ability of each species in mixtures to provide ecosystem services and then could be used as an indicator of the level the service production. When LERp exceeds 0.5 this means that a species in mixture has a better growth than in SC. Our results showed that globally the non-legume species appeared to be more advantaged in mixture than legumes species. It is especially true for turnip rape which reached the highest LERp but consequently the legume associated to this Brassicaceae appeared to be negatively affected. Similar results were obtained in literature for mixtures composed of a Brassicaceae species (*Brassica napus*, *Sinapis alba*, *Brassica juncea*, *Raphanus sativus*) with legume species (Szumigalski and Van Acker, 2008; Wortman et al., 2012b). We hypothesize that in addition to early development and growth, the turnip rape would also produce an allelopathic effect induced by the degradation of fine roots containing high concentration of glucosinolates, as demonstrated by our results, which could negatively affect the development of the associated species (Dam et al., 2008). Moreover it seems that the allelopathic effect on the associate legume would depend on the legume species. Our results revealed that the faba bean seems less sensitive to this effect than purple vetch or wild lentil. The faba bean would be thus a legume species to favor for a mixture containing a Brassicaceae species. However, the fact that in Lyon the legumes reached good level of development even in mixture with the same variety of turnip rape could be explained by duration of CC growing period which was longer than in other sites. This could have balanced the interactions in favor of slower growing species as legumes when turnip rape started to be less competitive after two months of growth.

Moreover, as suggested by Wortman et al. (2012b) and Smith et al. (2014), our results revealed that CC mixtures would produce more biomass per unit area as indicated by total LER values greater than 1.0 in Auzeville and Lyon sites. However in our study, this conclusion was true in the two sites were SMN availability at the sowing was low. As a consequence, CC mixtures have a better efficiency in abiotic resource use than SC when SMN availability at the beginning of the growth is low. Similar conclusion was found for cash crops

in mixture, especially cereal and grain legume intercropped such as durum wheat and winter pea (Bedoussac and Justes, 2010a). The CC mixture performances confirm the beneficial effect of a complementarity between legume and non-legume in mixtures, especially for the resource use efficiency greatly due to the principle of niche separation (e.g. Jensen, 1996b). To evaluate the interactions and the LERp of each species could help to choose the most adequate species according to the objective of the management of the fallow period and the soil and climate conditions. For example, in a site with low SMN availability and low autumnal and winter rainfall, it could be more interesting to sow a mixture favoring the green N manure effect. Our results are relevant for a growing period of approximately three months, however, as the interactions within mixture evolve over time (Bedoussac and Justes, 2010b), it may be interesting to investigate evolution of these interactions in CC mixtures for a longer growing period corresponding to CC destruction after winter and before spring crop sowing.

## **5 Conclusion**

Bispecific (legume and non-legume) mixtures were found more efficient to provide green N manure effect than non-legume SC even if legume SC still provided the best green N manure effect. The green manure effect provide by mixtures tend to be maximized when legume species is advantaged in mixture. However CC mixtures could also provide nitrate catch crop effect as much efficiently as non-legume SC by acquiring N from soil and reduce  $\text{NO}_3^-$  leaching. The effect tends to be maximized when the non-legume species is advantaged in mixture. A simultaneous production of both services was also produced by some mixtures, as for example, the phacelia\_faba bean mixture or the ryegrass\_purple vetch mixture, depending on the site. As ever shown the legume in mixture was able to acquire a significant amount of SMN in addition to  $\text{N}_2$  and then can produce a nitrate catch crop service indicating that it would be always better to sow a legume during fallow period than having the soil bare when considering the problematic of nitrate pollution. These mixture performances are maximized thanks to a good complementarity between legume and non-legume which allow a better resources valorization than sole crop, especially in systems with low N availability. The various mixtures provided a panel of compromise of the two ecosystem services which allow adapting mixtures choice according to the pedoclimatic site and to the fallow period management favoring the adequate services.

**Références indiquées à la fin du manuscrit.**

**Chapitre 3** : Estimation des fonctions  
écosystémiques produites par une espèce en  
culture pure ou en mélange bispécifique –  
Proposition de modèles conceptuels implémentés  
avec des GLM

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## **Plant functional traits combined with plant stand traits can predict agro-ecosystem functions provided by cover crop species and bispecific mixtures**

*Submitted to Agriculture, Ecosystems & Environment*

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### **Abstract**

Cover crops are used during fallow periods to produce ecosystem services, especially those related to N management in the environment and soil fertility. Examples include i) capturing residual mineral-N from soil to reduce nitrate leaching and groundwater pollution, mainly by non-legume species, and ii) improving N availability for the next main crop (green manuring), mainly by legume species. The magnitude of these services can be estimated from agro-ecosystem functions (AEF) such as crop growth rate, crop N acquisition rate and the C:N ratio of the cover crop, examined in this study. A wide range of cover crop species can potentially produce ecosystem services, and bispecific mixtures consisting of legume and non-legume species could be used to simultaneously produce the two N management services within the same cover crop. We investigated the ability of plant functional traits (measured at the plant level) coupled with plant stand traits (measured at the cover and plant-community levels) to predict the three AEFs, which underlie the ecosystem services produced by cover crops, either in sole crops or in mixtures. We developed a conceptual model for each AEF, which was described using general linear models. A four-step procedure was used: 1) calibrate sole crops under non-limiting conditions; 2) calibrate each species in bispecific mixtures under actual limiting conditions of water and N; 3) validate the complete-mixture model's predictions of the three AEF of all mixtures and the proportion of each species under actual conditions, and 4) validate the generality of sub-models and complete-mixture models to predict the AEF of cover crop mixtures consisting of species not used for calibration. The models based on both functional and stand traits were the most efficient (Efficiency or  $Eff=0.91$ ) in closely fitting the data and expressing the AEF provided by sole crop species under non-limiting conditions, which corresponds to the potential value of functions. For bispecific mixtures grown under actual limiting conditions, the use of the sole crop AEF and the difference in leaf functional traits, as indicators of plant strategies, were efficient in fitting and predicting the level of AEF provided by a cover crop species in the mixture. The availability of water and N in interaction with the plant and cover variables provided satisfactory prediction under actual conditions.

The models fitted for bispecific mixtures were also able to predict the legume proportion in the mixture (Eff=0.87) and thus to express species dominance and the interaction level between legume and non-legume species. The models were evaluated as satisfactory for crop growth rate and C:N ratio (Eff=0.28 and 0.76, respectively) for their generality in predicting the agro-ecosystem functions provided by other species in mixtures not used in the model calibration step, which illustrates the relevance of the approach.

## **1 Introduction**

Cover crops in agrosystems are grown during the fallow period, between the harvest of the main crop and sowing the next one, to provide ecosystem services, reduce negative environmental impacts of agriculture and improve production efficiency. Cover crops are commonly sown to prevent nitrogen (N) losses, especially to avoid water pollution caused by nitrate leaching. It is necessary that cover crops acquire mineral N from the soil before the drainage period to decrease nitrate leaching. This action is commonly called “catch crop effect” (Justes et al., 2012b; Kristensen and Thorup-Kristensen, 2004). Cover crops can provide other ecosystem services, such as producing the “green manure N effect” which releases mineral N into the next main crop through the mineralization of cover crop residues. This ecosystem service can be maximized by legumes through their efficient ability to fix atmospheric N<sub>2</sub> and then acquire more N and obtain a lower C:N ratio than non-legumes (e.g. Tonitto et al., 2006). The amount of N available for the next main crop is influenced by cover crop N acquisition and residue quality (i.e. the C:N ratio and/or the biochemical composition), which depend on factors such as climate, soil type, residual mineral N in the soil after harvesting, the preceding main crop and rooting depth of the cover crop species (Thorup-Kristensen et al., 2003). Biomass production and N content of the cover crop determine the C:N ratio of cover crop residues, which controls the dynamics and the rate of N release from residues incorporated into the soil (e.g. Jensen 1991; Justes et al. 2009). If well managed, the ecosystem services related to N can have a potential positive impact on the next cash crop production through internal N and nutrient recycling and reduce the use of fertilizers and their associated greenhouse gas and ammonia emissions (Justes et al., 2012b).

A wide range of cover crop species can potentially be used to manage and recycle N in arable cropping systems. In temperate regions, cover crops are sown in mid- to late summer and must grow rapidly during late summer, autumn and sometimes winter. Non-legume species (e.g. white mustard, turnip rape, oat, phacelia) are the most efficient species for capturing

nitrate from the soil, generally about twice as effective as legumes, even though legumes are also able to take up mineral N in the soil and decrease nitrate leaching (Meisinger et al., 1991; Tonitto et al., 2006). Unlike non-legumes, legume cover crops fix atmospheric N<sub>2</sub> and increase soil N availability for the next main crop after residue decomposition (e.g. Tonitto et al., 2006; Touchton et al., 1984). Mixing (equivalent to intercropping) legume and non-legume species as cover crops can lead to simultaneous acquire residual mineral-N that remains from the last cash crops and mineralized from soil organic matter. Additionally, introducing additional amounts of N from legume N<sub>2</sub> fixation reinforces the role of green manuring (Möller and Reents, 2009). The legume proportion of cover crop mixtures is a key factor for nutrient composition and N uptake because legumes increase N content and decrease the C:N ratio of cover crop mixtures that influence mineralization and N availability for the next cash crops (Kuo and Sainju, 1998; Thorup-Kristensen et al., 2003). In a bispecific mixture, the proportion of legume and non-legume species and the level of ecosystem services provided are influenced by characteristics of the intercropped species, such as their resource-use strategy, which may impact their competitiveness, but also by environmental factors such as N availability (e.g. Möller et al., 2008).

In this context of cover crops and mixtures, we intend to use the ecological concept of plant functional traits (PFTs), which has been commonly used to describe plant strategies (Diaz et al., 2004; Grime et al., 1997) and to test the impact of competition on community structure (Cahill et al., 2008; Fort et al., 2014; Grime et al., 1997; Kunstler et al., 2012). PFTs are defined as the morphological, physiological and phenological features, measurable at the plant level, that impact plant performances (Violle et al., 2007). They are known to be used as a method for assessing ecosystem functions such as primary productivity and nutrient cycling, especially in grasslands (Lavorel and Garnier, 2002). They also characterize the capacity of species for competitive dominance, especially specific leaf area (SLA) and leaf dry matter content (LDMC) (Westoby, 1998; Wilson et al., 1999). For cover crop species, SLA, LDMC and leaf area (LA) were useful in characterizing the ability to provide agro-ecosystem functions (AEFs) related to ecosystem services for N management, such as crop growth rate and crop N acquisition rate; however, some lack of precision was also reported (Tribouillois et al., submitted to PlosOne). It was also demonstrated that functional trait difference, defined as the trait distance between the target species (the species considered in the mixture) and an associated species, is a key driver explaining the intensity of interactions between species in mixtures (Fort et al., 2014; Kraft et al., 2014; Kunstler et al., 2012). Functional trait difference can express differences in strategies between species due to resource niche differentiation

(Chesson, 2000). The literature showed that leaf PFTs were robust between sites when measured under non-limiting conditions (Kazakou et al., 2014) and suggests that they can be generalized to other species to estimate their potential ecosystem functions. However, under “actual” field conditions with a wide range of available resources, functional trait values and the functional trait difference are influenced by environmental factors (Fort et al., 2014; Lavorel and Garnier, 2002).

This study aimed to provide conceptual models to express and predict the three main AEFs related to the production of ecosystem services for N management (nitrate capture and N green manuring) provided by cover crops, especially by mixtures during an autumnal fallow period. This includes i) crop growth rate (CGR), ii) crop N acquisition rate (CNR) and iii) the C:N ratio of the cover crop. The C:N ratio is actually an indicator of the AEF, but for clarity we classify it as the AEF itself. The models were based on both plant stand traits and PFTs. To this end, we applied a four-step approach for each AEF assessed:

- 1) Calibration of sole crops under non-limiting conditions for water and N resources. We tested the relevance of the fittings for expressing the potential AEF of sole crops based on i) functional traits only, ii) stand traits only, and iii) both functional and stand traits.
- 2) Calibration of bispecific mixtures under actual limiting conditions for water and/or N. We developed two independent sub-models to represent the behavior of each species in the mixture and to evaluate the dominance interactions between both species. One sub-model was developed for legume species and the other for non-legume species. We used three types of information as possibly influential variables: i) the potential AEF produced by sole crops under non-limiting water and N conditions for the targeted and associated species, ii) the leaf trait difference between the two intercropped species to represent inter-specific competition between both species in the mixture, as suggested by Fort et al. (2014) and Kunstler et al. (2012) and iii) the environmental factors (water and N availability) that represent stress functions and reduce the potential AEF.
- 3) Validation of the ability of the complete model (sum of both sub-models from step 2) to predict AEF production of the entire mixture, especially its ability to predict the dominance interactions of the legume proportion in the mixture.
- 4) Validation of the generality of the sub-models and complete models for predicting the AEF of a wide range of cover crop mixtures that include species not used in the calibration step.



## 2 Materials and methods

### 2.1 Experimental data

#### 2.1.1 Species choice

All species tested in our field experiments can be used as cover crops to produce ecosystem services for N management (Justes et al., 2012b). We evaluated 34 different species from six botanical families: Fabaceae, Brassicaceae, Poaceae, Asteraceae, Hydrophyllaceae and Polygonaceae (species list in Annexe A7). They were chosen to ensure a wide range of growth rates, N uptake, N<sub>2</sub> fixation for legumes, phenology, shoot and root architecture and taxonomic diversity.

#### 2.1.2 Experimental fields

Five field experiments were conducted from August to November 2012 on two sites in France: an Institut National de la Recherche Agronomique (INRA) site in Auzeville-Tolosane (43°31' N, 1°30' E), southwest France and an Arvalis – Institut du Végétal - Bignan site, western France (47°52' N, 2°46' W). Site descriptions and growth conditions are provided in Table 10.

Experiment 1 was performed to measure PFTs of the 34 species sowed as sole crops (SC); all details of this experiment are described in Tribouillois et al. (submitted to PlosOne). Each species was grown in plots in an 11m<sup>2</sup> pure-stand area under non-water- and non-N-limiting conditions. Irrigation occurred regularly and 100 N kg ha<sup>-1</sup> was provided to non-N fixing species at sowing. Data from this experiment were used for sole crop model calibration under non-limiting conditions.

Experiment 2 was performed at the Auzeville site in 2012. In this experiment the cover crops were grown under actual conditions (water and N limiting conditions) and as they would be normally used by farmers; therefore, no N fertilizer was added during the experiment. Irrigation was provided during the first week after sowing to ensure optimal plant emergence of homogeneous plant stands. No irrigation was used during cover crop growth. Since it was impossible to evaluate all combinations of the 34 species in a bispecific one-legume and one-non-legume mixture in a complete three randomized block design, we selected five legumes (crimson clover, purple vetch, wild lentil, forage pea and faba bean) according to their known different characteristics and five non-legumes (turnip rape, foxtail millet, bristle oat, Italian ryegrass and phacelia). We intercropped each legume with each non-legume to obtain 25 different mixtures. The sowing density in the mixtures corresponded to half that of the sole

crops for both species, which corresponds to a substitutive design (Jolliffe, 2000). Seeds of both species were mixed within the row to promote species interactions.

**Table 10** Experimental site description and growth conditions of the five field experiments. Variables in italics were used in mixture models.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Site location	Auzeville	Auzeville	Bignan	Auzeville	Auzeville
Data use	Sole crop calibration	Mixture sub-model calibration and mixture total model validation	Mixture sub-model calibration and mixture total model validation	Mixture sub-model calibration and mixture total model validation	Mixture sub-model and total models validation for species generality
Treatments	34 sole crops	25 mixtures	25 mixtures	16 mixtures	81 mixtures
Sowing date	16/08/12	16/08/12	17/08/12	22/08/13	16/08/12
Sampling date	12/10/12	26/10/12	13/11/12	22/10/13	29/10/12
Growth conditions	Non-limiting water and N	Actual	Actual	Actual	Actual
Soil texture	Clay loam	Clay loam	Silt loam	Loam	Clay loam
<i>Mineral nitrogen availability (kg ha<sup>-1</sup>) (AvN)</i>	44	53	112	44	45
Irrigation (mm)	170	60	0	50	60
Rainfall (mm)	44	80	323	82	80
ETP (mm)	210	237	159	193	237
Water at wilting point (mm)	132	132	87	132	132
Water in soil at sowing (mm)	179	179	281	166	180
Water available at sowing (mm)	197	47	194	34	48
<i>Water availability (mm) (AvW)</i>	69	-50	358	-27	-49
Daily mean temperature during experiment (°C)	19.1	19.1	13.5	18.4	19.1

The experimental design of 25 mixtures was replicated in Experiment 3 at another experimental site in Bignan in 2012. No irrigation occurred because sufficient rainfall ensured optimal plant emergence. This experiment was performed in a two block randomized design. Experiment 4 was performed in 2013 in another field experiment at the Auzeville site, where 16 promising mixtures were sown. These mixtures were identified from the 2012 experiments and were based on 14 species of legumes (crimson clover, Egyptian clover, purple vetch, common vetch, faba bean, and forage pea) and non-legumes (turnip rape, white mustard, Abyssinian mustard, foxtail millet, forage sorghum, bristle oat, Italian ryegrass, and phacelia). This experiment was performed in a three-block randomized design. Data from experiments 2, 3 and 4 were used to calibrate the legume and non-legume sub-models of the mixture models. They were also used to validate the complete model and the legume proportion in the mixture.

Experiment 5 was also performed at the Auzeville site in 2012 to evaluate a wide screening of bispecific mixtures. Two replicates of measurements by mixture were performed. Data from

this experiment were used as an initial step to validate mixture models for other species not used in the calibration step but whose functional traits were measured in Experiment 1.

### 2.1.3 Measurements

For each treatment of the five field experiments, we measured CGR, CNR and C:N ratio as three AEFs related to the ecosystem services for N management (CGR and CNR for capturing residual mineral-N in the soil and CNR and C:N ratio for the green manure effect): i) biomass production as CGR, expressed as the dry matter (DM) produced per ha per degree-day ( $\text{kg ha}^{-1} \text{DD}^{-1}$ ); ii) CNR, expressed as the amount of N in aboveground plant DM per ha per degree-day ( $\text{g ha}^{-1} \text{DD}^{-1}$ ); and iii) the C:N ratio at the date of sampling, which is the N content divided by C content in aboveground plant DM. These three AEFs were evaluated at the end of October or beginning of November, depending on the site (Table 10), and corresponding to an autumnal CC destruction after a 2-3 month growing period. For Experiment 5, CNR and the C:N ratio were evaluated for only the 22 mixtures (instead of 81) for which CGR was measured, due to limited labor availability. For the evaluated mixtures in all experiments, CGR and CNR were evaluated separately for each species (legume and non-legume). CGR and CNR were calculated as follows:

$$CGR = \frac{DM1-DM0}{T1-T0} \text{ and } CNR = \frac{Nacq1-Nacq0}{T1-T0}$$

where DM1 and DM0 is shoot DM ( $\text{kg ha}^{-1}$ ), and Nacq1 and Nacq0 is the amount of N acquired by plant shoots ( $\text{g ha}^{-1}$ ) at T1 and T0, respectively. DM0 and Nacq0 are equal to 0 because they correspond to DM and Nacq at the sowing date. The N acquired was estimated from shoot biomass DM and total N concentration. For shoot biomass, plant samples were dried in an oven at 80°C for 48 h and then weighed. Samples were then ground, and N and C content were measured by elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany). For sole crops in Experiment 1, the maximum root length depth was determined on 15 Oct 2012 by taking a 5-cm diameter soil sample 120 cm deep. Shoot height was measured with a ruler on 08 Oct 2012 and at flowering date. Leaf functional traits were measured on 10 Oct 2012 on the youngest mature leaf (lamina and petiole) free from herbivore or pathogen damage on 20 individual plants per species, according to the protocol of Cornelissen et al. (2003). All data measured in Experiment 1 for the 34 cover crops are provided in Annexe 7 (for details on plant leaf functional trait measurements, see Tribouillois et al., submitted to PlosOne).

## 2.2 Model description

### 2.2.1 Models for sole crops under non-limiting conditions

For sole crops, we tested the relevance of i) PFTs only, ii) plant stand traits only, and iii) both PFTs and plant stand traits combined for fitting the three AEFs (CGR, CNR and C:N ratio) produced by the 34 cover crop species under non-limiting conditions. We considered these sole crops' AEF production as “potential AEF” of each species because it expresses the maximum CGR or CNR and minimum C:N ratio under the most favorable conditions. Nine models were built and fitted (3 variables x 3 AEFs). We distinguished PFTs, which were normalized measurements of each species at the individual level, from plant stand traits, which were estimated at the community and cover level from a homogeneous stand of sole crop species (several individuals growing in intraspecific interactions). We tested eight PFTs and five plant stand traits as interesting model variables for predicting the AEFs (Table 11).

**Table 11 Variables tested for sole crop model optimization by category: plant functional traits and stand traits.**

Category	Variable	Unit	Description
Plant functional traits	SLA	m <sup>2</sup> kg <sup>-1</sup>	Specific leaf area
	LDMC	mg g <sup>-1</sup>	Leaf dry matter content
	LNC	mg g <sup>-1</sup>	Leaf nitrogen content
	LCC	%	Leaf carbon content
	Leaf C:N	unitless	Carbon to nitrogen ratio of one leaf
	Leaf area	cm <sup>2</sup>	Area of one leaf
	Flowering height	mm	Plant height at flowering stage
	Seed mass	mg	Mass of one seed
Stand traits	Cover height	cm	Mean height of plant cover
	Soil cover	%	Percentage of soil cover by plants vs. bare soil
	Cover N	%	Nitrogen content in aboveground cover biomass
	Cover C	%	Carbon content in aboveground cover biomass
	Root growth speed	mm DD <sup>-1</sup>	Root depth per degree-day

Our strategy was to test PFTs expressing the three main axes of plant strategies (Westoby, 1998). For example: i) leaf traits such as SLA, LDMC and LNC (Leaf Nitrogen Content) for resource acquisition and conservation strategies; ii) plant height at flowering for light-resource competitive advantage; and iii) seed mass for plant regeneration strategies.

### 2.2.2 Conceptual models for species in bispecific mixtures

Two sub-models comprised the complete model for each AEF evaluated. One sub-model was built for the legume species and another for non-legume species to consider differences in plant functioning. The sub-models were built separately to predict CGR, CNR and the C:N

ratio of the considered species (legume or non-legume) in a given bispecific mixture under actual conditions.

In the conceptual models, we hypothesized that the ability of a target species to provide the AEF in a given mixture would depend on its AEF in the sole crop grown under non-limiting conditions. Under actual conditions, this potential AEF would depend on environmental factors, especially water and N availability, which can stress plants. Interspecific interactions between the two intercropped species would influence AEF production of the target species in the given mixture. We hypothesized that the potential AEF of the associated species would describe these interactions, which might positively or negatively impact the growth of the target species in the mixture (e.g. the higher the potential CGR of the associated species, the stronger the competition exerted on the target species). The interspecific interactions would also be expressed by the interspecific distance between resource-use strategies due to leaf PFT differences. They were calculated as  $PFT_t - PFT_a$ , where  $t$  is the trait attribute of the target species and  $a$  is the trait attribute of its associated species in the given mixture, as proposed by Kunstler et al. (2012). We focused on leaf PFT differences of robust traits, which are SLA (DiffSLA), LDMC (DiffLDMC) and LA (DiffLA) (Kazakou et al., 2014; Tribouillois et al., submitted to PlosOne) and represent the resource-use strategies of plants. We also hypothesized that environmental factors would influence trait plasticity. We used environmental factors as individual input variables when testing models, but also in interaction with the AEF potential and leaf PFT differences. These factors were mineral N in the soil at sowing ( $AvN$ ) and water availability during the growing season ( $AvW$ ), which was calculated using a water budget.

To illustrate, e.g. for the CGR, the initial sub-models would be formulated as follows for non-legumes (nl) and legumes (leg) in the intercropped mixture (IC):

**CGR\_ICnl** = potential CGR\_SCnl + potential CGR\_SCleg + environmental factors + PFT\_SC differences + interactions [potential CGR\_SCnl x environmental factors + potential CGR\_SCleg x environmental factors + PFT\_SC differences x environmental factors].

**CGR\_ICleg** = potential CGR\_SCleg + potential CGR\_SCnl + environmental factors + PFT\_SC differences + interactions [potential CGR\_SCleg x environmental factors + potential CGR\_SCnl x environmental factors + PFT\_SC differences x environmental factors].

Parameters were calibrated independently for legume and non-legume species. Finally, the complete model for the entire mixture was expressed as follows:

**CGR\_whole-IC** = CGR\_ICnl + CGR\_ICleg

### 2.2.3 Generalized linear modeling

We used generalized linear modeling (GLM) to model the three AEFs. GLMs were fit using the statistical software package R (version 2.14.0). The stepwise regression procedure (StepAIC) was used to select variables for inclusion in the final model using Akaike's Information Criterion (AIC) as a variable selection criterion. The stepwise procedure was achieved with backward selection, which begins with all candidate variables. Resulting variables that correlated at  $P > 0.05$  were rejected. The GLM and stepAIC functions were found in the "Modern Applied Statistics with S" (MASS) package.

### 2.2.4 Validation of models representing bispecific mixtures

After calibrating the GLM models, we evaluated them in two steps. First, we evaluated the complete model's (legume and non-legume models summed) ability to predict the three AEFs in the whole bispecific mixture. We calculated the proportion of legumes in the whole mixture to reflect the changes in the dominant species induced by bispecific interactions. If the legume proportion exceeded 0.5, legume was the dominant species instead of non-legume species. Conversely, if the legume proportion was less than 0.5, the non-legume species was dominant in the bispecific mixture. For the C:N ratio, we predicted C:N for each species and estimated the weighted C:N of the whole mixture by the predicted CGR of each species in the mixture. Second, we evaluated the generality of the two sub-models and the complete models to predict AEFs production of different species or mixtures not used in the calibration step. For CGR, we considered two cases: i) neither of the two species had been used in calibration or ii) one of the two species had been used in calibration.

## 2.3 Statistical criteria

Statistical criteria were calculated for assessing the quality of model calibration and validation: relative root mean square error (RRMSE), bias and efficiency (Eff). These three criteria were calculated with the following equations:

$$RRMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n \left( \frac{P_i - O_i}{O_i} \right)^2} \quad Bias = \frac{1}{n} \sum_{i=1}^n (P_i - O_i) \quad Eff = 1 - \frac{\sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{o})^2}$$

where  $n$  is the number of observations,  $O_i$  and  $P_i$  are the observed and predicted values, respectively, and  $\bar{o}$  is the mean of observed values. RRMSE, based on the standard root mean square error, expresses error as a proportion, which is easily understandable to compare observed vs. predicted values. It ranges from 0 to 1, with 0 corresponding to no error. Bias

expresses model deviation from the  $x=y$  line. Eff describes model performance relative to the mean of observed data and ranges from  $-\infty$  to 1. Values close to 1 correspond to a perfect match between observed and predicted values. Efficiency can be negative when the observed mean is a better indicator than the model. We also calculated correlations between observed and predicted data with linear regressions and corresponding  $R^2$ . We evaluated the two sub-models (legume and non-legume) together to assess the overall ability of the model to predict the AEFs of a target species (legume or non-legume) in a given mixture.

### 3 Results and discussion

#### 3.1 Models of sole crops under non-limiting conditions

The three AEFs studied for the 34 cover crop species growing under non-limiting water and N conditions had the highest efficiencies (0.63-0.91) and lowest RRMSE (0.13-0.16) when both PFTs and plant stand traits were used as input variables (Figure 27; Table 12).

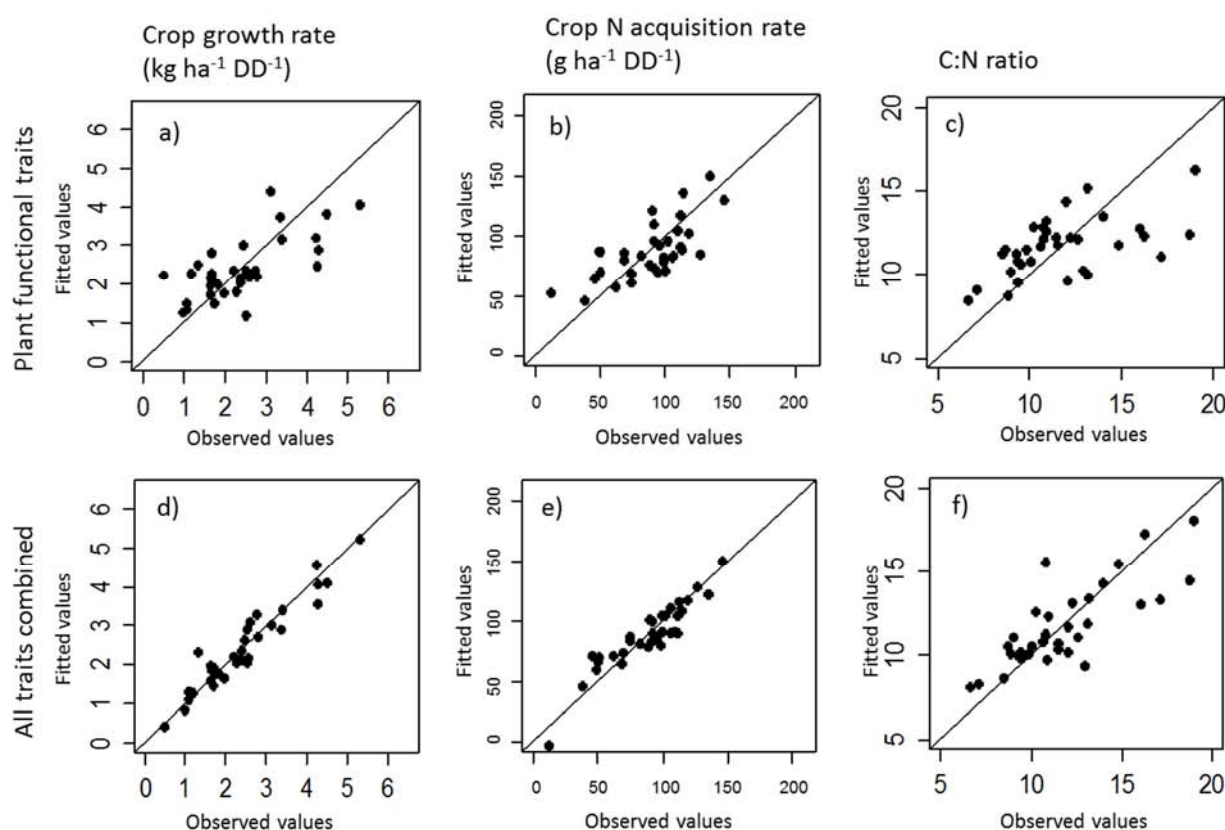


Figure 27 Observed vs. fitted agro-ecosystem functions using only plant functional traits: a) crop growth rate (CGR), b) crop N acquisition rate (CNR), and c) C:N ratio; and combining all traits (plant functional traits and stand traits) for d) CGR, e) CNR and f) C:N ratio. Solid lines represent the adjustment of data but also  $y=x$ , and DD is degree-day.

For models based only on PFTs, efficiencies were the lowest (0.32-0.49) and RRMSE were the highest (0.32 to 0.49). For models based only on plant stand traits, efficiencies were fairly

high (0.46-0.84) and RRMSE was rather low (0.18-0.20). For all models, the biases were close to 0; the highest bias was -0.08 for CNR based on PFTs (data not shown).

Concerning the models based only on PFTs, LA was the strongest significant variable in expression of CGR and CNR and therefore was the best PFT predictor for expressing AEFs, as also shown by Tribouillois et al. (submitted to PlosOne). However, when combined with plant stand traits, LA provided less information for AEFs for predicting CNR, and was no longer a significant variable for CGR. One explanation is that another variable, such as cover height, was more significant and correlated with LA ( $R^2=0.52$ , data not shown,). Therefore, cover height is more informative than LA.

**Table 12 Input co-variables, parameter values and statistical criteria of sole crop models to predict agro-ecosystem function, by category: plant functional traits, stand traits and all traits combined.**

Method used	Crop growth rate (kg ha <sup>-1</sup> DD <sup>-1</sup> )		Crop N acquisition rate (g ha <sup>-1</sup> DD <sup>-1</sup> )			Cover C:N ratio		
	Correlated variable	Parameter value	Correlated variable	Parameter value		Correlated variable	Parameter value	
Plant functional traits	(Intercept)	13.77 *	(Intercept)	0.00 ns		(Intercept)	15.48 ***	
	<i>Leaf area</i>	0.01 ***	<i>SLA</i>	1.87 ***		<i>LNC</i>	-0.14 *	
	<i>LDMC</i>	0.02 *	<i>Leaf area</i>	0.42 ***		<i>LDMC</i>	0.03 .	
	<i>Leaf C:N</i>	-0.84 *						
	<i>LNC</i>	-0.13 .						
		$R^2 = 0.49$		$R^2 = 0.49$			$R^2 = 0.32$	
		RRMSE = 0.33		RRMSE = 0.24			RRMSE = 0.21	
		Eff = 0.49		Eff = 0.48			Eff = 0.32	
Plant Stand traits	(Intercept)	0.00 ns	(Intercept)	0.00 ns		(Intercept)	8.17 ***	
	Cover height	0.03 ***	Soil cover rate	0.64 ***		Cover height	0.07 ***	
	Soil cover rate	0.02 ***	Cover height	0.59 ***				
	Cover N	-0.23 *						
			$R^2 = 0.85$		$R^2 = 0.63$			$R^2 = 0.46$
		RRMSE = 0.18		RRMSE = 0.20			RRMSE = 0.19	
		Eff = 0.84		Eff = 0.63			Eff = 0.46	
All traits combined	(Intercept)	10.98 ***	(Intercept)	257.30 *		(Intercept)	16.37 ***	
	Cover height	0.02 ***	Soil cover rate	0.70 ***		Cover height	0.08 ***	
	<i>Leaf C:N</i>	-0.60 ***	Cover height	0.40 **		<i>LNC</i>	-0.15 **	
	<i>LNC</i>	-0.10 ***	<i>Leaf C:N</i>	-17.20 **		<i>Leaf area</i>	-0.02 *	
	Cover N	-0.34 **	<i>Leaf area</i>	0.12 *				
	Soil cover rate	0.02 **	<i>LNC</i>	-2.40 *				
	Root growth speed	0.88 *	Root growth speed	25.30 .				
		$R^2 = 0.91$		$R^2 = 0.83$			$R^2 = 0.63$	
		RRMSE = 0.14		RRMSE = 0.13			RRMSE = 0.16	
		Eff = 0.91		Eff = 0.84			Eff = 0.63	

\*\*\* P-value<0.0001; \*\*P-value<0.01; \*P-value<0.05; .P-value<0.10; ns P-value non-significant  
“RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.

Concerning models based only on plant stand traits, cover height was the only significant trait for predicting the three AEFs. However, the percentage of soil covered was a significant variable explaining part of the variability of CGR and CNR. These results are consistent with those of Axmanová et al. (2012), who found that cover height and soil cover were two significant variables for predicting plant biomass production.



For the three AEFs, when PFTs and plant stand traits were both used in the same model, the most significant variables were plant stand traits, such as cover height for CGR and C:N ratio. Consequently, plant stand traits were better predictors than PFTs. This is consistent with other published equations that predict herbaceous biomass, in which plant stand height was also an influential variable (Axmanová et al., 2012; Elliott and Clinton, 1993; Redjadj et al., 2012). Root growth speed was also a significant variable for estimating CGR and CNR, which is consistent with the study of Kristensen and Thorup-Kristensen (2004), who found that root depth was a good indicator of catch-crop ability to capture nitrate. The leaf C:N and LNC PFTs were negatively correlated with CGR and CNR. This probably reflects N “dilution” in plant tissues, which indicates a decrease in N concentration in correlation with stand growth and biomass production (Greenwood et al., 1986; Lemaire and Salette, 1984). PFTs, such as height measured at flowering and LCC, never appeared as significant variables in explaining the variability of the three AEFs.

## **3.2 Species in mixtures under actual conditions**

### **3.2.1 Sub-model calibration and quality of fitting**

Models obtained for bispecific mixture cover crops consisted of two sub-models that correspond to two GLM equations, one for legumes and another for non-legumes. This strategy enabled a good adjustment of observed data for the three AEFs. The sub-models predicting CGR, CNR and C:N ratio of a given species in bispecific mixtures were globally well fitted to observed values with high efficiencies (0.93, 0.85 and 0.88, for CGR, CNR and C:N ratio, respectively) and almost no bias (0.00, 0.02, -0.01) (Figure 28a-b-c). All the data from the three experimental sites used for calibrations were fit well. For Experiment 3, some species in mixture had higher CGR (Figure 28b) and CNR (Figure 28d) than others; however, they were also well fitted. As an example of a parameterized sub-model, e.g. for CGR of a non-legume species in a mixture (or intercropping-IC), the sub-model developed was as follows (Table 13):

$$\text{CGR\_ICnl} = 4.46 - 2.41 \text{ CGR\_SCnl} - 0.08 \text{ AvN} + 0.01 \text{ AvW} - 0.05 \text{ DiffLDMC} + 0.05 (\text{CGR\_SCt} \times \text{AvN}) - 8.0\text{E-}3 (\text{CGR\_SCt} \times \text{AvW}) + 5.5\text{E-}5 (\text{DiffLA} \times \text{AvN}) + 1.1\text{E-}3 (\text{DiffLDMC} \times \text{AvN}) - 2.0\text{E-}4 (\text{DiffLDMC} \times \text{AvW}).$$

As expected, the potential AEF (e.g. CGR\_SCnl) measured for sole crops of a target species was a significant input variable (Table 13). In bispecific mixtures, the non-legume species usually induced stronger competition on the legume species, as shown for cereal-grain legume

intercrops. This is particularly true for medium or high N input conditions (Bedoussac and Justes, 2010b; Corre-Hellou et al., 2006). This could explain why, for legume species, the potential AEF of the associated species (e.g. CGR\_SCleg) was significantly correlated in the equations but was not included in non-legume equations, except for CNR. For CNR of non-legumes, the potential AEF of the associated legume species was significant probably because of legumes' ability to fix atmospheric N<sub>2</sub>, which increases their ability to access other abiotic resources, which increases interactions between the species.

**Table 13 Input co-variables and parameter values of sub-models to predict agro-ecosystem functions (AEF) for legume and non-legume species in bispecific mixture.**

Type of variable	Biomass production (kg ha <sup>-1</sup> DD <sup>-1</sup> )			N acquisition (g ha <sup>-1</sup> DD <sup>-1</sup> )			C:N ratio		
	Correlated variable	Parameter value		Correlated variable	Parameter value		Correlated variable	Parameter value	
		Non-Legume	Legume		Non-Legume	Legume		Non-Legume	Legume
(Intercept)	(Intercept)	4.46 ***	2.90 ***	(Intercept)	ns	96.96 ***	(Intercept)	94.65 ***	22.47 ***
Sole crop potential AEF	CGR_SCt	-2.41 ***	-1.91 ***	CNR_SCt	-1.21 **	-1.87 **	C:N_SCt	-7.10 ***	-1.27 ***
	CGR_SCa	ns	1.07 **	CNR_SCa	0.89 *	1.11 *	C:N_SCa	ns	0.12 *
Environmental factors	AvN	-0.08 **	-0.05 ***	AvN	ns	-1.08 ***	AvN	-1.65 ***	-0.30 ***
	AvW	0.01 ***	ns	AvW	ns	ns	AvW	0.32 ***	ns
PFT differences	DiffSLA	ns	0.16 ***	DiffSLA	ns	8.07 ***	DiffSLA	ns	-0.06 **
	DiffLA	ns	ns	DiffLA	ns	ns	DiffLA	ns	-0.01 ***
	DiffLDMC	-0.05 ***	-0.02 **	DiffLDMC	-1.02 ***	-1.24 ***	DiffLDMC	0.16 *	ns
Interaction sole crop potential AEF * environmental factors	CGR_SCt*AvN	0.05 ***	0.04 ***	CNR_SCt*AvN	0.03 ***	0.04 ***	C:N_SCt*AvN	0.15 ***	0.03 ***
	CGR_SCt*AvW	-8.0E-03 ***	-4.6E-03 **	CNR_SCt*AvW	-3.7E-03 **	-0.01 **	C:N_SCt*AvW	-0.03 ***	ns
	CGR_SCa*AvN	ns	-0.02 **	CNR_SCa*AvN	-0.02 **	-0.03 **	C:N_SCa*AvN	ns	ns
	CGR_SCa*AvW	ns	0.01 ***	CNR_SCa*AvW	2.5E-03 *	0.01 **	C:N_SCa*AvW	ns	ns
Interaction PFT differences * environmental factors	DiffSLA*AvN	ns	4.4E-04 ***	DiffSLA*AvN	-6.4E-03 ***	-0.15 ***	DiffSLA*AvN	ns	ns
	DiffSLA*AvW	ns	-2.9E-03 ***	DiffSLA*AvW	ns	0.03 ***	DiffSLA*AvW	ns	-2.6E-04 **
	DiffLA*AvN	5.5E-05 ***	ns	DiffLA*AvN	ns	ns	DiffLA*AvN	ns	ns
	DiffLA*AvW	ns	ns	DiffLA*AvW	ns	ns	DiffLA*AvW	1.2E-04 ***	-2.5E-05 *
	DiffLDMC*AvN	1.1E-03 ***	4.5E-04 **	DiffLDMC*AvN	0.02 ***	0.03 ***	DiffLDMC*AvN	-2.6E-03 *	ns
	DiffLDMC*AvW	-2.0E-04 ***	-8.6E-05 ***	DiffLDMC*AvW	-3.5E-03 ***	-4.7E-03 ***	DiffLDMC*AvW	-6.4E-04 *	ns

PFT is plant functional traits. CGR\_SCt (kg ha<sup>-1</sup> DD<sup>-1</sup>) and CNR\_SCt (g ha<sup>-1</sup> DD<sup>-1</sup>) correspond, respectively, to potential crop growth and crop nitrogen acquisition rate per degree-day (DD) measured under non-limiting conditions of target species in sole crop or of the associated species for CGR\_SCa (kg ha<sup>-1</sup> DD<sup>-1</sup>) and CNR\_SCa (g ha<sup>-1</sup> DD<sup>-1</sup>); C:N\_SCt and C:N\_SCa correspond, respectively, to the carbon to nitrogen ratio of aboveground biomass measured under non-limiting conditions of the targeted and associated species; AvN (kg ha<sup>-1</sup>) corresponds to mineral nitrogen availability in the soil at sowing and AvW (mm) to water availability during the growth period; DiffSLA (m<sup>2</sup> kg<sup>-1</sup>), DiffLA (cm<sup>2</sup>) and DiffLDMC (mg g<sup>-1</sup>) correspond to the difference between, respectively, specific leaf area (SLA), leaf area (LA) and leaf dry matter content (LDMC) values of target species and associated species values under sole crops measured under non-limiting conditions.

\*\*\* P-value<0.0001; \*\*P-value<0.01; \*P-value<0.05; ns P-value non-significant

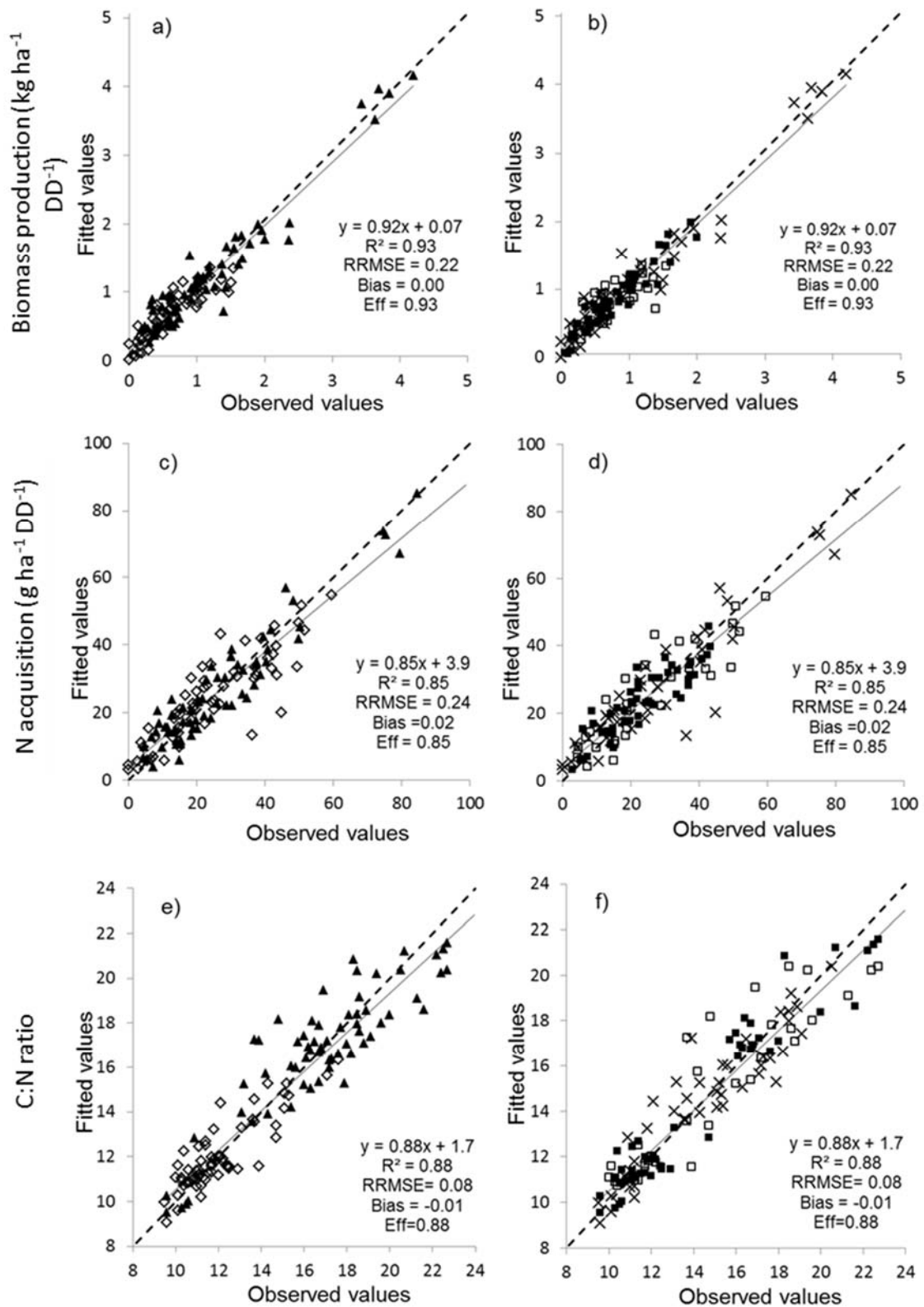
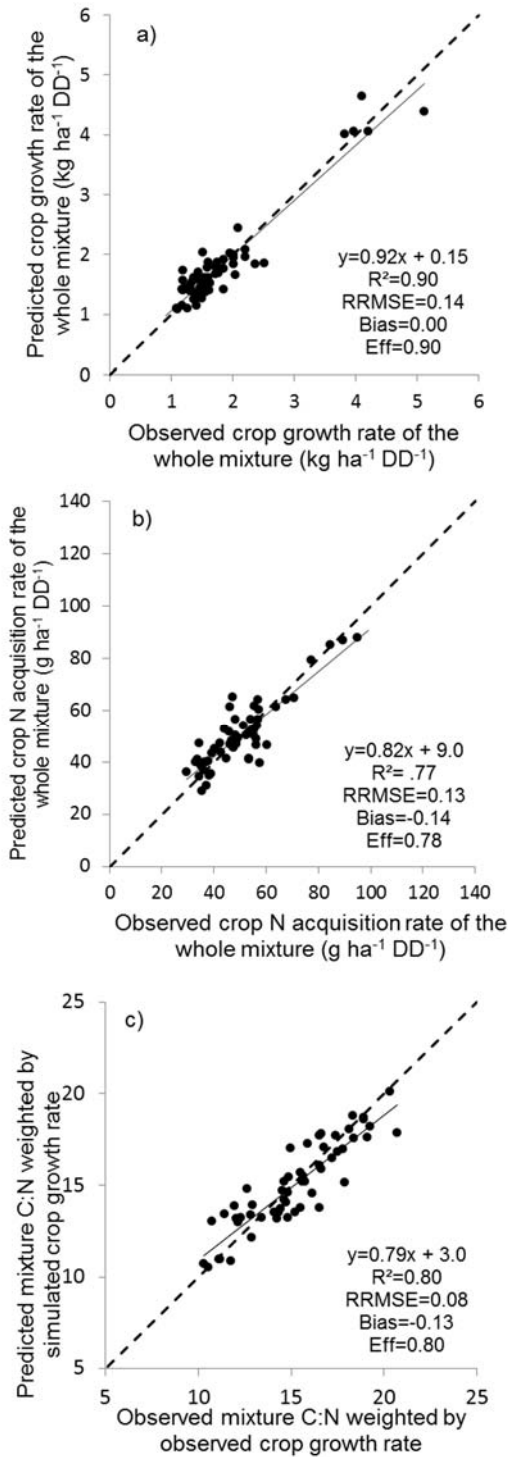


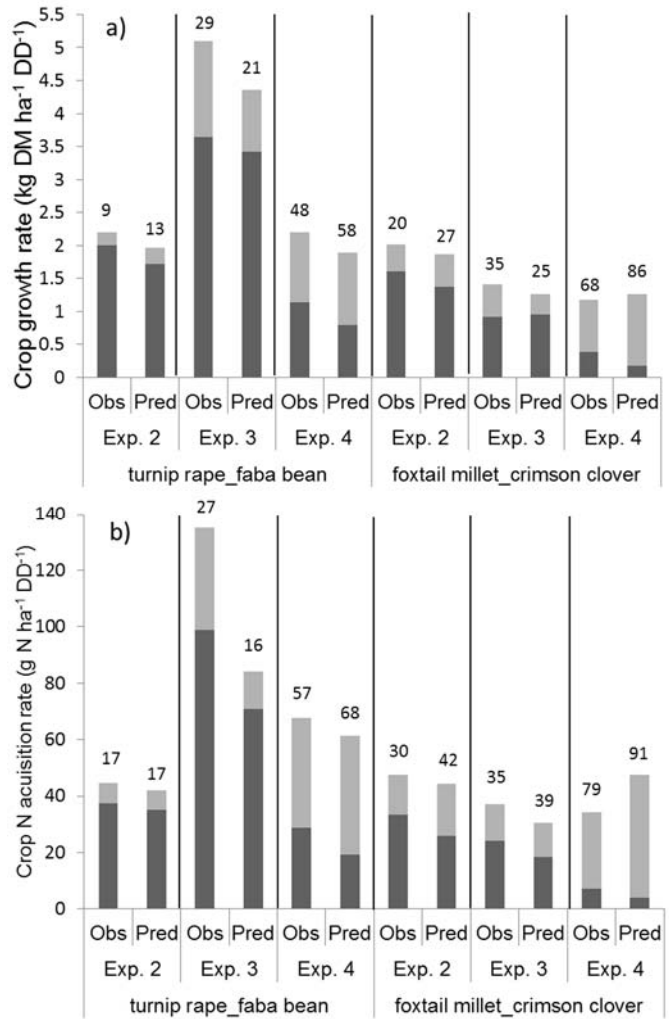
Figure 28 Fitting quality representation of the mixture sub-models for the three agro-ecosystem functions: crop growth rate (CGR), crop N acquisition rate (CNR) and species C:N ratio. Legume/non-legume fitting quality on a), c), and e) with black triangles corresponding to non-legumes and white diamonds to legumes. Experimental data set fitting quality on b), d), f) with black squares representing data from Experiment 2, black crosses Experiment 3 and white squares Experiment 4. Dashed lines represent  $y=x$  and solid lines the adjustment of data. “RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.

PFT differences were also significant input co-variables for most of the sub-models. The PFT differences can explain the interactions for resource use, especially the competition intensity between a target species and its associated species, which is consistent with results found in the literature (Fort et al., 2014; Kunstler et al., 2012). For non-legumes, DiffLDMC was a significant input co-variable for explaining CGR and CNR. For legumes, DiffSLA was a significant input co-variable for the two AEFs. SLA and LDMC are both negatively correlated functional traits (Garnier et al., 2001; McIntyre, 2008), which could explain the species' trade-off in resource-use strategy. A species with high SLA and low LDMC is able to quickly acquire N and produce biomass. Conversely, a species with a low SLA and high LDMC would have a more resources conservation strategy, first producing biomass and then acquiring N. A high LDMC or SLA difference indicates a great difference between a more conservative and a more acquisitive species, promoting stronger competition between species than a lower trait distance. This hypothesis of trait distance is widely discussed in ecological literature and both ideas are defended: several studies assume that trait distances between species enable coexistence (MacArthur and Levins, 1967; Stubbs and Bastow Wilson, 2004) while other studies argue that trait similarity (small distances) leads to species coexistence (Kraft et al., 2014; Mayfield and Levine, 2010). Our results are consistent with those of Kraft et al. (2014), who demonstrated that the best model for predicting plant biomass includes functional trait differences.

Finally, environmental factors such as water and N availability were significant input co-variables on their own or in interaction for expressing the AEFs (Table 13). AvN was significant in all models as an independent variable, even for legumes. One exception was for CNR of non-legumes, for which AvN significantly interacted with CNR\_SCt and CNR\_SCa. Environmental factors (AvN and AvW) also strongly interacted with PFT differences, especially with DiffLDMC (Table 13). These results support the hypothesis that PFT differences are impacted by growth conditions and affect competition intensity, as was recently suggested for roots of forage species (Fort et al., 2014). These factors in interaction with potential AEF and PFT differences could allow models to be adapted to sites with different soil and climate conditions that induce water and N stresses and therefore adapted to the behavior of species mixtures, such as the variation in dominant species.



**Figure 29** Observed vs. predicted values of the mixture complete model for a) crop growth rate (CGR) and b) crop N acquisition rate (CNR) of whole mixtures and c) observed vs. predicted C:N ratio of mixtures weighted by, respectively, observed and predicted CGR for each species in the whole mixture. Dashed lines represent  $y=x$  and solid lines the adjustment of data. “RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.



**Figure 30** Observed vs. predicted values for a) crop growth rate (CGR) and b) crop N acquisition rate (CNR) of each species of foxtail millet/crimson clover and turnip rape/faba bean mixtures at three experimental sites and years (Experiments 2, 3 and 4). Legume species are in gray and non-legume species in black. Numbers correspond to legume proportion in the whole bispecific mixture.

### 3.2.2 Validation of bispecific mixture models

#### 3.2.2.1 Validation of the whole mixture and legume proportion

Overall, complete models summing both sub-models were able to predict total CGR and CNR of whole mixtures with acceptable efficiency (0.90 and 0.78, respectively), and even the highest and lowest mixtures were well estimated (Figure 29a-b). For example, in Experiment 3, the turnip rape/faba bean mixture had the highest CGR in observations (5.10 kg DM ha<sup>-1</sup> DD<sup>-1</sup>) and predictions. Conversely, in Experiment 4, the foxtail millet/crimson clover mixture had one of the lowest CGR for both observations (1.18 DM ha<sup>-1</sup> DD<sup>-1</sup>) and predictions (Figure 30).

The models were also able to predict the proportion of biomass production and N acquisition (CGR and CNR, respectively) of legumes in a given mixture (Figure 31a-b). This legume proportion highlights the variation in competition and species dominance in a mixture as a function of the associated species and experimental site conditions (Ofori and Stern, 1987).

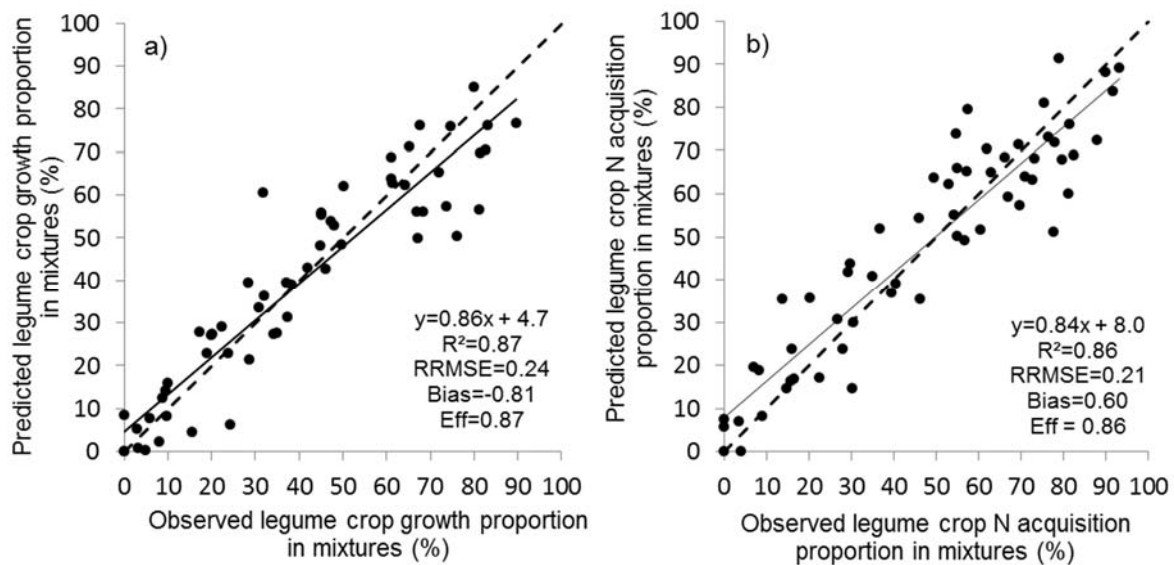


Figure 31 Observed vs. predicted legume proportion in total mixtures for a) crop growth rate (CGR) and b) crop N acquisition rate (CNR). Dashed lines represent  $y=x$  and solid lines the adjustment of data. “RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.

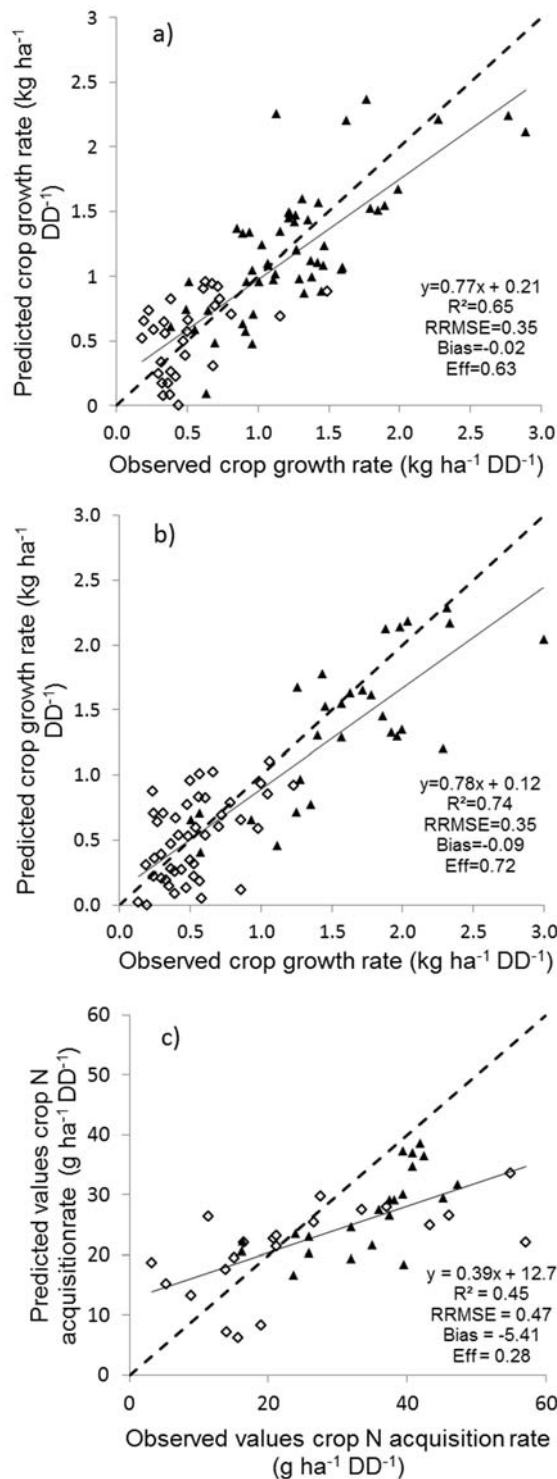
The predictions were satisfactory, with acceptable efficiencies (0.87 and 0.86, respectively), little bias (-0.81 and 0.60, respectively), and RRMSE of 0.24 and 0.21, respectively. The models were able to predict the wide range of legume proportions in bispecific mixtures, in which CGR varied under actual conditions from 0-0.9 (mean=0.41). They were well predicted: from 0-0.85 (mean=0.4). The observed legume proportion for CNR varied from 0-0.93 (mean=0.48), and the predicted proportion varied from 0-0.91 (mean=0.48). Two examples of the large variation in species dominance in mixtures were illustrated in Figure 30.

Experiment 4 was a site and a year more favorable to legumes (proportions  $> 0.5$  for both CGR and CNR). Conversely, Experiment 2 and Experiment 3 had legume proportions  $< 0.35$ . Satisfactorily, the models were able to predict these variations in species dominance for the three experiments despite the inversion of dominance. For both CGR and CNR, the low legume proportions could indicate that competition from non-legumes was strong and limited the legumes' ability to provide the AEF (e.g. faba bean with turnip rape in Experiment 2). Conversely, high legume proportions indicated that the non-legume grew poorly, probably due to unfavorable climatic or soil conditions or interspecific interactions that benefitted the legume (e.g. Experiment 4).

The C:N ratio of the whole mixture (weighted by CGR) (Figure 29c) was satisfactorily predicted according to the double prediction (prediction of CGR and C:N ratio for both species). The complete model had little bias (-0.13), a low RRMSE (0.08) and an acceptable efficiency of 0.80. Observed C:N values ranged from 10.3-24.7 (mean=15.3). Predictions varied within the same range, from 10.5-20.1 (mean=15.0). Predicting C:N ratio of the whole cover crop mixture well assessed the ability of its residues to release N after cover crop destruction (Kumar and Goh, 2002; Quemada and Cabrera, 1995). This would influence the amount of N available for the next main crop and the green manure service of the cover crop mixtures. A mixture with a low C:N ratio would promote rapid N release and avoid a pre-emptive competition for the next main crop (Francis et al., 1998; Justes et al., 2009; Trinsoutrot et al., 2000).

### **3.2.2.2 Validation of the models' generality for other species**

The sub-models were able to predict the CGR of species not used in calibration with acceptable efficiency (0.63), a RRMSE of 0.35, and almost no bias (Figure 32a). It was also possible to predict the CGR of species used in calibration but associated with species not used in calibration (Figure 32b) with almost the same level of accuracy: efficiency was 0.72, RRMSE was 0.45, and bias was -0.09. In both cases, legume CGR was less variable than that of non-legumes. The former ranged from 0.1-1.5 kg ha<sup>-1</sup> DD<sup>-1</sup> for observed data (0.0-1.0 kg ha<sup>-1</sup> DD<sup>-1</sup> for predicted values), while the latter ranged from 0.4-3.0 kg ha<sup>-1</sup> DD<sup>-1</sup> for observed data (0.1-2.4 kg ha<sup>-1</sup> DD<sup>-1</sup> for predicted values).



**Figure 32** Observed vs. predicted values for sub-model validation of a) crop growth rate (CGR) of species in mixtures for species not used in model calibration, b) CGR for species used in model calibration but associated with a species not used in model calibration and c) crop N acquisition rate (CNR) for both species and mixtures not used in model calibration. Black triangles correspond to non-legumes and white diamonds to legumes. Dashed lines represent  $y=x$  and solid lines the adjustment of data. “RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.

The CNR sub-models used for other species and mixtures (Figure 32c) predicted the CNR of species in mixture with an efficiency of 0.28, a RRMSE=0.47 and a bias of -5.4. This indicates that the model was partly efficient but not completely satisfactory. Despite this lack of accuracy, the sub-models were able to predict global trends. In general, the sub-models for CNR obtained acceptable statistical criteria for fitted adjustments and for prediction of legume proportions in mixture, but they were less efficient for CGR and the C:N ratio.

Application of the complete model to other species and mixtures yielded an efficiency of 0.30 (acceptable for an independent validation), a fairly low RRMSE of 0.21 and almost no bias (0.02) when predicting CGR (data not shown). The complete model was not able to satisfactorily predict CNR of the whole mixture for other species in terms of reproducing the variability of the whole CNR (Eff=-0.98, bias=-11.7); however, the RRMSE remained acceptable at 0.36 (data not shown). When N availability was relatively low, as in Experiment 5, the non-legume strongly competed for mineral N, which could explain the model’s lack of sensitivity. This increased the N<sub>2</sub> fixation rate of the associated legume species (Bedoussac and Justes, 2010a; Ghaley et al., 2005; Tofinga et al., 2009) and increased N acquisition due to niche complementarity or facilitation. The complete model was not able to consider this facilitation phenomenon, which



limited the CNR predicted for the mixture. This hypothesis is also supported by the complete model's ability to appropriately predict high CNR when N availability was high, as in Experiment 3 (the highest points in Figure 29b). When N availability is high, competition for mineral N is lower.

Finally, the C:N ratio was the AEF best predicted for other species and mixtures. The C:N ratio of mixtures, weighted by the CGR of each species (Figure 33), was predicted by the complete model with great accuracy and yielded acceptable model generality for other species and mixtures. Its efficiency was high (0.76), RRMSE low (0.13), with a small bias (-0.33). This complete model is useful for estimating a main N release variable for the next main crop and could help predict the green manure service.

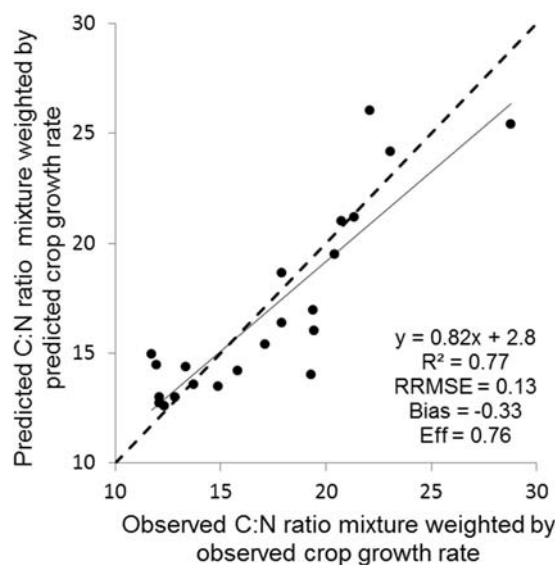


Figure 33 Observed vs. predicted values for the complete model of C:N ratio of total mixtures weighted by the crop growth rate (CGR) of each species. Dashed lines represent  $y=x$  and solid lines the adjustment of data. “RRMSE” means “relative root mean square error”, and “Eff” means “efficiency”.

The bispecific mixture models developed were able to predict AEFs using variables measured under non-limiting conditions along with environmental factors, as demonstrated by Duru et al. (2009) for predicting biomass production of herbage species in natural grasslands. Duru et al. (2009) also demonstrated the importance of integrating PFT and environmental factors, such as nutrient availability. They also highlighted the importance of including non-limiting conditions to improve predictions, which supports our results and conclusions.

One model application could be to predict *ex ante* the behavior of a given species, including those not tested here, in a bispecific mixture, for any species whose potential AEF and leaf functional traits are known. According to our results, potential AEF can be measured in simple field experiments, or predicted using the sole crop models developed if experiments

are not possible. Growing sole crops under non-limiting conditions to assess potential AEF and measuring leaf functional traits to calculate trait differences can be performed regardless of N and water conditions due to irrigation and fertilization. This provides a certain level of generality of the models for potential AEF based on sole crops when measured under non-limiting conditions, and for mixed cover crops when environmental conditions (water and N availability) can be estimated. This idea is supported by McIntyre and Lavorel (2007), who suggested that using a common language, such as PFTs, allows comparison of species' ability to provide ecosystem functions in different parts of the world by considering their responses to environmental factors. However, species generality in the bispecific mixture models was based on data from experimental fields where N availability was relatively low ( $45 \text{ kg N ha}^{-1}$ ) and competition for N occurred. It would be interesting to evaluate the validity of the models, especially in situations with higher levels of N availability, and validate the complete CNR model. It would also be interesting to expand validation to additional species and sites with contrasting soils and climates, especially the models' ability to predict variations in dominance under a wide range of bispecific mixtures. The models were useful for predicting AEF for an autumnal CC destruction corresponding to a growing period of approximately 2 or 3 months; however, it would be useful to determine the models' validity over longer growing periods. Another option would include developing a dynamic mechanistic model to predict interactions between two species growing in mixtures. It would be interesting to dynamically predict the key phenomena of competition, niche complementarity and facilitation of abiotic resources (e.g. light, water, N) to better predict the behavior and proportion of species in bispecific and plurispecific mixtures.

#### **4 Conclusion**

PFTs are considered a robust way to characterise species' behavior in ecosystems. It was informative to test their ability to predict rates of biomass production and N acquisition of cover crop species grown as sole crops and in mixtures. We demonstrated that PFTs must be combined with plant stand traits to obtain satisfactory prediction of cover crop species' behavior in sole crops to produce the potential AEF, such as CGR, CNR and C:N ratio, under non-limiting and under actual conditions for bispecific mixtures. Plant stand traits were necessary for obtaining accurate models. We also developed models that can predict the AEF of a target species in a given bispecific legume/non-legume cover crop mixture under actual conditions. These models are based on the potential AEF in sole crops of each species and on

leaf functional trait differences, which reveal plant strategies and the degree of competition between species. Environmental factors were also significant factors in the models and enabled them to be adapted to other experimental sites. They are based only on generalizable variables measured under non-limiting conditions, which might be too simplistic for cover crops generally grown under actual conditions. The models' predictions of species in bispecific mixtures were generally able to predict AEF, especially CGR and the C:N ratio of species not used for model calibration, which is initial evidence of their potential generality. However, additional validation on other sites with contrasting soils and climates and longer growing periods would be useful in continuing our work.

## **5 Acknowledgements**

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**Références indiquées à la fin du manuscrit.**



## **Chapitre 4 : Analyse en dynamique des interactions et des performances des mélanges de cultures intermédiaires en fonction de la gestion de l'interculture**

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## **1 Introduction**

Cover crops (CC) are sown during the fallow period to reduce nitrate leaching in order to avoid groundwater pollution but also to produce green manure effect: release mineral N available for the next crop to the soil (e.g. Thorup-Kristensen et al., 2003; Justes et al., 2012). The effects of CC have been widely studied in literature as sole crops (SC) -or monospecific crops- and appear to be contrasted regarding the species used (e.g. Stopes et al., 1996; Thorup-kristensen, 2001). Globally, even if all species can produce ecosystem services related to nitrogen (N) management, the legume species have shown to be more efficient to produce a green N manure effect due to their ability to acquire atmospheric N<sub>2</sub> by symbiotic fixation leading to large amount of N acquired and a low C:N ratio favoring a fast and a high proportion of N released from CC residues (e.g. Tonitto et al., 2006). On the contrary, the non-legume species would be more efficient to capture residual soil mineral nitrogen (SMN) and thus reduce nitrate leaching (e.g. Thomsen and Hansen 2014). An interesting way to produce simultaneously the two ecosystem services related to N management (catch crop and green manure) would be to intercrop legume and non-legume CC species in mixture. This type of CC mixtures have proved their effectiveness as reported in the literature (Kramberger et al., 2013; Kuo and Sainju, 1998; Möller et al., 2008; Ranells and Wagger, 1997; Summers et al., 2014; Tosti et al., 2012b). Intercropping (IC), or mixing species, is defined as the simultaneous growth of two or more species in a field during a significant period of their growth (Willey, 1979). This principle, mostly use for cash crops, is based on the resource complementarity use between the two species. To be efficient, the species must not compete for exactly the same resource niche and especially for the N resource (Bedoussac and Justes, 2010a; Jensen, 1996b; Ofori and Stern, 1987). In a previous paper (Chapitre 2), we demonstrated that some CC mixtures could favor the green manure effect in comparison with the non-legume SC and could be as much efficient as the non-legume SC to capture SMN and reduce nitrate leaching under the pedoclimatic conditions of France, in the case of autumnal destruction. However, the interactions between species are complex and evolve over time during CC cycle (Connolly et al., 1990). Several studies on cereal-legume cash crops provided analyses of interaction dynamics within bispecific mixtures which illustrated resource complementarity for light interception, nutrient availability but also mixtures performances especially for yield or N acquisition (Andersen et al., 2004; Bedoussac and Justes, 2010b; Corre-Hellou et al., 2006; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2006; Jensen, 1996b). The evolution of interspecific interactions over time may lead to various CC

mixtures performances according to the duration of the growing period and thus to the CC destruction date especially to provide green manure and SMN catching effects (Justes et al., 1999). However, to our knowledge, there is a lack of information in the scientific literature concerning the interactions and the performances in dynamics for CC bispecific mixtures. Thus, we propose to provide knowledge on functioning of the species in SC and in mixture in order to better understand and predict the behavior and the performances over time of such CC. The final goal is to provide information for adapting the species associated in mixtures by considering the length and the management objective of the fallow period as the date of CC destruction and incorporation. In this paper we analyzed i) the dynamic of growth of 14 CC species in SC and mixtures through autumnal and winter fallow periods; ii) the interspecific interactions and their potential advantages for species in mixture; iii) the CC mixtures performances as green manure and catch crops at three CC destruction dates and according to the favored service corresponding to different modes of management.

## 2 Material and methods

### 2.1 Experimental design

Thirty-one treatments were studied. They were composed of 16 bispecific mixtures, 14 corresponding SC and a bare soil without CC as a reference for N management and nitrate leaching during fallow period. The experimental design was randomized and replicated three times in elementary plots of 21m<sup>2</sup>. The mixtures were composed of one legume species and one non-legume species chosen for their diversity of botanical families, shoot and root architectures, frost sensibility and growth earliness, in order to ensure a large range of various mixtures. The mixture choice was made in order to evaluate different mixtures *a priori* adapted to four types of fallow periods management, based on the preceding experimental results (Chapitres 2 and 4) by choosing species couples which gave good results in the 2012 experiments. In all cases the sowing occurred in late summer, on the 22<sup>nd</sup> of August, after the harvest of the previous cash crop. To design the experiment, we have defined four cases of fallow period for which some mixtures were tested:

- i) **Short growing period**, for which the CC destruction occurred in late October, corresponding to a CC growing period of two months, e.g. for a grain legume / winter cereal succession. The objective is to produce a sufficient level of catch crop and green manure effects within few weeks during September and October. To address this purpose, we chose species having a fast growing in late summer. We evaluated four



- mixtures: 1) foxtail millet\_faba bean, 2) forage sorghum\_faba bean, 3) forage sorghum\_Egyptian clover and 4) turnip rape\_faba bean.
- ii) **Medium growing period**, for which the CC destruction occurred in beginning of December, corresponding to a CC growing period about 3.5 to 4 months, e.g. winter cereal / winter or spring pea or faba bean succession. The objective is also to maximize the catch crop and the green manure effects with destruction before winter. We chose species with medium growth speed and having a medium tolerance to frost (at least  $-5^{\circ}\text{C}$ ); we evaluated four mixtures: 5) bristle oat\_purple vetch, 6) bristle oat\_forage pea, 7) Abyssinian cabbage\_purple vetch and 8) white mustard\_purple vetch.
- iii) **Long growing period**, with destruction in late January or beginning of February (or later), corresponding to 5 to 6 months after sowing, e.g. winter cereal / spring crops (sunflower, sorghum, soybean...) succession. The objective is to maintain a green cover surviving to winter conditions and to produce catch crop and green manure along the fallow period. We chose species with medium to slow growth rate and a sufficient frost resistance; we tested four mixtures: 9) Italian ryegrass\_crimson clover, 10) Italian ryegrass\_forage pea, 11) phacelia\_crimson clover and 12) Abyssinian cabbage\_common vetch.
- iv) **Functions occurring in “relay”**, with also destruction in late January or beginning of February, corresponding to 5 to 6 months after sowing. The objective is to produce an early and strong catch crop effect thanks to an early growing non-legume species which is very frost sensitive and thus will be naturally killed by the first weak frost. The strategy is to associate a legume species with a slow growth which is not frost sensitive and can maintain a green cover during winter producing a high green manure effect. We evaluated four mixtures: 13) foxtail millet\_crimson clover, 14) foxtail millet\_purple vetch, 15) foxtail millet\_common vetch and 16) forage sorghum\_crimson clover.

However, it cannot be excluded that some mixtures tested for one case could be also adapted to other cases of fallow period management than those for which they were initially designed. This is the reason why we decided to evaluate all the 16 mixtures for all the four fallow period cases in this experiment which then allow giving more information for a future improving. Another key point is that the mixtures choice was also made in order to avoid strong allelopathic effect of crucifers on legume species; this explains why we limited the numbers of mixtures including crucifers. Thus, we have chosen the turnip rape to associate to

the legume which seemed to be the least legume species sensitive to this effect (Chapitre 2). We also limited the number of different species used in mixtures in order to be compatible with available experimental and human resources.

Finally, sixteen mixtures were tested. For comparing the performances of mixtures, we tested the pure species in SC, such as eight corresponding non-legume species and six legume species:

- two C<sub>4</sub> Poaceae: foxtail millet (*Setaria italica*) and forage sorghum (*Sorghum bicolor var. sudanense*),
- two C<sub>3</sub> Poaceae: bristle oat (*Avena strigosa*) and Italian ryegrass (*Lolium multiflorum*),
- three Brassicaceae: turnip rape (*Brassica rapa*), white mustard (*Sinapis alba*) and Abyssinian cabbage (*Brassica carinata*),
- one Hydrophyllaceae: phacelia (*Phacelia tanacetifolia*),
- six Fabaceae: faba bean (*Vicia faba*), Egyptian clover (*Trifolium alexandrinum*), crimson clover (*Trifolium incarnatum*), purple vetch (*Vicia benghalensis*), common vetch (*Vicia sativa*) and forage pea (*Pisum sativum*).

Each species in mixture was sown as the half density of its corresponding SC which corresponds to a substitutive mixture (Annexe A1). The sowing densities of SC were fixed by experts in order to obtain a homogenous cover. The seeds of both species were mixed on the row and the distance between two rows was 15 cm. No fertiliser was added during the experiment. Two irrigations were performed during the two first weeks to ensure a good and homogeneous plant emergence. Bare soil and cover crop plots were weeded by hand regularly.

## **2.2 Site description and weather conditions**

The experiment was carried out in the experimental field of INRA in Auzeville, southwest of France (47°52' N, 2°46' W) from 22 August 2013 to 7 February 2014. During the CC growing season the rainfall was 389 mm and the daily average temperature was 12.3°C, November was particularly rainy: 126 mm of rainfalls with a daily average temperature of 8.4°C (Figure 34). The first frost occurred on the 27<sup>th</sup> of November until -5.6°C. The experiment was carried out on a loamy clay soil. The preceding crop was durum wheat with a residual SMN availability on 120 cm depth of 44 kg ha<sup>-1</sup>.

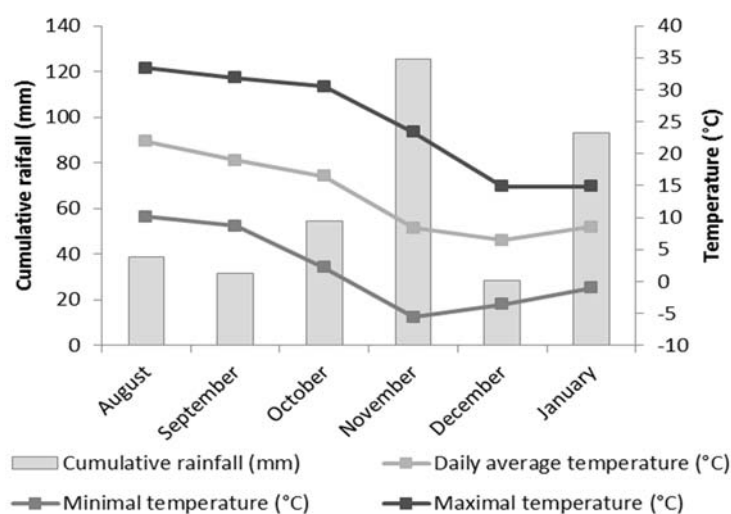


Figure 34 Monthly meteorological conditions during the cover crop growing period of the experiment.

### 2.3 Measurements

Three plant samplings were carried out corresponding to different dates of CC destruction. The first one occurred on 22<sup>nd</sup> October to evaluate the CC performances corresponding to a short growing period (two months of growth – minimal fallow period length in the French legislation) before a winter crop. The second date was on the 2<sup>nd</sup> of December, illustrating a medium growing period with a CC destruction before winter. The third date was on the 30<sup>th</sup> of January, corresponding to a long growing period and a long fallow period in which winter cover is attempted to be maintained.

At each sampling date, the shoots of all treatments and replicates were harvested on a 1m<sup>2</sup> area (1m by 1m). For mixtures, the biomass of each species was separated in two samples of each species which stayed separated for the next analyses. The samples were dried at 80°C during 48h and weighted. Then they were grinded and total-N and C concentrations were analysed by elemental analyses based on the Dumas method (Elementar MicroVario Cube, Germany). For the 30<sup>th</sup> of January, the faba bean, foxtail millet, forage sorghum, Egyptian clover and the purple vetch were totally senescent and forage pea was largely senescent. The biomass staying in the shoot part was harvested but it was not possible to sample leaves fallen on the soil and thus the C and N contents must be considered carefully in the data analysis.

Soil samples were taken from each treatment and replicate, including bare soil, during the week following the plant harvest. They were sampled on 0 to 120cm depth with a hydraulic coring and separated in four layers of 30 cm depth. Six cores were taken to constitute one soil sample in order to take into account soil variability. The samples were then analysed in

laboratory to determine water and mineral-N contents of each 30cm soil layer. Soil mineral content was evaluated after KCl extraction and analysed by colorimetric reactions in a continuous flow auto-analyser (Skylar 51000, Skalar analytic, Erkelenz, Germany). However, because of limited technical resources, the soil samplings of December were performed on 10 mixtures instead of the 25; on white mustard and Italian ryegrass in SC to illustrate two contrasted species and on all legume species in SC.

In addition to these samplings, several measurements were performed in order to analyse the growth dynamic. The cover height of each species in mixture and SC was measured manually regularly (weekly at the beginning then bi-monthly or monthly at the end) from emergence to 19<sup>th</sup> of November. Moreover, the fraction of the photo-synthetically active radiation (PAR corresponding to 400-700 nm wavelengths) absorbed by the cover crop was evaluated by radiative balance using with sensors PAR-80 (Decagon Devices, Pullman, WA) and measurements every ten minutes during the experiment. For the detailed methodology and calculation of the PAR fraction absorbed by cover, see Bedoussac and Justes (2010). Finally, root depth dynamic was measured on SC by introducing transparent Plexiglas tubes inclined at 45° in the soil that allowed measuring the root depth growth until approximately 100 to 120 cm according to soil heterogeneity. The evaluation of the root growth dynamics was done by images capture acquired by a circular scanner (CI-600 Digital Root Imager, CID Bioscience) regularly introduced in the tubes (weekly of bi-monthly).

## **2.4 Analyses and simulations**

The interspecific interactions occurring in bispecific mixtures were evaluated with a commonly indicator used in intercropping studies: the land equivalent ratio or LER (Bedoussac and Justes, 2011; Malézieux et al., 2009; Vandermeer, 1989). It is defined as the amount of land area that would require to grow SC to achieve the same level of productivity as was attained by a mixture (Willey, 1979). This indicator allows evaluating the biomass production of a mixture relatively to the biomass production of the corresponding SC. For the detailed calculation and explanation of LER see Chapitre 2.

In order to evaluate the CC mixtures and species performances to achieved ecosystem services related to N management, we used the soil-crop model STICS (Brisson et al., 2008) which has already been parameterised and satisfactory evaluated to simulate SMN mineralization from CC residues (Justes et al., 2009) and to predict nitrate leaching under CC in sole crops (Constantin et al., 2012).

The STICS soil-crop model was used to simulate (Figure 35):

i) The amount of nitrate leached from sowing to the end of drainage period (31<sup>st</sup> of March) under bare soil and species in SC over the all CC growing period. Initialization was done based on soil measurements of moisture (%), amount of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the soil layers of 0-30cm, 30-60cm, 60-90cm and 90-120cm on the sowing date (22<sup>nd</sup> of August 2013).

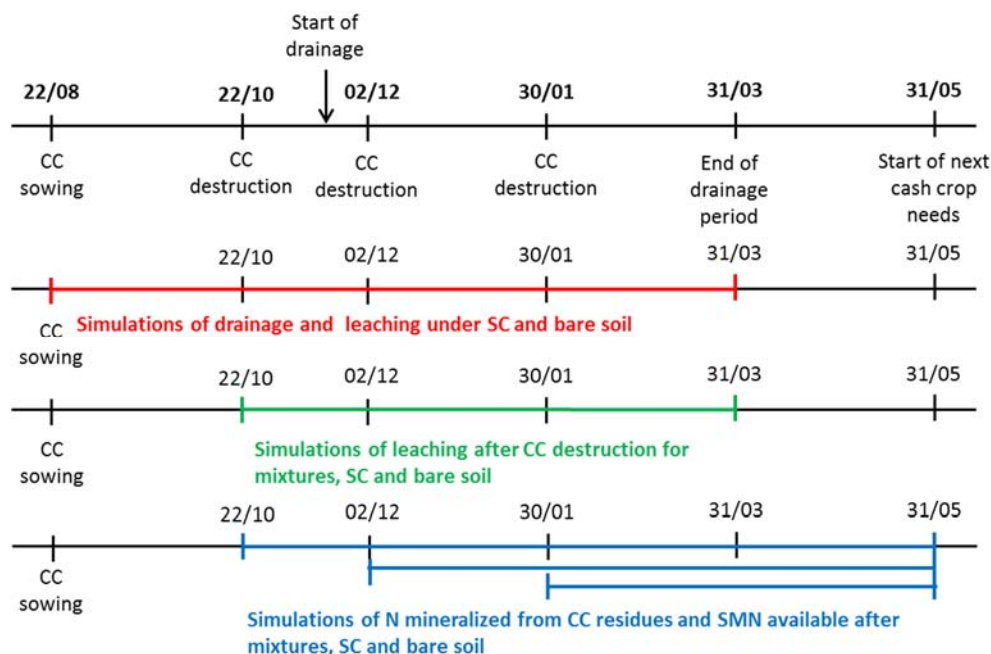


Figure 35 Schematic representations of the different simulations made with the STICS model to evaluate the ecosystem services related to N management produced by cover crops (CC) in sole crops (SC) or bispecific mixtures and bare soil.

ii) For the mixtures and corresponding SC, the model was used to simulate the nitrate leaching only for the scenario after CC incorporation at the first date of plant and soil sampling on the 22<sup>nd</sup> of October by initializing simulations using biomass ( $\text{t ha}^{-1}$ ), C:N ratio and soil water and mineral measurements at this date. We took this decision because simulation of plant functioning for mixtures was not sufficiently good to be sure that the STICS outputs being correct to ensure nitrate leaching under mixture covers during growing period. The end of simulation was on the 31<sup>st</sup> of March after which no leaching was simulated. We did not simulated nitrate leaching after CC destruction dates on 2<sup>nd</sup> of December and on 30<sup>th</sup> of January because nitrate leaching might occurred before the destruction date but we were not able to estimate it.

iii) For mixtures and corresponding SC, the amount of N mineralized from CC residues to the 31<sup>st</sup> of May and the SMN available at the 31<sup>st</sup> of May, corresponding approximately to the beginning of the next main crop N needs. These simulations were performed for the three CC destruction and incorporation dates in order to evaluate the best destruction date allowing the

best green manure effect. The inputs variables were the biomass ( $\text{t ha}^{-1}$ ) and the C:N ratio at the corresponding date, and soil water and mineral measurements.

Data were evaluated with analysis of variance with level of significant  $P < 0.05$  and considering the mixture or the species as a factor to explain each variable. If significant treatment differences were detected we performed *a posteriori* a Newman-Keuls tests. Statistical analyses were performed with STATGRAPHICS Centurion XV software (version 15.2.06).

### 3 Results

#### 3.1 Dynamic of growth and nitrogen acquisition of cover crops

##### 3.1.1 Sole crops behavior

Concerning the SC growth over time, the legume species produced globally more biomass than non-legume species, especially in early growing period, in the conditions of this experiment. Foxtail millet, forage sorghum and faba bean reached their maximal biomass early, around October or late November (Figure 36a-b); in fact, the  $C_4$  Poaceae and the Egyptian clover were totally destroyed by the first frost ( $-5.6^\circ\text{C}$  on 27<sup>th</sup> of November) and the faba bean has been heavily attacked by anthracnose. The purple vetch obtained also its maximal biomass early and did not grow more in late autumn because it has proved sensitive to the frost while forage pea and common vetch obtained their highest biomass in December. White mustard and turnip rape obtained also their maximal biomass around December and then started to be senescent. Phacelia and Abyssinian cabbage could maintain their cover during winter. Finally, bristle oat, Italian ryegrass and crimson clover reached their highest biomass in January and were thus survival to winter in the conditions of South-Western France (Auzeville). The legumes species acquired large amount of N from 89 to 115  $\text{kg ha}^{-1}$  in shoots at the early growing date, except the two clovers which had a slower growth after sowing (Figure 36c). In December they also maintained N acquisition from 103 to 116  $\text{kg ha}^{-1}$ , except faba bean and purple vetch. Finally in February, only the crimson clover acquired a large amount of 115  $\text{kg N ha}^{-1}$ . Common vetch and forage pea which maintained partially their cover accumulating both 71  $\text{kg N ha}^{-1}$  in shoots. Concerning the non-legume species, they all acquired in shoots around 24  $\text{kg ha}^{-1}$  in early growing period, except phacelia which acquired more; sorghum and ryegrass acquired less (Figure 36d). In December, the three Brassicaceae and phacelia acquired the largest amount of N at this date, from 52 to 75  $\text{kg ha}^{-1}$  in shoots and did not accumulated more N during winter growing period. Italian ryegrass

increased its N acquisition over time, even during winter period, but in January it finally accumulated less N in shoots than Abyssinian cabbage, turnip rape and phacelia.

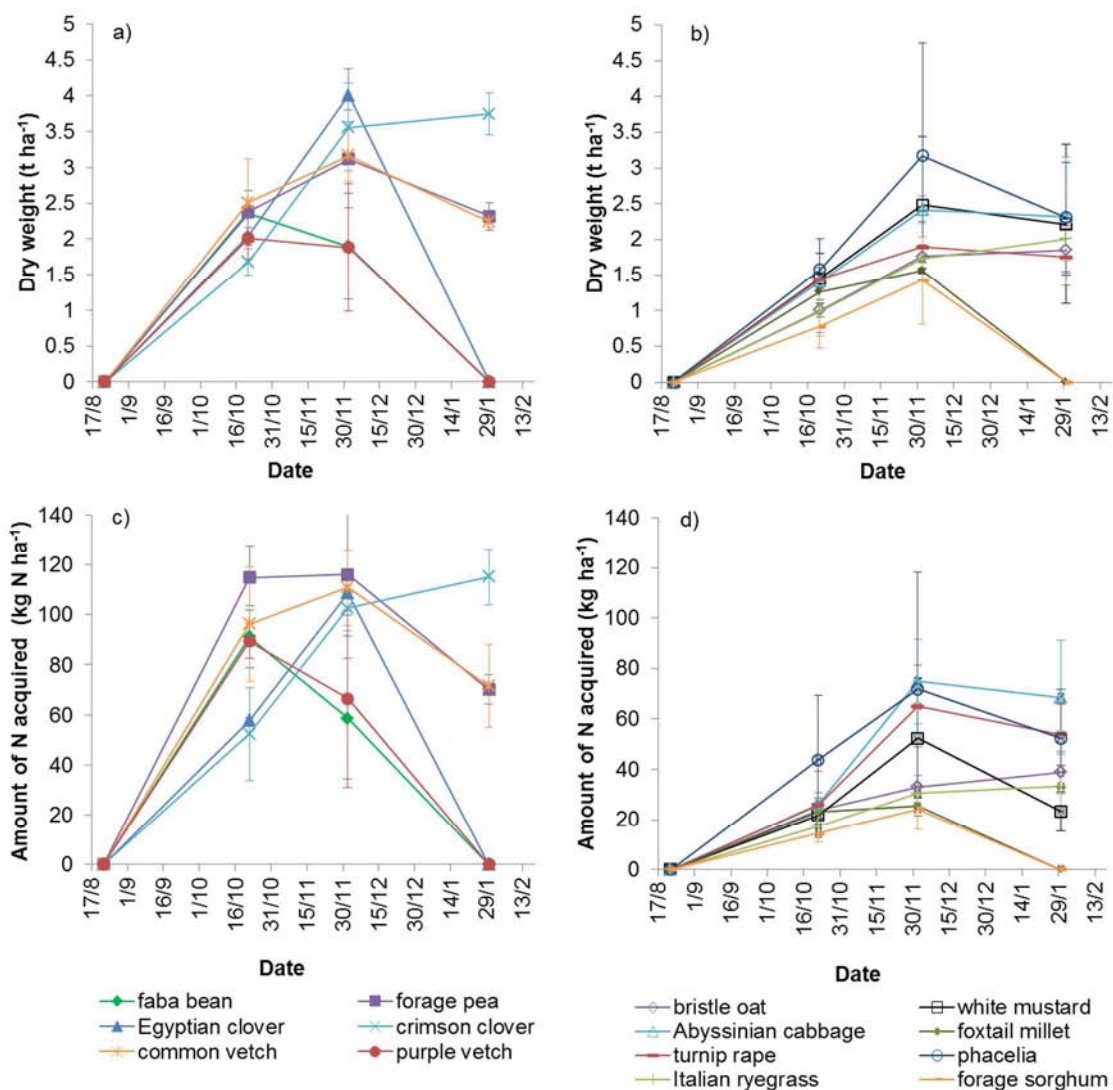


Figure 36 Evolution of shoot dry weight production ( $t\ ha^{-1}$ ) and amount of N acquired in shoot ( $kg\ N\ ha^{-1}$ ) from sowing to different sampling dates of cover crop species in sole crops for a-c) legume species and b-d) non-legume species.

### 3.1.2 Cover crop mixtures

We also evaluated the performances of the species mixtures (or intercrops, IC) according to the three same sampling dates than for SC and the results are detailed in Table 14.

For the early date, on the 22<sup>nd</sup> October, the significant highest total biomasses were obtained by mixtures of turnip rape\_faba bean and white mustard\_purple vetch. The mixtures which acquired the most of N were Abyssinian cabbage\_common vetch, turnip rape\_faba bean. The mixtures with foxtail millet associated with common and purple vetches were also efficient for growth and led to obtain low C:N ratios. The two mixtures with bristle also provided

low C:N ratio (12). At contrary, mixtures composed with crimson clover obtained the lowest total biomass and were the less efficient to acquired N for short fallow period, and they also presented C:N ratios slightly higher than the other mixtures.

**Table 14 Biomass production, N acquisition and C :N ratio measured for the 25 mixtures and sole crops (SC) averaged at the three date of cover crop destruction. Values are means  $\pm$  standard deviation.**

Treatments	Shoot dry weight (t ha <sup>-1</sup> )			Amount of N acquired (kg ha <sup>-1</sup> )			C:N ratio weighted by biomass (kg ha <sup>-1</sup> )		
	22/10	2/12	30/1	22/10	2/12	30/1	22/10	2/12	30/1
white mustard_purple vetch	2.4 $\pm$ 0.6	3.8 $\pm$ 0.7	2.1 $\pm$ 0.7	69 $\pm$ 13	96 $\pm$ 32	40 $\pm$ 7	16 $\pm$ 2	19 $\pm$ 7	nd
Abyssinian cabbage_common vetch	2.3 $\pm$ 0.2	3.5 $\pm$ 0.6	3.1 $\pm$ 0.7	80 $\pm$ 2	126 $\pm$ 29	92 $\pm$ 20	11 $\pm$ 1	11 $\pm$ 1	15 $\pm$ 2
Abyssinian cabbage_purple vetch	1.9 $\pm$ 0.3	3.1 $\pm$ 0.4	2.0 $\pm$ 0.4	57 $\pm$ 15	105 $\pm$ 23	66 $\pm$ 12	15 $\pm$ 2	13 $\pm$ 1	nd
turnip rape_faba bean	2.5 $\pm$ 0.4	2.4 $\pm$ 0.7	2.4 $\pm$ 0.3	77 $\pm$ 13	85 $\pm$ 28	92 $\pm$ 7	14 $\pm$ 1	12 $\pm$ 1	nd
bristle oat_forage pea	1.9 $\pm$ 0.1	2.9 $\pm$ 0.2	2.1 $\pm$ 0.4	69 $\pm$ 6	92 $\pm$ 10	62 $\pm$ 10	12 $\pm$ 1	15 $\pm$ 1	17 $\pm$ 3
bristle oat_purple vetch	1.8 $\pm$ 0.1	2.5 $\pm$ 0.1	2.1 $\pm$ 0.4	63 $\pm$ 6	81 $\pm$ 4	59 $\pm$ 7	12 $\pm$ 1	14 $\pm$ 0	nd
foxtail millet_faba bean	2.0 $\pm$ 0.4	2.1 $\pm$ 0.2	1.4 $\pm$ 0.4	70 $\pm$ 8	58 $\pm$ 8	40 $\pm$ 9	13 $\pm$ 1	16 $\pm$ 1	nd
foxtail millet_crimson clover	1.8 $\pm$ 0.1	3.2 $\pm$ 0.7	3.5 $\pm$ 0.4	49 $\pm$ 3	79 $\pm$ 11	96 $\pm$ 13	15 $\pm$ 1	17 $\pm$ 2	nd
foxtail millet_common vetch	2.0 $\pm$ 0.3	2.9 $\pm$ 1.1	2.7 $\pm$ 0.4	74 $\pm$ 9	98 $\pm$ 35	88 $\pm$ 14	11 $\pm$ 0	12 $\pm$ 0	nd
foxtail millet_purple vetch	2.0 $\pm$ 0.5	2.1 $\pm$ 0.5	1.5 $\pm$ 0.1	72 $\pm$ 11	67 $\pm$ 10	44 $\pm$ 3	12 $\pm$ 1	14 $\pm$ 2	nd
Italian ryegrass_forage pea	1.9 $\pm$ 0.1	3.3 $\pm$ 0.7	2.7 $\pm$ 0.7	66 $\pm$ 7	102 $\pm$ 28	77 $\pm$ 23	13 $\pm$ 0	15 $\pm$ 1	15 $\pm$ 2
Italian ryegrass_crimson clover	1.6 $\pm$ 0.2	2.8 $\pm$ 0.3	3.6 $\pm$ 0.6	47 $\pm$ 7	73 $\pm$ 10	95 $\pm$ 19	16 $\pm$ 3	16 $\pm$ 1	16 $\pm$ 1
forage sorghum_faba bean	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1	1.3 $\pm$ 0.2	72 $\pm$ 4	69 $\pm$ 11	35 $\pm$ 4	16 $\pm$ 1	16 $\pm$ 1	nd
forage sorghum_Egyptian clover	1.8 $\pm$ 0.2	3.1 $\pm$ 0.1	1.9 $\pm$ 0.1	52 $\pm$ 9	82 $\pm$ 6	54 $\pm$ 4	16 $\pm$ 1	18 $\pm$ 1	nd
forage sorghum_crimson clover	1.6 $\pm$ 0.1	2.6 $\pm$ 0.4	3.5 $\pm$ 0.3	44 $\pm$ 6	74 $\pm$ 10	101 $\pm$ 13	16 $\pm$ 3	16 $\pm$ 2	nd
phacelia_crimson clover	1.6 $\pm$ 0.2	3.4 $\pm$ 0.1	3.4 $\pm$ 0.4	43 $\pm$ 3	78 $\pm$ 1	94 $\pm$ 8	15 $\pm$ 2	17 $\pm$ 1	14 $\pm$ 1
Average SC non-legume	1.5 $\pm$ 0.2	2.2 $\pm$ 0.5	2.2 $\pm$ 0.6	31 $\pm$ 7	50 $\pm$ 20	48 $\pm$ 17	23 $\pm$ 4	21 $\pm$ 5	22 $\pm$ 11*
Average SC legume	2.3 $\pm$ 0.3	3.0 $\pm$ 0.9	2.2 $\pm$ 0.9	88 $\pm$ 23	95 $\pm$ 24	67 $\pm$ 28	12 $\pm$ 3	14 $\pm$ 2	13 $\pm$ 1*

\* Measured only on 6 species for non-legume and on 2 species for legume species corresponding to species not totally senescent. 'nd' is not determined because of too important frost damages and decomposition of the mixture.

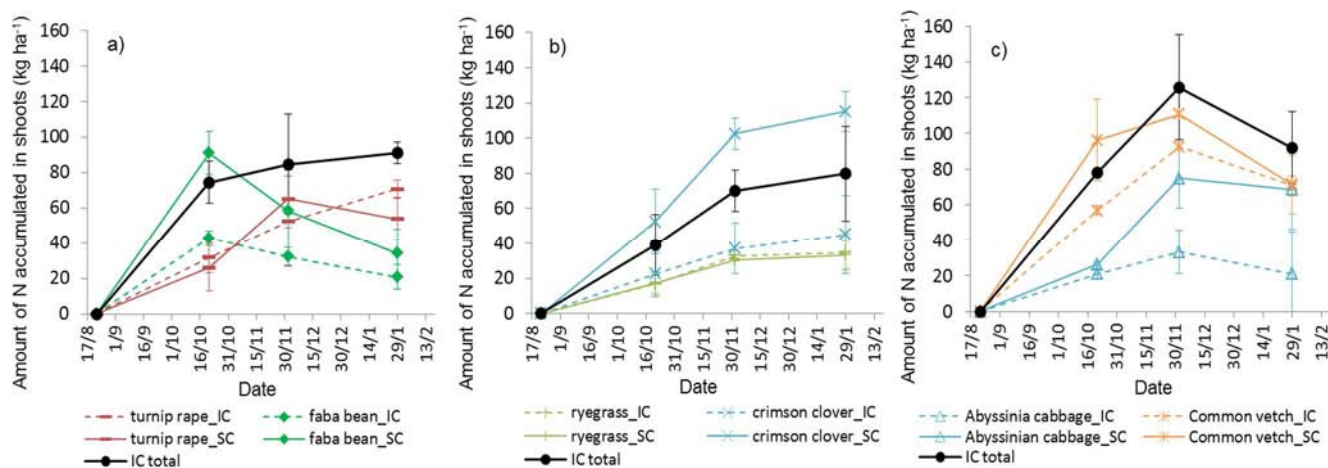
On the 2<sup>nd</sup> of December, the white mustard\_purple vetch mixture still had the significant highest biomass but with the significant highest C:N ratio. The mixtures composed with Abyssinian cabbage and those composed with common vetch also obtained high level of biomass, high amount of N acquired and had the significant lowest C:N ratio (12). Mixtures with forage pea also accumulated high amount of N in shoots but with an intermediate C:N ratio (15). At contrary, the three mixtures with C<sub>4</sub> Poaceae such as foxtail millet\_faba bean, foxtail millet\_purple vetch and forage sorghum\_faba bean presented the significant lowest biomass and thus a low amount of N acquired.

Finally, on the 30<sup>th</sup> of January, all mixtures composed with crimson clover produced the significant highest biomass production, especially for the mixture with Italian ryegrass which reached 3.6 t ha<sup>-1</sup>. Secondly, mixtures with common vetch also allowed maintaining their shoot biomass during winter, especially with Abyssinian cabbage. Thus, the most efficient mixtures for N acquisition were those with crimson clover or with common vetch. For non-



legume species, mixtures with phacelia, turnip rape and Italian ryegrass allowed to acquire the highest amount of N in shoots at the end of January.

The dynamical N acquisition in shoots of each species in mixture was analyzed in comparison to the total mixture and the corresponding SC, in particular for three examples illustrating contrasted behaviors in mixture (Figure 37).



**Figure 37** Evolution of the amount of N acquired (kg ha<sup>-1</sup>) by each species in mixture (IC for intercrop), in sole crop (SC) and by the mixture in total (IC total). Three examples of mixtures: a) turnip rape and faba bean; b) Italian ryegrass and crimson clover; c) Abyssinian cabbage and common vetch.

The turnip rape\_faba bean mixture acquired in mid-October about 77 kg N ha<sup>-1</sup> which was not significantly different to those acquired by faba bean SC but significantly higher than those acquired by turnip rape SC (P=0.002). The two species contributed to approximately the same amount of N acquired in the mixture, but during winter the turnip rape maintained its cover mainly green while faba bean being senescent due to a strong anthracnose attack. This mixture thus seems efficient to acquire a large amount of N early in two months of cover crop growth and became less efficient during the end of autumn and winter but still maintained the N accumulated in shoots significantly higher than SC (Figure 37a).

At the opposite, Italian ryegrass\_crimson clover mixture acquired N more regularly in dynamic over time even during winter (Figure 37b). Even if the total mixture acquired significantly less N in shoots than crimson clover SC, the mixture cover significantly acquired more N than ryegrass SC since the beginning of December. Globally, at each date, the two species contributed similarly to the total mixture.

Finally, the Abyssinian cabbage\_common vetch mixture presented a N acquisition maximized by the mixture in December with a total of 126 kg N ha<sup>-1</sup> in shoots which was a very good performance (Figure 37c). Indeed, the total mixture acquired more N than Abyssinian cabbage SC but also more than common vetch SC even if the differences were not significant

( $P=0.07$ ). In this mixture, the legume acquired the largest amount of N compared to the non-legume species.

## **3.2 Dynamics of the interspecific interactions and species complementarity**

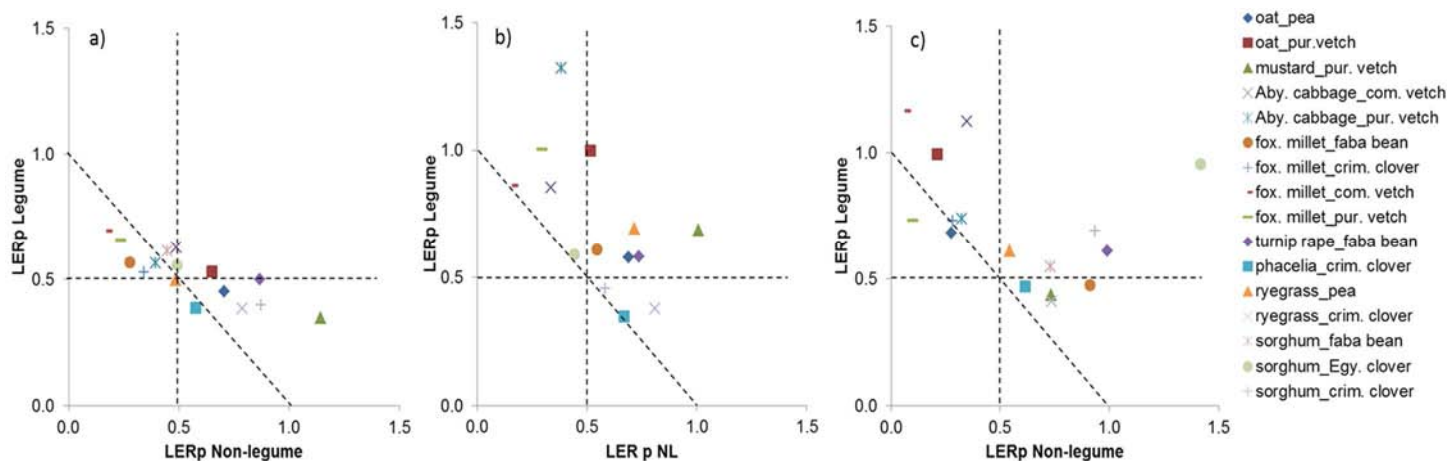
### **3.2.1 Evolution of land equivalent ratio over time**

When total LER is significantly  $>1$  means that the mixture had a better resources acquisition and then valorization for growth than the corresponding SC, due to an interspecific complementary in mixture. In Figure 38, the results showed that, in mid-October, only seven mixtures presented a total LER significantly  $>1$ , such as especially mixtures with Brassicaceae and oat. Three mixtures had a total LER close to 1 and six mixtures got total LER below 1, especially mixtures with foxtail millet (Figure 38). Instead, almost all mixtures reached total LER  $\geq 1$  in early December at the end of January. In December, 13 mixtures reached total LER significantly higher than 1 and two obtained total LER non significantly different than 1. In the end of January, 12 mixtures reached significant total LER  $>1$ , three were close to 1 and one was significantly lower than 1 (foxtail millet\_purple vetch).

On the 22<sup>nd</sup> of October, numerous mixtures presented a LERp of non-legume  $>0.5$  and a LERp of legume  $<0.5$ , meaning that the non-legume species was advantaged in mixture whereas the associated legume was disadvantaged in comparison to the corresponding SC. Only two mixtures maximized both legume and non-legume species with LERp $>0.5$ , however the legume LERp was close to 0.5. The turnip rape and the white mustard reached high LERp of 0.87 and 1.05 respectively, indicating that they were strongly advantaged in mixture. Conversely, foxtail millet strongly suffered in association as indicated by an average of LERp=0.24, probably because of climatic conditions (warm temperatures instead of hot temperatures needed for foxtail millet) which were more favorable to growth for the legume species which then took advantage and induced a strong competition on foxtail millet for light and other abiotic resources capture.

In early December, legume and non-legume species behavior were more variable but the LERp of both species were mostly higher than 0.5, indicating that the complementarity in mixture was increased with the time of growth. The best example is white mustard which was particularly favored (LERp=1.0) in association with purple vetch, leading to a total LER of 1.7. The two vetches were also advantaged in mixture with an average of LERp=0.87 and 0.84 for purple and common vetches, respectively, leading to average of total LER of 1.4 and 1.2.

Finally, it seems that during winter, for species staying relatively green (low to moderate senescence), the common vetch was particularly favored with an average of  $LER_p=1.15$ , leading to total LER of 1.7 especially with Abyssinian cabbage. The crimson clover was also favored during winter with an average of  $LER_p$  of 0.64 that was increased until 0.73 when it was associated with a senescent species such as foxtail millet.



**Figure 38** Partial land equivalent ratio ( $LER_p$ ) of legume and non-legume species associated in mixture calculated from dry weight on Exp. 1 on a) 22<sup>nd</sup> of October, b) 2<sup>nd</sup> of December and c) 30<sup>th</sup> of January. Vertical and horizontal dotted lines indicate limit values for  $LER_p$  (0.5) in the case of neutral interaction between the species. And the diagonal dotted line indicates the limit value of the total LER for which a mixture is equal to sole crops.

### 3.2.2 Light acquisition complementarity

These advantages in mixture can be due to light acquisition complementarity maximized by a cover height increased in mixtures compared to SC that allowed a better shoot architecture complementarity. For example, the turnip rape associated with faba bean reached a cover height higher than SC. However, the faba bean height did not show any significant difference compared to its SC (Figure 39a). This can be explained by the erected architecture of the faba bean which allowed a good light interception even with associated species. The turnip rape in mixture was constrained to increase its height by having more erected leaves to achieve its light interception. However, in the case of the white mustard\_purple vetch mixture, both species maximized their height, especially purple vetch which doubled its cover height in mixture compare to SC by climbing to the stem of the mustard that allowed the legume to absorb sufficient incoming radiation in mixture; this underline the plasticity of these species for adapting their shoot architecture to light competition (Figure 39b).

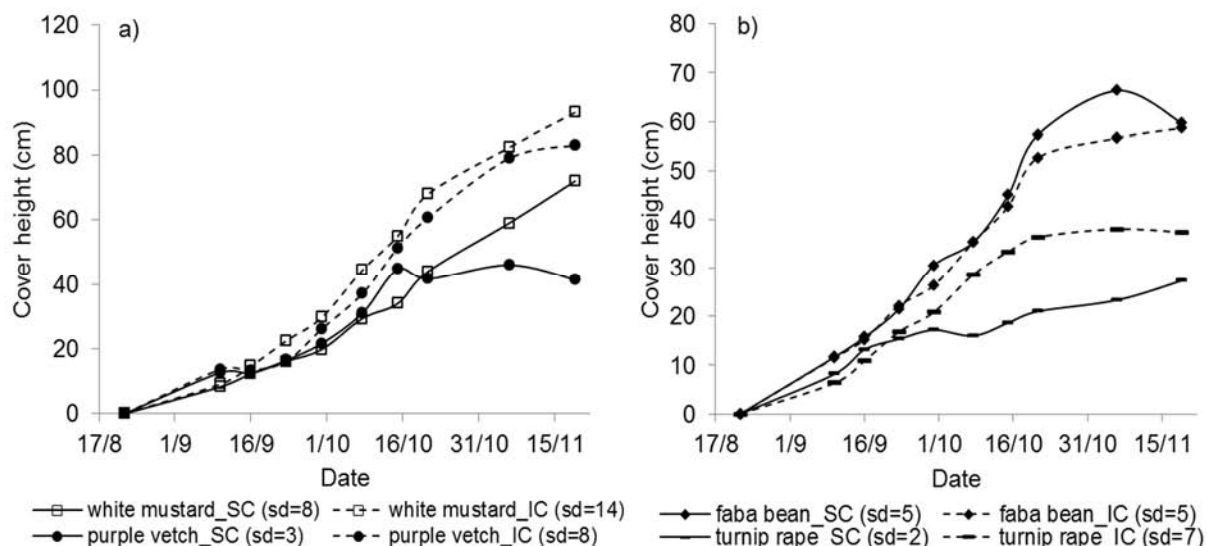


Figure 39 Evolution of cover height over time of each species in mixture (IC) and in sole crop (SC). Two examples of mixtures: a) turnip rape and faba bean; and b) white mustard and purple vetch. “sd” is average standard deviation for the nine measurement dates.

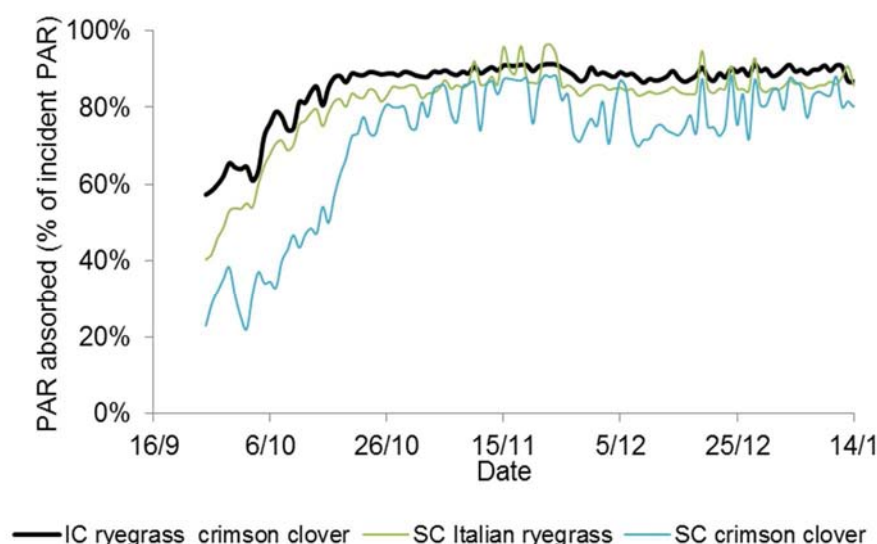
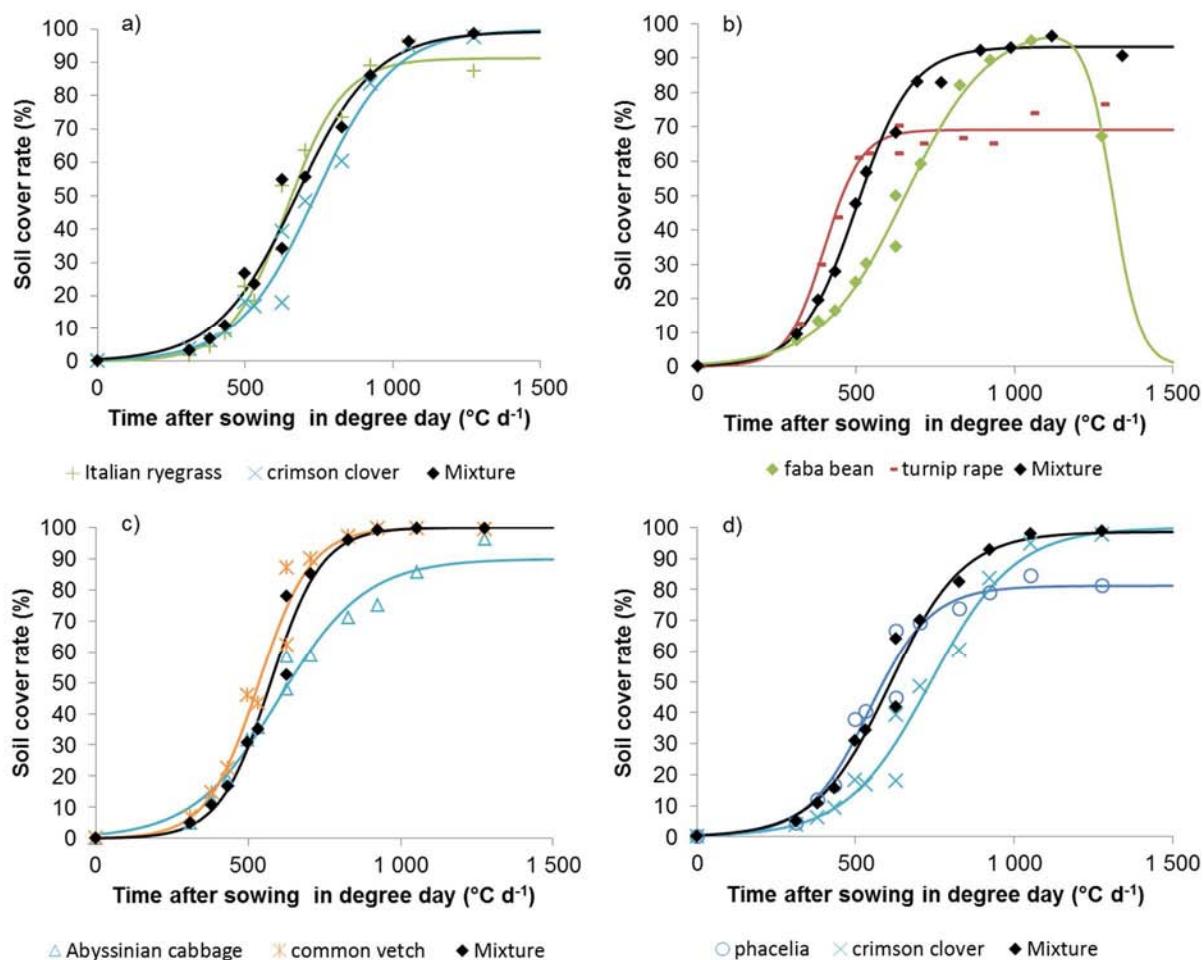


Figure 40 Evolution of photo-synthetically active radiation (PAR) fraction absorbed by the whole mixture Italian ryegrass and crimson clover and by the corresponding species in sole crops.

In order to analyze the complementarity of both species in mixture for the light absorption, dynamical light acquisition was measured in order to calculate the fraction of photosynthetically active radiation (PAR) absorbed by the cover. An example is given here for the Italian ryegrass\_crimson clover mixture for the whole growing period (Figure 40). Our results showed that globally the integrated PAR absorbed by the whole mixture was slightly higher than ryegrass SC and significantly higher than crimson clover SC during all the growing period. The fraction of PAR absorbed by the mixture was first very superior to those

absorbed by the crimson clover SC. During the first weeks, Italian ryegrass probably contributed the most in the mixture since its SC absorbed higher PAR than crimson clover SC showing the ability of the mixture to maximize resource acquisition and allowed a good complementarity for light absorption over time.



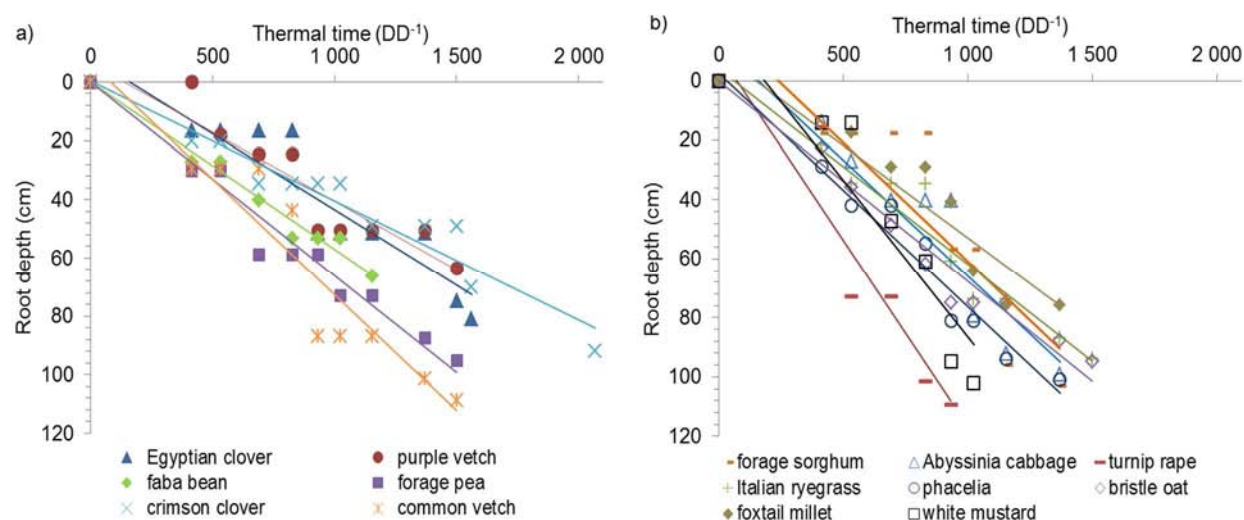
**Figure 41** Dynamic of soil cover rate over time for four mixtures and corresponding sole crops : a)Italian ryegrass\_crimson clover ; b) turnip rape\_faba bean ; c) Abyssinian cabbage\_common vetch ; d) phacelia\_crimson clover. Values are the observed data and curves are regressed non-linearly against thermal time using the following logistic model :  $SCR_t(\%) = SCR_{max} \times \frac{\exp(K1 \times (t - K2))}{1 + \exp(K1 \times (t - K2))}$ . As illustration, the corresponding dates for some thermal times are “0 DD<sup>-1</sup>”=22/08/13; “500 DD<sup>-1</sup>”=17/09/13; “1000 DD<sup>-1</sup>”=16/10/13 and “1500 DD<sup>-1</sup>”=25/11/13.

Cover crop mixtures globally maximized light acquisition thanks to a temporal complementarity as also illustrated by the soil cover rate in Figure 41 for four contrasted mixtures. In agreement with the measurements of PAR absorbed by the Italian ryegrass\_crimson clover mixture (Figure 40), the soil covering of the mixture reached the same level than non-legume SC, and the crimson clover covering was delayed (Figure 41a). The differences between species were higher for other mixtures also presenting complementarity for light resource in mixture. For example, for the turnip rape\_faba bean

mixture, soil cover rate was slightly more rapid for the turnip rape SC than in mixture which was also quite rapid. The faba bean allowed to reached higher maximal soil covering in mixture, and, after faba bean disease attack which destroyed the leaves, the turnip rape maintained the cover rate (Figure 41b), due to a better N status and growth of each cruciferous plant in mixture than in SC (data not shown). For Abyssinian cabbage\_common vetch and phacelia\_crimson clover mixtures (Figure 41c-d), the soil covering was similar to the fastest SC species. In the case of the first mixture, the fastest species was the common vetch, during the whole period, instead for phacelia\_crimson clover mixture, it was firstly similar to the fastest SC (phacelia) and later to the slowest SC which had the best soil covering later period (crimson clover); thus the mixture had the highest integrated soil covering for the whole period.

### 3.3 Dynamic root growth in soil depth for sole crops

For methodological reasons, the root growth in soil depth was measured only in SC, because we were not sure to correctly identify the roots of each species in mixture.



**Figure 42** Evolution of the root depth over time expressed in degree day (DD) for a) legume species and b) non-legume species. The statistics of the regressions are shown in Table 15. The corresponding dates for the thermal time are “0 DD<sup>-1</sup>”=22/08/13; “500 DD<sup>-1</sup>”=17/09/13; “1000 DD<sup>-1</sup>”=16/10/13; “1500 DD<sup>-1</sup>”=25/11/13 and “2000”=04/02/14. The corresponding slope coefficients and R<sup>2</sup> of the linear trend lines are presented in Table 15.

The turnip rape and white mustard were the two species having the fastest root growth in depth in the soil (Figure 42 and Table 15); these two cruciferous reached the depth of 1 meter in approximately 1.5 months after sowing. On the contrary, the legume species had globally slower root growth in depth than non-legume species, particularly crimson clover which only reached half a meter in December. The common vetch was the fastest depth rooting legume species and was even earlier to reach deep rooting than some Poaceace such as bristle oat, Italian ryegrass or forage sorghum.

For SC, a negative linear relationship was found ( $R^2=0.46$ , data not shown) between the root depth reached on the 22<sup>nd</sup> of October (first date of sampling) and the amount of SMN measured at the same date, revealing the relevance of choosing species with early rooting growth to allow an early nitrate catching, especially for an early CC destruction.

**Table 15 Slope coefficient and  $R^2$  of the linear trend line of Figure 42 corresponding to the dynamic of the depth rooting speed and to the quality of adjustment. DD is degree day.**

Species	Slope coefficient (cm DD <sup>-1</sup> )	R <sup>2</sup>
Egyptian clover	0.052	0.87
purple vetch	0.047	0.87
faba bean	0.057	0.97
forage pea	0.066	0.96
crimson clover	0.041	0.93
common vetch	0.079	0.90
phacelia	0.079	0.96
bristle oat	0.068	0.96
white mustard	0.106	0.85
foxtail millet	0.062	0.88
Abyssinian cabbage	0.078	0.88
turnip rape	0.126	0.89
Italian ryegrass	0.066	0.95
forage sorghum	0.080	0.79

### **3.4 Estimation of ecosystem services related to N management**

#### **3.4.1 Mixtures performances to “catch” soil mineral N during growing period and to reduce nitrate leaching**

The simulations indicated that drainage and nitrate leaching at 1.2 m depth would began only mid–November (data not shown) and then after the first plant and soil sampling (22<sup>nd</sup> October) that make the results of nitrate leaching simulation for mixtures relevant.

Firstly, we simulated nitrate leaching and drainage under covers during the whole growing period, only for SC because of no effective model to simulated leaching under bispecific mixtures (Table 16). As expected the highest nitrate leaching was simulated for the bare soil with about 44 kg N ha<sup>-1</sup> leached during the whole fallow period, that is to say the end of the drainage period was simulated in mid-March 2014. The SC non-legume CC species obtained the lowest amount of NO<sub>3</sub><sup>-</sup> leached and NO<sub>3</sub><sup>-</sup> concentration in drainage water and indicated a very good efficiency for the nitrate catch crop service (Table 16). The drainage was reduced of 42 mm in average, from 27 to 53 mm, with CC in comparison to bare soil. Even if nitrate leaching was low under all CC, its level and the NO<sub>3</sub><sup>-</sup> concentration in water drained were

higher under legume species (average of 34 mg NO<sub>3</sub><sup>-</sup> L<sup>-1</sup>) than under non-legume species (average of 9 mg NO<sub>3</sub><sup>-</sup> L<sup>-1</sup>).

**Table 16 Simulated sole crops and bare soil performances related to nitrate leaching, water drainage and nitrate concentration over the simulation period: from cover crop sowing to the end of drainage period (31<sup>st</sup> of March).**

Sole crop species	Cumulated amount of NO <sub>3</sub> <sup>-</sup> leached (kg ha <sup>-1</sup> )	Cumulated amount of water drained (mm)	Concentration of NO <sub>3</sub> <sup>-</sup> in drainage water (mg L <sup>-1</sup> )
white mustard	3	205	7
turnip rape	1	178	2
bristle oat	2	204	5
foxtail millet	7	201	16
forage sorghum	7	187	17
Italian ryegrass	6	181	13
phacelia	1	197	2
purple vetch	14	182	35
common vetch	6	189	15
forage pea	13	180	33
faba bean	12	185	30
Egyptian clover	15	182	36
crimson clover	25	193	57
bare soil	44	232	84

Secondly, we evaluated bispecific mixture to reduce nitrate leaching by STICS simulations after destruction on the 22<sup>nd</sup> of October. Because no drainage occurred before this date, it can be consider by default as the minimum effect of catch crop effect at this date. In fact, by staying alive during a longer time, the mixtures would probably slightly reduce nitrate leaching especially if N uptake would occur after the 22<sup>nd</sup> of October in mixtures. Then the catch crop service provided by mixtures could probably be slightly improved in case of later destruction in early December and end of January. To assess this effect we measured the SMN at the three destruction dates, as the depletion of SMN susceptible to be leached.

The amount of SMN measured at the end of October was significantly lower under mixtures than under bare soil. The SMN was also lower under mixtures than under SC legumes, showing the ability of bispecific mixtures to reduce the SMN susceptible to be leached. However, in average for all mixtures (31 kg SMN ha<sup>-1</sup>), the SMN was slightly higher under mixtures than under non-legume SC which were the most efficient covers to decrease the SMN before the drainage period (Table 17). The turnip rape\_faba bean and the white mustard\_purple vetch mixtures had a low measured SMN close to those of SC non-legumes. These two mixtures allowed significantly decreasing SMN in a few weeks and thus the simulated nitrate leaching was efficiently reduced during the fallow period. At the opposite,



the mixtures composed with Italian ryegrass and foxtail millet had the highest SMN amount in October, constituting the least efficient mixtures to prevent nitrate leaching after early CC destruction.

**Table 17 Performances of the 16 mixtures, the average of sole crops and bare soil for mineral-N measured in soil at the cover crop destruction, and for simulated nitrate leached, simulated N mineralization from cover crop residues and simulated amount of mineral-N in soil for the next crop. 'CC' is cover crop; 'SC' is sole crop.**

Treatments	Amount of mineral N measured in soil at CC destruction (kg ha <sup>-1</sup> )			Amount of NO <sub>3</sub> <sup>-</sup> leached after CC destruction (kg N ha <sup>-1</sup> )	Amount of N mineralized from CC residues (kg N ha <sup>-1</sup> )			Amount of soil NO <sub>3</sub> <sup>-</sup> for next crop (kg N ha <sup>-1</sup> )		
	22/10	2/12	30/1	22/10	22/10	2/12	30/1	22/10	2/12	30/1
white mustard_purple vetch	25±7	20±8	31±14	10±2	21±6	22±24	ns	78±4	76±19	ns
Abyssinian cabbage_common vetch	28±7	23±10	31±21	15±5	36±2	54±15	27±11	90±2	108±16	82±25
Abyssinian cabbage_purple vetch	27±4	nd	23±7	12±1	21±8	40±11	ns	78±6	95±11	ns
turnip rape_faba bean	16±8	18±2	17±6	9±2	29±7	34±11	ns	82±6	87±12	ns
bristle oat_forage pea	27±8	nd	33±12	15±3	29±4	30±6	17±3	84±4	86±6	77±8
bristle oat_purple vetch	35±10	29±17	15±4	16±7	27±5	28±2	ns	84±6	84±8	ns
foxtail millet_faba bean	36±11	nd	31±10	16±3	27±4	16±3	ns	83±1	78±3	ns
foxtail millet_crimson clover	26±9	30±9	16±7	12±4	16±3	20±1	30±7*	72±4	78±3	75±11*
foxtail millet_common vetch	41±9	nd	24±18	22±8	34±4	38±14	33±6*	91±2	99±13	84±11*
foxtail millet_purple vetch	42±11	41±6	23±8	21±4	30±1	22±3	ns	89±6	89±3	ns
ryegrass_forage pea	45±4	14±8	16±9	21±4	27±3	32±11	22±8	85±2	80±9	68±17
ryegrass_crimson clover	32±6	24±6	13±1	16±3	14±6	21±5	29±1	72±5	76±8	67±6
sorghum_faba bean	37±8	nd	26±15	23±4	31±4	21±5	ns	87±5	76±5	ns
sorghum_Egyptian clover	31±11	25±8	14±5	14±4	18±4	20±4	ns	77±7	76±2	ns
sorghum_crimson clover	24±12	nd	18±7	15±7	14±7	21±3	36±6*	71±7	77±3	84±9*
phacelia_crimson clover	26±8	13±3	12±8	11±5	14±1	20±2	29±1	70±2	71±1	71±6
Average SC non-legume	23±4	14±0	14±3	9±2	5±6	11±12	25±9	63±4	61±17	68±7
Average SC legume	42±5	37±14	29±8	23±3	37±16	35±11	39±5	98±12	97±13	83±6
Bare soil	65±31	45±16	31±9	44±7	/	/	/	74±5	60±3	51±6

\* Only the part of the winter survival legume was estimated because the associated non-legume was very frost sensitive, destroyed on 27<sup>th</sup> November and then degraded. 'nd' is not determined because soil mineral N was not measured for all treatment on 2<sup>nd</sup> of December. 'ns' is not simulated because of too important frost damages and decomposition of the mixture.

On the 2<sup>nd</sup> of December, the bispecific mixtures of phacelia\_crimson clover, ryegrass\_forage pea and those with Brassicaceae presented the lowest amounts of SMN. On the contrary, mixtures composed with foxtail millet had the highest SMN amount and thus would lead to have the highest nitrate leaching than all mixtures.

Finally, in the case of a winter destruction after the 30<sup>th</sup> of January, no significant differences were found between mixtures for the SMN amount (P=0.22) even if the mixtures phacelia\_crimson clover and Italian ryegrass\_crimson clover had the lowest amount of measured SMN. It is consistent with the fact that these two mixtures were not senescent

contrarily to the majority of other mixtures and then could uptake N over time and decreased regularly the SMN even during winter.

### **3.4.2 Green manure effect of bispecific cover crop mixtures**

The green manure effect provided by bispecific CC mixtures was estimated as the amount of N released from CC residues incorporated after destruction and soil incorporation simulated for the three dates of plant sampling. The simulations indicated a net N release from residues which allowed almost always compensating the pre-emptive competition for SMN (nitrate reduction due to N uptake) and finally led to higher SMN amount than bare soil. The N availability for next cash crop was better than for the bare soil, indicating the occurrence of a significant green manure effect which was however variable according to the mixtures (Table 17).

The simulations indicated that the best green manure effect was obtained with the date of destruction in December. Indeed, at this date the amount of N mineralized from mixtures residues would be in average 27 kg N ha<sup>-1</sup> leading to a SMN of 91 kg NO<sub>3</sub><sup>-</sup> ha<sup>-1</sup> at the end of May. In the case of the earliest destruction date simulation, an average of 24 kg N ha<sup>-1</sup> were mineralized from CC mixtures residues that led to an amount in average of 81 kg NO<sub>3</sub><sup>-</sup> ha<sup>-1</sup> of SMN available for the next main crop. Finally the simulation indicated an average of 25 kg N ha<sup>-1</sup> mineralized from mixture residues and 73 kg N ha<sup>-1</sup> of SMN with a CC destruction in late January.

For a destruction and incorporation on the 22<sup>nd</sup> of October, the two mixtures composed with common vetch as legume species would allow the significantly highest amount of N released and N-NO<sub>3</sub><sup>-</sup> in soil for the next cash crop, compared to mixtures composed with other legume species (Table 17). Afterwards the mixtures with faba bean, forage pea and purple vetch which would provide a N release and N-NO<sub>3</sub><sup>-</sup> availability significantly higher than mixtures with the two clovers. No significant difference (P=0.06) was found according to the non-legume species associated, even if the mixture with phacelia and Italian ryegrass were simulated as the least efficient green manures with an early autumnal destruction.

For a CC destruction on the 2<sup>nd</sup> of December, the Abyssinian cabbage\_common vetch mixture was significantly (P=0.02) the best mixture for green manure with 54 kg N ha<sup>-1</sup> of estimated mineralized from CC residues and 122 kg N ha<sup>-1</sup> in soil available for next cash crop, which was the only mixture which would provide the same amount of N available than legume SC. The three mixtures composed with Abyssinian cabbage and purple or common vetch and the

foxtail millet\_commun vetch would release the highest amount of mineralized N from residues and SMN for the succeeding cash crop.

For the latest date of destruction on the 30<sup>th</sup> of January, only few mixtures had majority of green cover and were still photo-synthetically active. Among these mixtures, Italian ryegrass\_crimson clover, phacelia\_crimson clover and Abyssinian cabbage\_common vetch would provide the most amount of N mineralized. However, even with the mixtures that maintained growth during winter, the amount of SMN available was lower with this late destruction date compared to an earlier destruction. Moreover, the mixtures composed with a winter survival legume and a very frost sensitive non-legume ( $C_4$  Poaceae), only the harvestable part of the legume was included in the simulation because it was difficult to harvest dead biomass already partly decomposed on the soil. Consequently, for these mixtures producing services in “relay”, it cannot be excluded that the simulated N mineralized and thus the SMN available for succeeding cash crop were underestimated. However these mixtures seem to allow the best SMN availability and would thus be the most efficient to provide green manure effect.

#### **4 Discussion**

The interspecific interactions for intercrops producing grains as cash crops have been reported in literature to evolve over time (Andersen et al., 2004; Bedoussac and Justes, 2010b; Connolly et al., 1990). However, no previous published study reported an analysis of the dynamics of interactions over time for CC species in mixtures. The LER calculated for the three dates of sampling indicated that the interactions between the two species also evolved over time. The balance between complementarities and competitions became positive after 3.5 months of growth corresponding to the second sampling at the end of autumn. Indeed, the non-legume species seemed to be advantaged in mixture in early growing period, while winter resisting legumes species took advantage later especially during winter. This result is consistent with Bedoussac and Justes (2010a) who found similar conclusion for durum wheat\_winter pea mixtures. This is thus suggesting that some bispecific CC mixtures were found efficient to produce early nitrate catching and in the same time produced a better green manure effect for the succeeding cash crop, in comparison to non-legume SC which are well known to produce efficient nitrate catching (Thorup-Kristensen et al., 2003). The dynamical analysis allowed to understand CC mixture functioning and then gave new information for adapting the species choice in mixture. Indeed, the different CC species studied showed various abilities to reach an early growth or to maintain their growth during autumn

explaining why some mixtures were found more efficient as soon as the growth duration continued until the beginning of December. Species such as foxtail millet, forage sorghum, Egyptian clover and purple vetch did not resist to weak and moderate frost (-1 to -5.6°C), and faba bean was strongly attacked by a disease attack. This information indicated that these species should be mainly used for early CC destruction. However, the two C<sub>4</sub> Poaceae (foxtail millet and forage sorghum) did not provide the best efficiency to capture large amount of SMN and thus to reduce efficiently nitrate leaching. This may be explained because of relatively moderate temperatures which occurred in September. Consequently an earlier sowing would be more appropriate for these species in order to increase the thermal time needed to obtain significant growth and thus to increase their performances as nitrate catch crops. However, mixtures with faba bean, forage pea and purple vetch were found efficient to provide green manure effect with an early CC destruction due to their rapid growth and N acquisition. Moreover, even if the common vetch can maintain its growth and N<sub>2</sub> fixation during winter, mixtures containing this species were very efficient to provide green manure effect in the case of the latest CC destruction.

For an early nitrate catching, species with fast rooting development may be the best choice (Thorup-Kristensen, 2001). Our results suggest that crucifers –especially turnip rape and white mustard tested here– are the fastest species to provide deep rooting growth able to catch nitrate in the deeper soil layers. This result is consistent with Thorup-kristensen (2001) who found that the radish, also a crucifer, was the fastest species tested to obtain deep roots. The faster the species develops its roots in depth, the lower is the residual SMN before the drainage starting and then the more efficient the species is for reducing nitrate leaching, as it was also supported by Kristensen and Thorup-Kristensen (2004). Moreover, our results showed that drainage and NO<sub>3</sub><sup>-</sup> concentration in water drained were reduced by CC compare to bare soil, and that NO<sub>3</sub><sup>-</sup> concentration was higher under legume SC than non-legume SC which is consistent with the literature (Justes et al., 2012b; Thorup-Kristensen, 2001). Based on this result, it can be hypothesized that a bispecific legume\_non-legume mixture would allow an intermediate NO<sub>3</sub><sup>-</sup> concentration. The mixtures containing crucifers were thus the most efficient to reduce nitrate leaching for a short fallow period. Indeed, the turnip rape\_faba bean and the white mustard\_purple vetch may produce both catch crop and green manure effects in a short fallow period or early CC destruction (Table 18). These mixtures had a good resource complementarity between both species, especially for the resource-light access. In the case of the white mustard\_purple vetch mixture, the vetch was able to climb thanks to its

tendrils on the erected stem of the mustard, leading to an efficient “tutor effect” (Mariotti et al., 2006).

**Table 18 Summary of cover crop mixtures performances to reach the level of catch crop effect of the average of non-legume sole crops corresponding to the reference of 100% (calculated based on simulated amount of NO<sub>3</sub><sup>-</sup> leached after 22/10 cover crop destruction, and based on the amount of soil mineral N measured at the destruction date of 2/12 and 30/1) and the level of green manure effect of the average of legume sole crops corresponding to the reference of 100% (calculated based on simulated soil mineral N available for next main crop). Bold values are the most efficient mixtures corresponding to ≥70% of non-legume sole crops level achievement for catch crop effect and ≥80% of legume sole crops level achievement for green manure effect.**

Treatments	Catch crop effect			Green manure effect		
	22/10	2/12	30/1	22/10	2/12	30/1
white mustard_purple vetch	<b>90%</b>	<b>71%</b>	45%	<b>80%</b>	78%	ns
Abyssinian cabbage_common vetch	57%	62%	45%	<b>92%</b>	<b>111%</b>	<b>98%</b>
Abyssinian cabbage_purple vetch	<b>71%</b>	nd	61%	<b>80%</b>	<b>98%</b>	ns
turnip rape_faba bean	<b>95%</b>	<b>79%</b>	<b>82%</b>	<b>84%</b>	<b>90%</b>	ns
bristle oat_forage pea	59%	nd	42%	<b>86%</b>	<b>89%</b>	<b>86%</b>
bristle oat_purple vetch	53%	48%	<b>91%</b>	<b>86%</b>	<b>87%</b>	ns
foxtail millet_faba bean	55%	nd	45%	<b>85%</b>	<b>80%</b>	ns
foxtail millet_crimson clover	<b>70%</b>	46%	<b>89%</b>	74%	<b>80%</b>	<b>90%*</b>
foxtail millet_common vetch	39%	nd	58%	<b>93%</b>	<b>102%</b>	<b>100%*</b>
foxtail millet_purple vetch	41%	34%	61%	<b>91%</b>	<b>92%</b>	ns
Italian ryegrass_forage pea	42%	<b>103%</b>	<b>90%</b>	<b>87%</b>	<b>82%</b>	81%
Italian ryegrass_crimson clover	52%	59%	<b>111%</b>	74%	78%	81%
forage sorghum_faba bean	38%	nd	53%	<b>89%</b>	78%	ns
forage sorghum_Egyptian clover	60%	57%	<b>100%</b>	78%	78%	ns
forage sorghum_crimson clover	56%	nd	<b>76%</b>	73%	79%	<b>101%*</b>
phacelia_crimson clover	<b>76%</b>	<b>111%</b>	<b>112%</b>	72%	73%	<b>85%</b>

\* Only the part of the winter survival legume was estimated because the associated non-legume was destroyed by frost the 27<sup>th</sup> November and then was partly decomposed on the 30<sup>th</sup> January. ‘nd’ is not determined because soil mineral N was not measured for all treatment on 2<sup>nd</sup> of December. ‘ns’ is not simulated because of too important frost damages and decomposition of the mixture.

In our situation, the optimal date of destruction for almost all mixtures in order to obtain a simultaneous the two ecosystem services, especially green manuring, was in early December. This allowed to obtain the highest SMN available for the succeeding cash crop, which it produced the best interaction’s complementarity. This complementarity and probably some facilitation explain why some mixtures produced simultaneously the two ecosystem services effectively (Table 18). For the latest destruction date, the mixture phacelia\_crimson clover produced the best catch crop and also a good green manure effect. Indeed, the phacelia was a little bit slower to grow than white mustard or turnip rape and then achieved its maximal growth in December, but this species maintained its cover during winter and then was efficient to produce a nitrate catch crop effect during winter. The two mixtures composed of Abyssinian cabbage and vetch and also mixtures composed with forage pea and common vetch allowed to the mixtures acquiring large amount of N and thus to provide high green N manuring, corresponding to at least 86% of the average of legume SC.

Winter is also a sensitive period of nitrate leaching since strong rainfall and thus drainage can occur due to low actual evapotranspiration (Meisinger et al., 1991). During this period, maintaining a plant cover can be an efficient way to reduce  $\text{NO}_3^-$  losses while increasing the future green N manure effect by increasing N acquisition and also soil erosion protection (Martin, 1999; Van Schöll et al., 1997). Our results suggested that only a few number of species tested were able to reach their maximal growth during winter in our conditions. They were  $\text{C}_3$  Poaceae bristle oat and Italian ryegrass, and crimson clover and thus allow decreasing efficiently the SMN which can potentially be leached. Phacelia, Abyssinian cabbage and common vetch can also maintain a green cover during winter. A destruction in winter and at a date not too late in spring is probably the best solution to avoid negative effect for next cash crop due to low C:N ratio and thus limiting the pre-emptive effect of catch crop caused by an eventual N organization after destruction (Thorup-Kristensen, 1994).

Moreover, the results showed that an interesting type of mixture could provide ecosystem services related to N management in “relay” during long fallow period with an initial efficient nitrate catch crop effect followed by a reinforced green N manure effect due to the ability of some legume species to acquire N during winter thanks to  $\text{N}_2$  fixation. This type of mixture would be effective with a frost sensitive non-legume species able to catch large amount of SMN early in summer and autumn in order to avoid nitrate leaching and which would be naturally be destroyed by the first slight frost ( $-1^\circ\text{C}$ ). For example, these frost sensitive species could be some  $\text{C}_4$  Poaceae as demonstrated in this study, but with an earlier sowing, or other species as niger (*Guizotia abyssinica*) or buckwheat (*Polygonum fagopyrum*) which are also very frost sensitive (Kalinová and Moudrý, 2003; Kandel et al., 2004) that we had confirmation with our experimental observations in the experiments carried out in 2012 on a large CC species screening. The sensitive species to weak frost should be associated with a slow or moderate early growing legume species, in order to avoid a too strong initial competition, and that has the ability to maintain its growth during winter (frost and winter diseases resistant). The best legume species tested in our experimented to perform in those conditions was crimson clover. However, the cover crop survival to winter is not always determined for the species but can also be dependent on the cultivar, as showed for hairy vetch (*Vicia villosa*) by Wilke and Snapp (2008). Then in order to complete our study an analysis of various cultivars could be interesting in order to design the most effective mixtures in “relay”. Moreover, we showed that some mixtures were found complementary also for the light absorption according to the green cover occupied by each species over time. An early light absorption by the  $\text{C}_4$  Poaceae and then a late light absorption by the clover after the

death of the foxtail millet killed by frost could maintain an efficient light absorption by the crimson clover. This result is consistent with Bedoussac and Justes (2010a) who found that in a bispecific legume and non-legume species, the light absorption was more efficient for the whole mixture than the non-legume SC (wheat). They also found that this light complementarity occurred in the case of an earlier growth of the wheat leading to a best light absorption then, in later stages, the light absorption of the mixture was due to better light absorption of the legume (pea).

Finally, the choice of species in mixture may thus be adapted in function of:

- 1) The date of CC destruction which can be managed according to the duration of the fallow period. For example, for a short fallow period, the turnip rape\_faba bean mixture was the best cover in order to simultaneously catch nitrate in soil and produce a positive green N manure effect, even in a short growing period.

- 2) The targeted ecosystem services researched during the fallow period. For example, for a long fallow period, it can be expected to reduce both nitrate leaching and protect soil against erosion during winter (or maintain a favorable soil structure). However, the winter soil covering which could maintain nitrate catching effect longer can lead to a strong pre-emptive competitive for water and N (Thorup-Kristensen and Dresbøll, 2010). In that case, it is needed to increase the green N manure effect and then to have a legume species which could growth and acquire N from biological N<sub>2</sub> fixation during winter in order to increase the amount of N content in the cover without having a high C:N ratio at the date of destruction.

- 3) The pedoclimatic conditions. For example, as reported by Thorup-Kristensen and Dresbøll (2010) in case of sandy soils in Denmark, the CC should be incorporated later than in deep loamy soils because the heavy rainfall occurring generally during winter could lead to low N availability for the main cash crop, due to too early N release from CC residues that a part of nitrate could be leached during the same fallow period.

*In fine*, in our conditions, various mixtures showed a simultaneous production of both ecosystem services related to N management maintained efficient over time because of resources complementarity and probably some facilitation effects, as also discussed in Chapitre 2 for turnip rape\_faba bean mixture. Some mixtures also showed good performances for the three dates of destruction and thus can be well adapted for all fallow period situations that could be useful when the destruction date must be postponed in case of rainy periods limiting the days for cover crop incorporation.

The present study brings some new knowledge on the dynamics interspecific interactions during CC growing period that can help to understand and predict CC performances of the

same mixture for various destruction dates. However, it would be useful to assess these results and hypothesis explaining mixture functioning in other contrasted pedoclimatic conditions, in order to validate these conclusions. Modelling could then be a relevant method for analyzing and simulating other pedoclimatic scenarios. Nevertheless, it is necessary to dispose of a well parameterized and validated model, simulating correctly growth and N acquisition in soil of a wide range of CC species in mixture. This remains a challenge to get soil-crop model parameterized for a large number of CC species. Evidently, concerning the nitrate leaching during fallow period, it would be interesting to be able to simulate nitrate transfer during the CC growing in bispecific mixture. However, even if the STICS model comprises an intercropping version (Brisson et al., 2004; Corre-Hellou et al., 2009), it is not able to correctly simulate the growth of CC in mixtures at the moment.

## **5 Conclusion**

Our results showed that various CC species can be used in bispecific mixture during fallow period in order to simultaneously and efficiently i) “catch” SMN and thus reduce nitrate leaching, and ii) produce effective green N manure effect for the next cash crop. However, these species showed different abilities of early growth, frost sensitivity or to maintain winter growth explaining why the species choice to associate in mixture is not easy to do. Another difficulty is that the compromise of ecosystem services targeted can varied according to the date of destruction. It is then difficult to obtain the best complementarity for resources as nutrients or light without knowing the climatic conditions. However, our results suggested that the best complementarity for the both species mainly occurred at the end of autumn (early December) in our experimental conditions, indicating that a CC destruction at this date may provide the best N availability for the next cash crop. Mixtures containing crucifers would reduce early nitrate leaching and maintaining legume growth during winter may favor green manure effect for the succeeding crop with a late CC destruction (late January). The next step of such work would be to have a generic soil-crop model correctly parameterized for cover crop mixtures in order to simulate dynamic processes of water and nitrogen budgets in soil occurring simultaneously during the fallow period and species interactions for growth and N acquisition.

**Références indiquées à la fin du manuscrit.**



## Discussion générale, conclusions et perspectives

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## **1 Synthèse des principaux résultats de la thèse**

L'objectif de ce travail de thèse était de caractériser un grand nombre d'espèces pouvant être utilisées comme cultures intermédiaires et d'évaluer leurs performances en mélanges bispécifiques pour produire des services écosystémiques liés à la gestion de l'azote. Ce travail de recherche a comme ambition d'apporter des éléments objectifs pouvant aider au choix des espèces à associer en mélange pour produire les services écosystémiques recherchés dans le cas d'un semis des cultures intermédiaires en fin d'été, pour la gestion de l'azote. Pour cela, nous nous sommes appuyés sur une démarche mobilisant différents concepts et outils. Ainsi une originalité de ce travail est d'avoir testé la pertinence du concept de traits fonctionnels, emprunté à l'écologie fonctionnelle, pour pouvoir faire du screening rapide des espèces pour caractériser leur stratégie de capture des ressources. Par ailleurs, nous avons aussi réalisé des expérimentations dans différentes conditions, au champ en conditions réelles et en laboratoire en conditions contrôlées, et nous nous sommes appuyés sur la modélisation pour accroître la généralité de nos résultats expérimentaux.

### **1.1 Caractérisation des espèces utilisées en culture intermédiaire**

*Q1 : Les traits fonctionnels et les méthodes écologiques associées permettent-ils de caractériser et différencier les diverses espèces utilisées en cultures intermédiaires en qualifiant leur stratégie d'acquisition des ressources ?*

L'un des objectifs de cette étude consistait à caractériser et différencier, en mobilisant le concept de traits fonctionnels, un grand nombre d'espèces de culture intermédiaire par rapport à leur aptitude à se développer et à acquérir l'azote au cours de l'interculture automnale. Nos résultats ont montré que les 36 taxons de cultures intermédiaires étudiés, incluant une large gamme de 34 espèces issues de différentes familles botaniques (Fabacées, Poacées, Brassicacées, etc.), présentaient tous une stratégie d'acquisition rapide des ressources selon les références acquises en écologie pour des espèces présentes dans les écosystèmes. Malgré la diversité mesurée en terme de vitesse de croissance et d'acquisition d'azote de ces espèces, les traits fonctionnels et les stratégies écologiques associées n'ont pas permis une différenciation aussi fine que nous l'espérions. Cependant, pour certaines espèces, notamment les crucifères, les méthodes issues de l'écologie les ont clairement identifiées comme très compétitrices, comme nos mesures agronomiques l'indiquent. Bien que la surface et la teneur en matière sèche d'une feuille aient présenté des corrélations significatives avec les fonctions écosystémiques recherchées (vitesse de croissance et d'acquisition d'azote), nous avons dû

constater un manque de précision quant à une caractérisation fine des stratégies de capture des ressources des cultures intermédiaires par les traits foliaires. Ceci pourrait être expliqué par un effet de la domestication de ces espèces. En effet, ces espèces sont issues de programmes de sélection, plus ou moins importants, ce qui a probablement modifié leur syndrome de traits, en favorisant univoquement une stratégie d'acquisition rapide des ressources.

*Q2 : La réponse de la germination des espèces utilisées en culture intermédiaire à une large gamme de températures, notamment élevées, peut-elle discriminer les espèces pour leur aptitude germinative en conditions de semis d'été ?*

Nous avons caractérisé les 36 taxons de cultures intermédiaires analysés par la méthode des traits fonctionnels foliaires pour connaître leur aptitude germinative en réponse aux températures et calculer leurs températures cardinales, en particulier les températures optimales et maximales de germination. En effet, une étude portant sur les cultures intermédiaires semées en fin d'été avait montré que la température dans les premiers centimètres du sol peut être élevée (35°C), notamment dans le Sud de la France, ce qui pouvait réduire la capacité de germination de ces cultures (Constantin et al., Submitted). De plus, les températures cardinales et la température de base de germination ( $T_b$ ), pourraient être utilisées comme une première approximation des autres températures cardinales de développement et de la croissance des plantes (Parent and Tardieu, 2012). La connaissance des valeurs de ces températures cardinales pourrait permettre de paramétrer les modèles, notamment le modèle STICS (Brisson et al., 2008) que nous avons utilisé pour estimer les services écosystémiques. Ainsi, cette étude a fourni ces valeurs qui sont difficiles à extraire de la littérature, pour un large panel d'espèces de cultures intermédiaires. Nos résultats ont montré que la majorité des espèces, dont notamment les Brassicacées, les Graminées  $C_4$  ou encore le fenugrec, était bien adaptée aux conditions de semis d'été avec une température optimale de germination (29°C en moyenne) élevée alors que certaines espèces, telles que la phacélie ou la vesce commune, ont des températures optimales (22°C) mais surtout maximales (30°C) nettement plus faibles, pouvant être limitantes en cas de semis d'été.

L'analyse de ces aptitudes germinatives a permis de proposer des groupes fonctionnels au-delà des familles botaniques sur la base des températures cardinales. Ce regroupement permet une identification des différentes aptitudes germinatives plus aisée et permet ainsi d'adapter le choix des espèces aux conditions climatiques et/ou à leur semis en mélange. En effet, associer en mélange des espèces appartenant à des groupes contrastés pourrait, par exemple, réduire le risque de faible levée au champ ou donner un certain avantage compétitif à certaines espèces

par rapport aux services écosystémiques attendus durant l'interculture. Cependant, il serait pertinent d'associer une caractérisation de la sensibilité de la germination de ces espèces à différents niveaux de stress hydriques, qui peut aussi être un facteur limitant fort en conditions de semis d'été. Des expérimentations sur ce facteur hydrique sont en cours d'achèvement dans le cadre d'une collaboration avec la plateforme de génotypage du GEVES à Angers, mais n'ont pas pu être intégrées à ce manuscrit de thèse. Ces résultats seront disponibles prochainement et permettront de compléter cette caractérisation fonctionnelle de la germination des 36 taxas en vue de l'écriture d'une publication incluant ce facteur.

## **1.2 Les performances et les interactions des espèces de cultures intermédiaires en mélanges bispécifiques pour gérer l'azote dans le cas d'une destruction automnale des couverts**

*Q3.1 : Les mélanges sont-ils aussi performants que les cultures pures pour simultanément piéger l'azote minéral et produire un effet « engrais-vert » quelles que soient les conditions pédoclimatiques ?*

Les expérimentations au champ mises en place en 2012 sur trois sites pédoclimatiques différents (Auzeville, Bignan et Lyon) ont mis en évidence que les mélanges bispécifiques légumineuse/non-légumineuse de cultures intermédiaires permettaient de réduire la quantité d'azote minéral du sol résiduel et la lixiviation de nitrate après une destruction automnale des couverts, ceci par rapport à un sol nu. Néanmoins, comme attendu, l'efficacité de piégeage de nitrate est variable en fonction des mélanges d'espèces. Pour les trois sites, l'effet « piège à nitrate » correspondant à une réduction de la quantité d'azote lixiviée, s'est révélé quasi aussi efficace pour certains mélanges que les cultures monospécifiques de non-légumineuses couramment utilisées pour produire ce service. Il faut noter que les couverts de légumineuses ont certes été moins efficaces que les mélanges ou les cultures monospécifiques de non-légumineuses, mais ont montré un effet significatif et non négligeable sur la réduction de la lixiviation en comparaison au sol nu. Cela confirme qu'il est préférable, vis à la vis de la pollution nitrique, d'avoir un couvert de légumineuse à forte vitesse de croissance en fin d'été que de laisser le sol nu en interculture. L'effet « engrais vert » produit par les mélanges a été intermédiaire entre celui produit par les cultures monospécifiques de légumineuses et de non-légumineuses. Ces résultats sont en accord avec ceux trouvés dans la littérature bien qu'ils aient été obtenus dans d'autres pédoclimats (Hauggaard-Nielsen et al., 2003; Möller et al., 2008; Tosti et al., 2012a, 2012b).

*Q3.2 : Les interactions entre les espèces en mélange favorisent-elles la mutualisation des services écosystémiques visés ?*

La performance des mélanges dépend de la complémentarité entre les espèces notamment vis à vis de la capture des ressources du milieu. De façon simplifiée, la dominance d'une espèce par rapport à l'autre va orienter le niveau de service écosystémique produit. Ainsi, une légumineuse favorisée en mélange aura tendance à favoriser l'effet engrais vert fourni par celui-ci. A l'opposé, si la non-légumineuse est favorisée, le mélange produira un effet « piège à nitrate » plus important. Toutefois, nous avons pu identifier des mélanges bispécifiques permettant d'atteindre des performances avec une excellente capacité de piégeage du nitrate et un effet « engrais vert » proche de celui produit par la moyenne des cultures monospécifiques correspondantes. Cette efficacité du mélange est due à une complémentarité de niche et/ou de la facilitation dans la capture des ressources abiotiques. Les meilleures complémentarités ont été principalement obtenues sur les deux sites où la disponibilité en azote minéral était faible au semis de la culture intermédiaire, ce qui est en concordance avec les conclusions issues de la littérature scientifique sur les cultures associées de vente, comme par exemple en mélange de blé dur et de légumineuses à graines (Bedoussac and Justes, 2010a; Hauggaard-Nielsen et al., 2009; Naudin et al., 2010).

### **1.3 Proposition d'un modèle conceptuel pour estimer les fonctions écosystémiques de couverts de cultures intermédiaires en mélange bispécifique**

*Q4 : Est-il possible de concevoir un modèle générique pour prédire les fonctions écosystémiques à partir de variables simples ?*

*Q4.1 : Les traits fonctionnels sont-ils pertinents pour modéliser les fonctions écosystémiques ?*

*Q4.2 : Les variables « agronomiques » comme les traits de couvert sont-elles nécessaires pour obtenir une prédiction fiable ?*

Nos résultats ont montré que les traits fonctionnels utilisés seuls, bien que robustes et expliquant une partie de la variabilité des fonctions, n'ont pas permis d'obtenir une prédiction étroite et aussi précise que souhaitée. Ainsi, pour gagner en précision dans l'explication de cette variabilité de réponse, nous avons montré que les modèles les plus efficaces devaient combiner des variables du couvert (variables « agronomiques »), généralement plus significatives pour expliquer la variabilité de réponse, et des traits fonctionnels pour gagner en précision de prédiction. A contrario, la différence de traits entre les espèces, s'est révélée être

efficace pour représenter le niveau d'interaction pouvant s'exercer entre les deux espèces, ce qui a permis de prédire de façon satisfaisante la production des fonctions d'une espèce en mélange en fonction de l'espèce à laquelle elle était associée.

*Q4.3 : Quelle est la généralité du modèle conceptuel et son domaine de validité, notamment pour une large gamme d'espèces cultivées en mélange bispécifique ?*

Dans le Chapitre 3, nous proposons un modèle conceptuel implémenté en modèle statistique de type GLM ; ce type de modèle a été utilisé pour chacune des trois fonctions indicatrices de services écosystémiques de la gestion de l'azote (production de biomasse, acquisition d'azote et C/N). Chaque modèle a été appliqué pour estimer le niveau de ces fonctions produit par les espèces en mélanges dans le cas d'une destruction automnale. Les modèles GLM proposés ont été construits à partir de trois types de variables : 1) le niveau potentiel de ces indicateurs par l'espèce considérée en culture pure, 2) la différence de traits fonctionnels foliaires entre l'espèce considérée et l'espèce associée et 3) les facteurs du milieu : disponibilité en eau et en azote. Bien que nous ayons démontré que le modèle numérique pouvait permettre de prédire le comportement d'autres mélanges avec des espèces non utilisées dans la calibration, ce travail méritera une validation plus large afin de connaître le niveau de généralité du modèle conceptuel.

Par ailleurs, les modèles GLM ont été construits à partir de variables que l'on peut obtenir pour une large gamme d'espèces, mais à condition de réaliser des expérimentations en conditions non-limitantes en eau et en azote. Il faut aussi être en mesure de caractériser a priori les niveaux de disponibilité en eau et en azote du site. La validation de ces modèles pour prédire les fonctions produites par d'autres espèces en mélanges (mélanges non utilisés pour le paramétrage) a mis en évidence un potentiel de généralité, notamment pour la prédiction de la production de biomasse et du C/N. En revanche, une plus large validation est nécessaire pour tester plus largement les modèles notamment sur d'autres sites pédoclimatiques. Enfin, il restera aussi à l'adapter à d'autres durées d'interculture.

#### **1.4 Evaluation des performances des mélanges bispécifiques de cultures intermédiaires en fonction du mode de gestion de l'interculture**

*Q5 : Comment évoluent la production des services de gestion de l'azote et les interactions entre les deux espèces en fonction de la date de destruction des couverts ?*

Nous nous sommes intéressés à l'évolution des performances des mélanges de cultures intermédiaires en fonction du temps et de la date de destruction des couverts. Pour cela, nous avons sélectionné, notamment à partir des résultats des essais de 2012, des mélanges susceptibles d'être performants dans le cas de quatre types d'interculture (date de destruction et objectifs) puis nous les avons testés en essai expérimental au champ en 2013. L'évaluation à trois dates de destruction des mélanges a montré des évolutions temporelles des performances permettant d'apporter des éléments de réflexion pour adapter le choix des espèces à associer en fonction de la situation pédoclimatique et le mode de gestion de l'interculture.

*Q5.1 : Quels mélanges sont les mieux adaptés en fonction du mode de gestion de l'interculture ?*

L'analyse de la croissance aérienne, de l'acquisition d'azote, des reliquats de l'azote minéral du sol, couplée avec les simulations réalisées avec le modèle de culture STICS, ont montré que certaines espèces en mélange sont mieux adaptées que d'autres à une situation donnée d'interculture. Ainsi, dans le cas d'une destruction avant l'hiver des couverts, des mélanges de type crucifère/légumineuse (ex : navette/féverole ou moutarde blanche/vesce pourpre) ont montré des capacités de croissance et de piégeage d'azote nitrique du sol très efficaces. A contrario, le mélange d'une non-légumineuse sensible au gel (ex : moha ou sorgho) avec une légumineuse se développant en hiver (ex : trèfle incarnat) permet de produire des services écosystémiques en relais dans le cas d'une interculture longue et d'une destruction en sortie d'hiver. Certaines caractéristiques des espèces sont donc critiques (capacité de germination à haute température, sensibilité au gel, vitesse d'enracinement et de croissance aérienne) et doivent donc être prises en compte pour optimiser les mélanges d'espèces selon la situation dans laquelle la culture intermédiaire sera implantée.

*Q5.2 : Les interactions entre les espèces en mélanges permettent-elles une complémentarité pour le partage des ressources dans le temps ?*

Cette adaptation des mélanges en fonction de la gestion de l'interculture est possible grâce à une complémentarité entre les espèces qui évoluent au cours du temps pour le partage des ressources. Nos résultats suggèrent que la meilleure complémentarité pour les deux espèces se produit après 3 à 4 mois de culture dans nos conditions expérimentales. La complémentarité peut notamment s'exprimer pour l'accès à la lumière comme, par exemple, un effet tuteur de



la moutarde blanche pour la vesce pourpre permettant à la vesce d'accéder à la lumière malgré la hauteur de la moutarde.

Bien que ces résultats montrent l'intérêt d'adapter le choix des espèces en mélange en fonction de la date de destruction et du pédoclimat, il serait nécessaire de disposer d'un modèle dynamique, par exemple comme STICS, correctement paramétré et validé pour simuler en dynamique les flux d'eau et d'azote pendant la période de croissance des cultures intermédiaires en mélange. Les résultats des simulations pourraient permettre de réaliser une évaluation *ex ante* des performances des mélanges en fonction des conditions pédoclimatiques et de progresser plus rapidement dans l'optimisation de l'assemblage des espèces. La version actuelle du modèle STICS-intercropping n'a pas donné des résultats suffisamment satisfaisants pour réaliser ce travail de simulation avec les mélanges d'espèces de cultures intermédiaires.

## 2 Discussion générale et conclusions

### 2.1 Quels apports de l'écologie fonctionnelle à la problématique des cultures intermédiaires ?

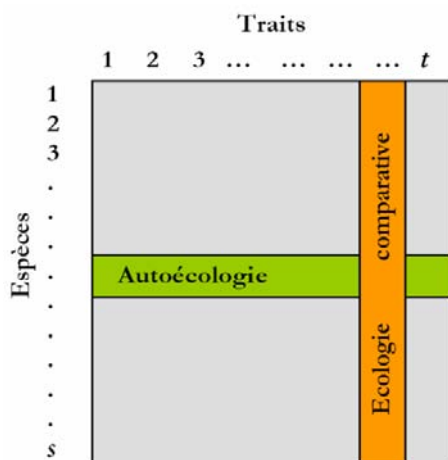


Figure 43 Représentation schématique d'une matrice du nombre d'espèces x le nombre de traits, proposée par Keddy (1992b). L'approche de l'écologie comparative s'oppose à l'autoécologie.

Dans le cadre de cette thèse nous avons mobilisé les théories de l'écologie fonctionnelle à travers le concept de « traits fonctionnels » et des méthodes associées pour caractériser les stratégies des espèces. Cette approche est déjà couramment utilisée en écologie des communautés (e.g. Violle et al., 2007) mais son application en agronomie et notamment aux cultures intermédiaires est originale. Cette approche se base sur des caractéristiques

mesurées au niveau de l'individu, appelés « traits fonctionnels » (Violle et al., 2007) ; elle a permis de classer de nombreuses espèces des écosystèmes en fonction de leur stratégie, notamment d'utilisation des ressources (Grime, 1979; Grime et al., 1997; Wright et al., 2004). L'approche par les traits fonctionnels telle que mobilisée dans ce travail de thèse a montré certains avantages, notamment méthodologique pour faciliter la compréhension générique des fonctions physiologiques lorsqu'on travaille sur une large gamme d'espèces. En effet, les travaux en

agronomie (autoécologie) ont généralement pour but de caractériser finement le fonctionnement d'un nombre limité d'espèces alors que l'écologie fonctionnelle (écologie comparative) cherche à caractériser simplement un grand nombre d'espèces dans un objectif de proposer une théorie générique pour une large gamme d'espèces et d'écosystèmes (Garnier and Navas, 2013; Keddy, 1992) (Figure 43). Il reste à explorer une voie médiane où, avec un nombre bien choisi de traits fonctionnels, il serait possible de caractériser suffisamment précisément un assez grand nombre d'espèces que l'on chercherait à qualifier vis-à-vis de la production de tel ou tel service écosystémique. C'est la démarche que nous avons mise en œuvre dans ce travail de thèse.

Les groupes fonctionnels permettent de classer les espèces par rapport à une aptitude commune partagée par les espèces au sein d'un même groupe (Gitay and Noble, 1997; Lavorel et al., 2007). Nous avons mobilisé cette méthode pour discriminer les aptitudes germinatives des 36 taxons de cultures intermédiaires par rapport à la température. Cela a permis de réduire la diversité des réponses en distinguant cinq groupes contrastés, en partie indépendants de leur famille botanique (Chapitre 1.2). Nous souhaitons également pouvoir regrouper les 36 taxons de cultures intermédiaires sur la base des traits fonctionnels foliaires afin de distinguer la diversité des espèces en termes d'aptitudes à se développer et à acquérir l'azote plus ou moins précocement. Nous avons choisi l'étude de quatre traits foliaires couramment utilisées en écologie fonctionnelle par rapport à leur lien avec les stratégies des plantes, notamment d'utilisation des ressources (Grime et al., 1997; Westoby, 1998; Wright et al., 2004). L'hypothèse formulée était que les espèces partageant le même syndrome de trait, et donc appartenant à un même groupe fonctionnel, présentaient les mêmes stratégies d'utilisation des ressources et ainsi auraient un comportement similaire en mélange. La validation de cette hypothèse pourrait permettre d'extrapoler les performances d'une espèce en mélange à toute autre espèce appartenant au même groupe fonctionnel. Si ce concept était pleinement validé, cela constituerait une avancée significative pour caractériser rapidement et suffisamment précisément le fonctionnement des espèces et ainsi évaluer rapidement leur aptitude à produire les fonctions écosystémiques recherchées. Cela pourra ainsi être utilisé *in fine* pour adapter le choix des espèces à associer sans avoir à réaliser des expérimentations coûteuses et longues pour acquérir les résultats en expérimentation au champ.

Ainsi, nous avons cherché à tester si le regroupement fonctionnel des 36 taxons de cultures intermédiaires réalisé parallèlement sur les traits fonctionnels et les variables indicatrices de la fonction écosystémique visées conduisait à la même caractérisation. Ce double classement hiérarchique, calculé indépendamment, est illustré dans la Figure 44 sur la base des vitesses

de croissance et d'acquisition de l'azote (Figure 44a) et avec les traits fonctionnels foliaires (Figure 44b).

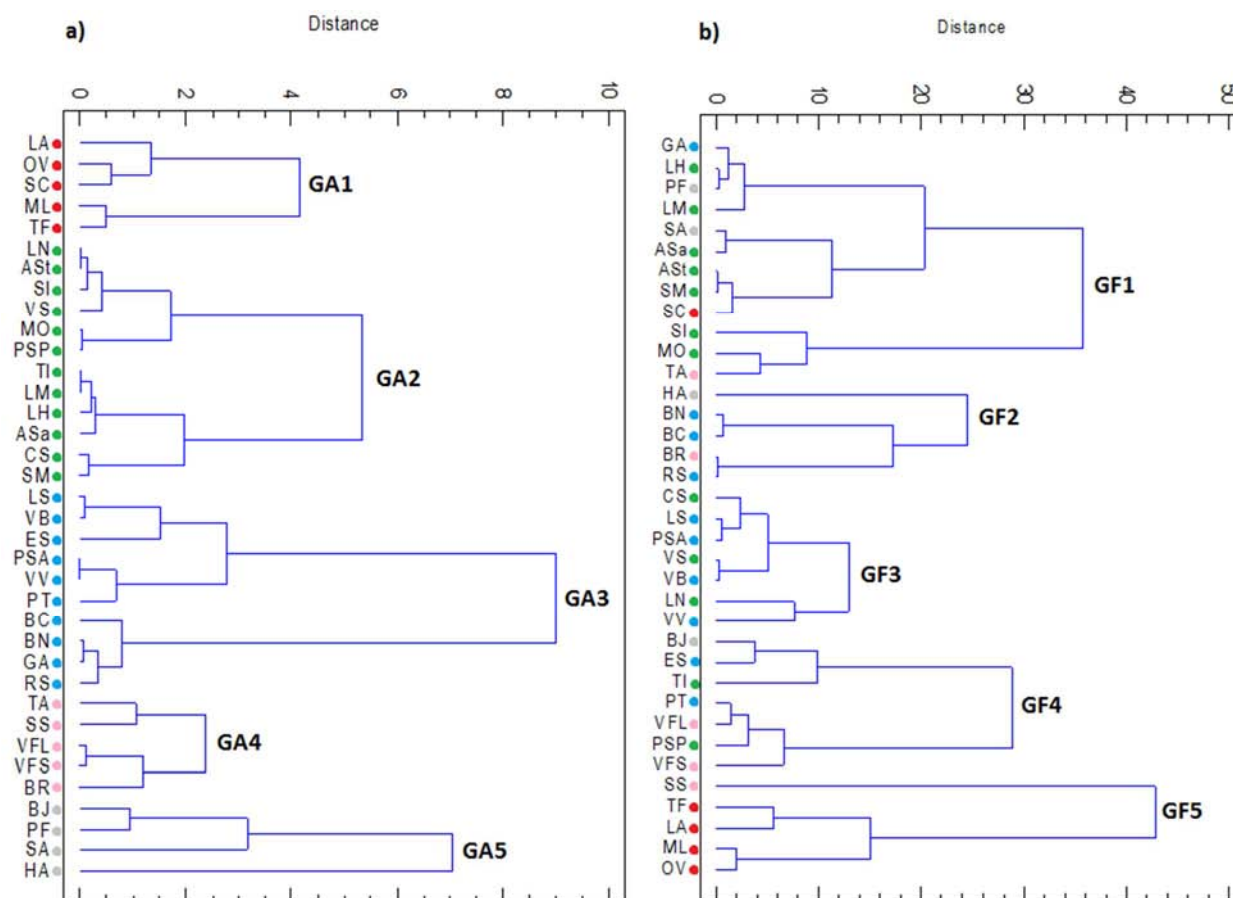


Figure 44 Regroupements des 36 taxons de cultures intermédiaires sur la base a) de groupes agronomiques (GA) constitués à partir du crop growth rate et du crop N acquisition rate, et b) de groupes fonctionnels (GF) constitués à partir de la specific leaf area, leaf dry matter content, leaf area et leaf N content. Les différentes couleurs représentent les espèces constituant les cinq groupes agronomiques.

Tableau 19 Valeurs moyennes des variables et des traits des espèces composant les groupes. CGR signifie « crop growth rate », CNR « crop N acquisition rate », LA « leaf area », LDMC « leaf dry matter content », LNC « leaf N content » et SLA « specific leaf area ».

Groupes agronomiques	CGR (kg DM ha <sup>-1</sup> DD <sup>-1</sup> )	CNR (g N ha <sup>-1</sup> DD <sup>-1</sup> )	Groupes fonctionnels	LA (cm <sup>2</sup> )	LDMC (mg g <sup>-1</sup> )	LNC (mg g <sup>-1</sup> )	SLA (m <sup>2</sup> kg <sup>-1</sup> )
GA1	1.0	33.0	GF1	34	145	48	34
GA2	1.9	75.0	GF2	194	98	41	21
GA3	2.8	125.0	GF3	25	119	59	44
GA4	2.9	92.0	GF4	66	103	53	31
GA5	4.8	125.0	GF5	37	162	35	24

On constate que certaines espèces ayant des aptitudes « agronomiques » similaires sont classées dans le même groupe fonctionnel basé sur les traits foliaires, ce qui est un premier résultat intéressant. C'est, par exemple, le cas de la majorité des espèces du GA1, regroupant les espèces ayant les croissances et les acquisitions d'azote les plus tardives (principalement des légumineuses) (Tableau 19) qui se retrouvent également ensemble dans le GF5 indiquant que ces espèces partagent le même syndrome de traits foliaires (Tableau 20). En revanche, on

note, principalement, des classements avec peu de convergence, comme par exemple, les espèces du GA3, regroupant notamment des légumineuses et des crucifères relativement rapides à se développer et acquérant des quantités importantes d'azote, qui se retrouvent distribuées dans quatre groupes fonctionnels différents. C'est également le

GA \ GF	1	2	3	4	5
1	1	0	0	0	4
2	7	0	3	2	0
3	1	3	4	2	0
4	1	1	0	2	1
5	2	1	0	1	0

**Tableau 20 Répartition du nombre de taxons de cultures intermédiaires dans les différents groupes agronomiques (GA) et groupes fonctionnels (GF)**

cas du GA4 dont les espèces se retrouvent aussi distribuées dans quatre groupes fonctionnels différents. De plus, des incohérences de regroupement apparaissent comme, par

exemple, le sorgho fourrager (SS), espèce au développement très rapide, qui se retrouve avec le même syndrome de traits que les légumineuses aux développements tardifs et ayant une faible vitesse de croissance. Nous avons vérifié que la méthode statistique ou le choix du nombre de groupes fonctionnels déterminé n'était pas la cause de ces résultats.

Nos résultats indiquent que le regroupement sur la base des traits fonctionnels foliaires permet de différencier une partie de la diversité des espèces de cultures intermédiaires mais que les traits foliaires ne sont pas suffisants pour réaliser des regroupements convergents et ainsi constituer des groupes fonctionnels en forte cohérence avec le comportement agronomique des espèces testées. Cela indique que l'information « fonctionnelle » contenue dans l'analyse des traits foliaires est insuffisante et que d'autres traits fonctionnels, comme par exemple ceux décrivant le fonctionnement racinaire ou l'architecture aérienne des plantes pourraient permettre de mieux caractériser le comportement des espèces testées. On peut faire l'hypothèse que la densité de racines par unité de volume de sol (RLD : root length density), la longueur spécifique racinaire (SLR : specific root length) ou encore le diamètre racinaire pourraient apporter des informations supplémentaires utiles notamment pour caractériser des espèces de cultures intermédiaires dont l'exploration racinaire est un élément important pour l'acquisition d'azote (Thorup-Kristensen, 2001). En effet, les travaux de Craine (2006), Eissenstat (2008) et Mommer et al. (2011) ont notamment mis en évidence des relations entre ces traits racinaires et l'acquisition d'azote par les plantes et peuvent également différencier les stratégies des espèces (Fort et al., 2012). Néanmoins ces traits racinaires sont nettement plus difficiles à mesurer en routine que les traits foliaires, notamment en conditions au champ. Par ailleurs, des traits fonctionnels relatifs à la stature et à l'architecture de la plante pourraient aussi être des traits pertinents à étudier, notamment pour la différenciation des stratégies d'accès à la lumière et ainsi des phénomènes de compétition qui peuvent s'opérer dans le cas des mélanges d'espèces. Par exemple, la hauteur d'une plante, considérée par

rapport à celle des plantes voisines, peut être un indicateur pertinent d'un avantage compétitif par rapport à l'accès à la lumière (King, 1990; Westoby et al., 2002). Les résultats du Chapitre 3 appuient cette hypothèse puisque le travail de modélisation a montré que d'autres informations, en plus des traits fonctionnels foliaires, sont nécessaires pour estimer les fonctions écosystémiques. Ainsi, la hauteur du couvert mesurée à la même date que les deux variables de vitesse de croissance et d'acquisition de l'azote est une des variables apportant le plus d'information pour expliquer la variabilité du fonctionnement des espèces de cultures testées. Le compromis entre « piège à nitrate » et « effet engrais vert » doit être produit en peu de temps de croissance et avec des contraintes pédoclimatiques variables, ce qui rend exigeant l'objectif à atteindre et explique en partie que les traits fonctionnels foliaires ne soient pas, à eux seuls, suffisants pour caractériser les espèces de cultures intermédiaires.

## **2.2 Vers un choix raisonné des mélanges bispécifiques de cultures intermédiaires ?**

Un des objectifs de l'implantation de culture intermédiaires en interculture est de piéger l'azote minéral du sol résiduel après la récolte de la culture de vente précédente et celui issu de la minéralisation « naturelle » de l'azote organique du sol, ceci afin d'éviter que l'azote nitrique ne soit lixivié lors de la période automnale et hivernale de drainage. Le semis de culture intermédiaire est désormais obligatoire pour réduire la pollution des eaux par les ions nitrate dans les zones vulnérables en interculture longue et avant une culture de printemps. Cela représente donc une grande partie des surfaces agricoles des grandes cultures françaises. L'optimisation des cultures intermédiaires est donc aujourd'hui un enjeu fort avec la mise en œuvre du 5<sup>ème</sup> programme d'action de la « Directive Nitrates ». Au-delà de l'aspect strictement réglementaire, les cultures intermédiaires peuvent produire d'autres services écosystémiques, notamment celui d'« engrais vert », en plus de l'effet de couverture du sol ou de stockage de carbone dans le sol. L'effet « engrais vert » permet de restituer de l'azote minéral pour la culture de vente suivante après destruction des cultures intermédiaires. Cet effet « engrais vert », bien que souvent modeste, permet de favoriser une meilleure nutrition azotée ou de réduire les doses d'intrants azotés qui seront apportés normalement sur la culture (Justes et al., 2012a; Thorup-Kristensen et al., 2003). Le point central de ce travail de thèse était d'analyser les performances des mélanges bispécifiques, associant une légumineuse à une non-légumineuse, pour favoriser une production simultanée de ces deux services et de limiter l'effet potentiellement négatif de la culture intermédiaire dû à une compétition pré-emptive de l'azote pour la culture principale suivante (Thorup-Kristensen, 1993). L'analyse des performances et des interactions, se produisant en dynamique entre les espèces en

mélange, a pour objectif finalisé d'aider au choix des espèces à associer en fonction de la situation considérée (sol, climat, durée d'interculture, type de succession de cultures principales). Pour cela, nous avons pu analyser les résultats obtenus dans différents sites expérimentaux. Nos résultats ont montré que certains mélanges bispécifiques (ex. navette fourragère\_lentille noirâtre ou moha\_vesce pourpre) peuvent avoir une efficacité pour réduire la lixiviation proche de celui des cultures pures de non-légumineuses, mais qu'ils ne permettaient pas toujours de produire simultanément un « effet engrais » vert aussi élevé que celui produit par les légumineuses, et vice-versa. Ainsi, dans le cas d'un semis en août et d'une destruction automnale des couverts sur trois sites pédoclimatiques français contrastés (Sud-Ouest: Auzeville ; Centre-Est: Lyon ; Nord-Ouest: Bignan), aucun mélange n'a permis d'obtenir simultanément le niveau maximum de services « piégeage de nitrate » et « engrais vert » produits par les espèces monospécifiques de non-légumineuses (effet piège à nitrate) et de légumineuses (effet engrais vert). Nous avons toutefois observé des mélanges où des phénomènes de complémentarité de niche et de facilitation se sont produits et ont ainsi permis d'atteindre des compromis entre les deux services intéressants d'au moins 80% d'atteinte de chacun des deux services visés (ex. raygrass italien\_vesce pourpre ou phacélie\_féverole). Par ailleurs, et comme attendu, des comportements de certaines espèces en mélange ont montré des résultats différents en fonction des sites pédoclimatiques. Ainsi, pour un même mélange des inversions de compétition entre la légumineuse et la non-légumineuse ont été observées entre les sites, ce qui induit des performances différentes dans le compromis en service de piégeage du nitrate et d'effet engrais vert. Cela indique que le choix des espèces et des mélanges doit se raisonner en fonction des conditions pédoclimatiques, conditions qu'il est nécessaire de caractériser a priori pour pouvoir raisonner le choix des deux espèces à associer. Au cours de notre travail, il n'a pas été possible de proposer de règle simple et générique d'assemblage des espèces car les interactions entre espèces se produisent différemment selon les niveaux de disponibilité des facteurs abiotiques et des conditions climatiques. Toutefois, la modélisation basée sur les modèles GLM a permis de prédire le comportement de deux espèces en mélange, y compris les cas d'inversion de dominance, mais à condition de caractériser a priori les niveaux de disponibilité en eau et en azote minéral du sol. Au final, notre travail confirme que le choix des mélanges et des espèces à associer doit se raisonner en fonction de plusieurs facteurs pédoclimatiques : i) du type de sol qui peut être plus ou moins drainant, et ii) du climat en fonction de la hauteur des précipitations favorisant le drainage mais aussi des températures pouvant par exemple, limiter le développement des espèces ou détruire celles qui sont très sensibles au gel. Enfin, nos résultats montrent que le choix des

mélanges doit également être raisonné en fonction du mode de gestion de l'interculture et notamment de la date de destruction des couverts. D'un point de vue opérationnel, on peut retenir les résultats clés suivants pour constituer les mélanges bispécifiques :

- i) Dans le cas d'une destruction précoce, intervenant mi à fin octobre dans le cas d'une interculture courte suivi d'un semis d'une culture d'hiver ou pour des raisons de travail du sol profond (sols argileux), les deux espèces doivent avoir un développement très rapide pour éviter une forte dominance d'une espèce sur l'autre ; c'est par exemple le cas du mélange navette fourragère et féverole ou moutarde blanche et vesce pourpre. Il faut aussi noter que le choix de la crucifère n'est pas anodin compte tenu des possibles effets allélopathiques sur la légumineuse.
- ii) Dans le cas d'une interculture longue avec une destruction des cultures intermédiaires avant l'hiver (au plus tard mi-décembre), il devra être privilégié un mélange composé avec des espèces ayant un développement suffisamment rapide mais sans qu'il ne soit nécessairement proche entre les deux espèces. Dans ce cas l'espèce ayant une croissance plus lente ne devra pas être sensible au gel à faible température afin d'être en capacité de maintenir soit l'un effet « piège à nitrate », soit d'accroître l'effet « engrais vert » par une croissance et fixation d'azote se produisant durant tout l'automne ; c'est par exemple le cas du mélange moutarde éthiopienne et vesce commune.
- iii) Dans le cas d'une interculture longue avec une destruction des cultures intermédiaires en sortie d'hiver, il est souhaitable de produire les deux services de gestion de l'azote tout en maintenant une couverture végétale du sol. Dans ce cas il convient d'associer deux espèces ayant une résistance aux conditions hivernales (peu de sénescence ou bonne résistance au gel) ; c'est le cas par exemple du mélange raygrass et trèfle incarnat. On peut aussi vouloir préférer que ces services se produisent en relais avec une intensité initiale forte de « piègeage des nitrates » au début du cycle de la culture intermédiaire, et un effet prolongé et accru de l'effet « engrais vert » dans un second temps. Il conviendra de semer un mélange associant une non-légumineuse au développement précoce en automne favorisant le piègeage des ions nitrate très précocement et efficacement mais qui sera très sensible au premier gel (dès -1 ou -2°C), ce qui détruira l'espèce naturellement, même dans le Sud-Ouest de la France. Dans ce cas, la légumineuse associée devra être résistante au gel et aux conditions hivernales et aura une capacité de croissance et d'acquisition de l'azote significative en fin d'automne et durant l'hiver, une fois la non-légumineuse détruite. Une destruction naturelle à l'automne permettra à la légumineuse de se développer plus efficacement avec moins de compétition et ainsi produire un effet

engrais vert plus tardif tout en maintenant une protection du sol. Ce comportement a notamment été observé pour le mélange moha (ou sorgho) et trèfle incarnat (Figure 45).



**Figure 45** Exemple de l'évolution du mélange moha/trèfle incarnat permettant des services écosystémiques en «relais» grâce à un développement précoce du moha puis du maintien d'un couvert hivernal grâce au trèfle incarnat résistant au gel. Photographies prises a) le 4 octobre 2012 et b) le 8 janvier 2013.

Par ailleurs, le choix des mélanges est également à adapter en fonction du pédoclimat et de l'itinéraire technique de gestion de l'interculture. Nous avons évalué un panel de différents mélanges proposant tous un compromis plus ou moins équilibré entre l'effet « piège à nitrate » et l'effet « engrais vert ». Dans une situation avec de forts reliquats d'azote minéral après la récolte du précédent, ou par exemple après une culture protéagineuse dont la disponibilité en azote minéral est généralement élevée pendant l'automne, il sera préférable de choisir un mélange favorisant l'effet « piège à nitrate », d'autant plus si le sol est filtrant et/ou le climat hivernal habituellement très pluvieux. A l'opposé, dans une situation à faible disponibilité en azote minéral (faible reliquat récolte et incorporation des résidus de récolte) et dans des conditions de faible drainage, on pourra choisir un mélange favorisant l'effet « engrais vert » afin d'éviter l'effet de compétition pre-emptive pour la culture principale suivante. L'annexe A6 présente l'ensemble des résultats obtenus pour les 25 mélanges testés dans les expérimentations de 2012. Le niveau d'atteinte des services de « piège à nitrate » et « engrais vert » a été calculé pour les mélanges en référence à la moyenne des services des cultures pures correspondantes ; ce calcul a été fait pour les trois sites pédoclimatiques contrastés. Ces résultats indiquent que le comportement du mélange et son efficacité sont clairement fonction du site et du compromis entre les deux services écosystémiques qui est recherché.



### **2.3 Modélisation dynamique des mélanges bispecifics de cultures intermédiaires**

L'évaluation du niveau d'atteinte des services écosystémiques a été en partie réalisée grâce à des simulations réalisées avec le modèle de culture STICS, notamment pour estimer la quantité d'ions nitrate lixiviés, la quantité d'azote minéralisé à partir des résidus de cultures intermédiaires ou encore la quantité d'azote minéral disponible pour la culture suivante de printemps. Il s'agit donc ici d'estimations, et bien que le modèle STICS ait été validé aussi bien en sol nu qu'avec des cultures intermédiaires, il pourrait être intéressant d'effectuer des mesures plus directes de ces services notamment grâce à la mise en place de plaques lysimétriques pour mesurer la quantité d'eau drainée et sa concentration en nitrate (effet « piège à nitrate »). On pourrait également mesurer l'absorption en azote de la culture principale suivante, notamment avec des témoins « 0 azote » (effet « engrais vert »). Cependant, ces mesures expérimentales sont lourdes en temps de travail, en coûts analytiques et en investissements matériels. De plus, nous avons également vu les difficultés des expérimentations au champ où les contraintes biotiques ne sont pas toujours bien maîtrisées : invasions d'adventices, maladies (anthracnose), ravageurs (tenthredes) etc. Il existe aussi des imprécisions dans les mesures et certaines analyses dues à la sénescence des plantes et à la variabilité spatiale de répartition de l'azote minéral dans le sol initialement. Par ailleurs il n'est pas toujours matériellement possible de synchroniser les dates de prélèvements entre différents sites expérimentaux. Une alternative est donc le recours à la modélisation, notamment au moyen de modèles dynamiques correctement paramétrés et validés. Cela permet notamment d'analyser un grand nombre de traitements, sur de nombreux sites pédoclimatiques tout en faisant varier les itinéraires techniques comme c'est le cas pour le modèle de culture STICS (Brisson et al., 2008). Le modèle STICS présente également l'avantage d'avoir de nombreuses variables de sorties pouvant permettre une analyse en dynamique des processus sous différents angles, notamment en cours de végétation puis après la destruction des couverts. La démarche de modélisation permet aussi de simuler une diversité de scénarios ce qui pourrait constituer une base d'étude numérique pour quantifier les effets « piège à nitrate » et « engrais vert » des mélanges bispecifics de cultures intermédiaires. Pour la simulation de minéralisation des résidus de culture intermédiaire après incorporation, le modèle STICS est bien adapté (Justes et al., 2009). Pour la croissance et l'acquisition d'azote, le formalisme du modèle est a priori pertinent (Brisson et al., 2008), mais il reste cependant à l'adapter à une large gamme d'espèces, ce qui a pu être réalisé pour une partie des espèces monospécifiques testées. Le modèle STICS comporte une version pour simuler des associations d'espèces qui prend en compte les interactions pour l'absorption de

la lumière, de l'eau et de l'azote minéral du sol des deux espèces associées en fonction de leur vitesse de développement, de croissance aérienne et racinaire (Brisson et al., 2004; Corre-Hellou et al., 2009). Cependant, il doit être paramétré et validé correctement pour la diversité des espèces de cultures intermédiaires afin de s'assurer de sa validité dans différents pédoclimats, notamment quant à sa capacité à simuler des inversions de dominance en mélanges bispécifiques. Ce travail de paramétrage de STICS-intercropping n'a malheureusement pas pu être réalisé durant la thèse pour les mélanges bispécifiques car des problèmes de fonctionnement informatique (en cas de gel d'une des deux espèces par exemple) ont été identifiés et corrigés trop tardivement. Nous avons toutefois vérifié que le modèle avait le potentiel de simulation de mélange bispécifique avec le mélange raygrass d'Italie et trèfle incarnat ; le travail sera cependant à réaliser pour d'autres espèces de cultures intermédiaires.

### **3 Perspectives de recherche**

Les travaux présentés dans cette thèse ont permis d'apporter des éléments d'analyse sur les performances des cultures intermédiaires en mélanges bispécifiques, en comparaison aux cultures monospécifiques, cependant cela ouvre également de nouvelles questions et perspectives de recherche.

Nous nous sommes intéressés aux mélanges de cultures intermédiaires composés de deux espèces, et cela a permis d'avoir une première évaluation de couverts plurispécifiques. Dans un premier temps, cela permet d'analyser les interactions entre les espèces et facilite le travail de modélisation des cultures associées car ce sont seulement deux espèces qui interagissent dans le peuplement végétal. Cependant, il pourrait être pertinent d'étudier également des mélanges composés de plus de deux espèces, intégrant toujours au moins une légumineuse afin de pouvoir obtenir une mutualisation des services écosystémiques liés à la gestion de l'azote. Associer trois ou quatre espèces, ou plus, pourrait également diminuer les risques liés à une mauvaise implantation, limiter la destruction d'une partie du couvert par les bioagresseurs, ou encore renforcer la production de services écosystémiques supplémentaires (protection du sol, réduction des adventices, favoriser la pollinisation...). Il sera également intéressant d'étudier les phénomènes d'interactions dans ce type de mélange. Smith et al (2014) et Wortman et al (2012) ont notamment évalué des mélanges comprenant jusqu'à huit espèces de cultures intermédiaires, dont des légumineuses, qui permettaient une meilleure valorisation des ressources que les cultures pures ( $LER_{total} > 1$ ). Les semenciers français

proposent déjà, à l'heure actuelle, de nombreux mélanges composés de plusieurs espèces sur la base de connaissances expertes mais aussi de résultats expérimentaux. La question du nombre d'espèces à associer pour obtenir une « assurance » de croissance de la culture intermédiaire et de produire les services écosystémiques visés pourrait donc être analysée.

Par ailleurs, nous nous sommes focalisés sur l'évaluation des cultures intermédiaires au niveau de l'espèce mais plusieurs variétés par espèce sont généralement disponibles. Des différences peuvent être relevées entre certaines variétés d'une même espèce, notamment pour la sensibilité au gel (Kalinová and Moudrý, 2003; Wilke and Snapp, 2008), les teneurs en glucosinolates (Gimsing and Kirkegaard, 2006) pouvant induire des effets d'allélopathie plus ou moins forts, ou encore certaines variétés ont des effets particuliers comme par exemple des propriétés « nématocides » pour certaines crucifères (Mojtahedi et al., 1991). Une évaluation au niveau variétal semble donc également nécessaire pour affiner le choix des espèces à assembler.

La proportion de chaque espèce par rapport à sa culture pure, conditionnant la densité de semis, est probablement un facteur significatif de la performance des mélanges. Nous avons évalué des mélanges uniquement de type « substitutif » en semant 50% de la densité de la culture monospécifique pour les deux espèces. Cependant, de multiples combinaisons sont possibles et pourraient être raisonnées en fonction de l'objectif recherché, à condition que cet « effet densité » soit bien caractérisé en termes de compétition intraspécifique. Quelques études ont mis en évidence des différences de performances en fonction de la proportion de semis de chaque espèce, mais elles se limitent à un nombre restreint de mélanges de cultures intermédiaires étudiés, notamment les mélanges raygrass italien ou seigle / trèfle incarnat (Karpenstein-Machan and Stuelpnagel, 2000; Kramberger et al., 2014, 2013b). Il est évident qu'il reste encore beaucoup à faire dans ce domaine.

Les travaux de cette thèse étaient orientés vers l'évaluation des mélanges de cultures intermédiaires pour produire des services écosystémiques liés à la gestion de l'azote, cependant, de nombreux autres services écosystémiques tels que la réduction de l'érosion, la réduction des adventices ou la pression des ravageurs sont reportés dans la littérature pour les cultures pures de cultures intermédiaires (Justes et al., 2012a). Les effets des mélanges de cultures intermédiaires sur le stockage de carbone, les émissions de gaz à effet de serre, ou encore sur les autres minéraux tels que le phosphore par exemple, sont autant de pistes de recherche qu'il reste à investiguer en relation avec les performances des cultures intermédiaires en peuplement plurispécifique.



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# Annexes

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Annexe A6. Mixtures performances to provide catch crop and green manure effects for the three experimental sites. The catch crop effect is presented as the achievement level by mixtures in reference to the average of non-legume sole crops based on the amount of N leached after cover crop destruction. The achievement level of the green manure effect was estimated for the mixture in reference to the average of the legume sole crops based on the amount of N mineralized from cover crop residues.

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### Annexe A1. Densité de semis en culture pure et en mélange des 36 taxons de cultures intermédiaires étudiés.

Famille	Nom Espèce	Nom latin	Poids de mille grains (g)	Densité semis culture pure (kg/ha)	Densité semis culture pure (plantes/m <sup>2</sup> )	Densité semis en mélange (kg/ha)	Densité semis en mélange (plantes/m <sup>2</sup> )
Composée	nyger	<i>Guizotia abyssinica</i>	3.3	8	242	4	121
Composée	tournesol	<i>Helianthus annuus</i>	48	20	42	10	21
Crucifère	caméline	<i>Camelina sativa</i>	1.3	3	231	1.5	115
Crucifère	colza fourrager	<i>Brassica napus</i>	2.7	4	148	2	74
Crucifère	moutarde blanche	<i>Sinapis alba</i>	8	8	100	4	50
Crucifère	moutarde brune	<i>Brassica juncea</i>	3	3	100	1.5	50
Crucifère	moutarde éthiopienne	<i>Brassica carinata</i>	5	7.5	150	3.75	75
Crucifère	navette fourragère	<i>Brassica rapa</i>	3.7	5	135	2.5	68
Crucifère	radis fourrager	<i>Raphanus sativus</i>	13	10	77	5	38
Crucifère	roquette	<i>Eruca sativa</i>	1.3	4	308	2	154
Graminée	avoine printemps	<i>Avena sativa</i>	39.4	75	190	37.5	95
Graminée	avoine rude	<i>Avena strigosa</i>	16.1	35	217	17.5	109
Graminée	moha	<i>Setaria italica</i>	2.2	15 (2012); 30 (2013)	682 (2012); 1364 (2013)	7.5 (2012); 15 (2013)	341 (2012); 682 (2013)
Graminée	raygrass hybride	<i>Lolium hybridum</i>	3.4	20	588	10	294
Graminée	raygrass italien	<i>Lolium multiflorum</i>	2.7	15	556	7.5	278
Graminée	seigle forestier	<i>Secale multicaule</i>	18.8	30	160	15	80
Graminée	seigle hiver	<i>Secale cereale</i>	32.3	50	155	25	77
Graminée	sorgho	<i>Sorghum bicolor var. sudanense</i>	13.8	15	109	7.5	54
Hydrophyllacée	phacélie	<i>Phacelia tanacetifolia</i>	1.8	7 (2012); 8 (2013)	389 (2012); 445 (2013)	3.5 (2012); 4 (2013)	195 (2012); 223 (2013)
Légumineuse	fenugrec	<i>Trigonella foenum-graecum</i>	16	35	219	17.5	109
Légumineuse	féverole var. LAURA	<i>Vicia faba var. LAURA</i>	443	220	50	110	25
Légumineuse	féverole var. SSNS	<i>Vicia faba var. SSNS</i>	360	180	50	90	25
Légumineuse	gesse	<i>Lathyrus sativus</i>	176	60	34	30	17
Légumineuse	lentille	<i>Lens nigricans</i>	21.5	35	163	17.5	81
Légumineuse	lupin bleu	<i>Lupinus angustifolius</i>	179.4	80	45	40	22
Légumineuse	mélilot	<i>Melilotus officinalis</i>	2.5	12.5	500	6.25	250
Légumineuse	minette	<i>Medicago lupulina</i>	1.5	10	667	5	333
Légumineuse	pois fourrager var. ASSAS	<i>Pisum sativum ASSAS</i>	169	100	59	50	30
Légumineuse	pois fourrager var. PFX	<i>Pisum sativum PFX</i>	214	108	50	54	25
Légumineuse	sainfoin	<i>Onobrychis viciifolia</i>	23	160	696	80	348
Légumineuse	trèfle alexandrie	<i>Trifolium alexandrinum</i>	3	25	833	12.5	417
Légumineuse	trèfle incarnat	<i>Trifolium incarnatum</i>	4.7	15	319	7.5	160
Légumineuse	vesce commune	<i>Vicia sativa</i>	53.8	50	93	25	46
Légumineuse	vesce pourpre	<i>Vicia benghalensis</i>	41.4	50	121	25	60
Légumineuse	vesce velue	<i>Vicia villosa</i>	26.7	30	112	15	56
Polygonacée	sarrasin	<i>Polygonum Fagopyrum</i>	25	40	160	20	80

**Annexe A2.** List of references of published data used to establish the SLA-LDMC relationship on 2176 wild species.

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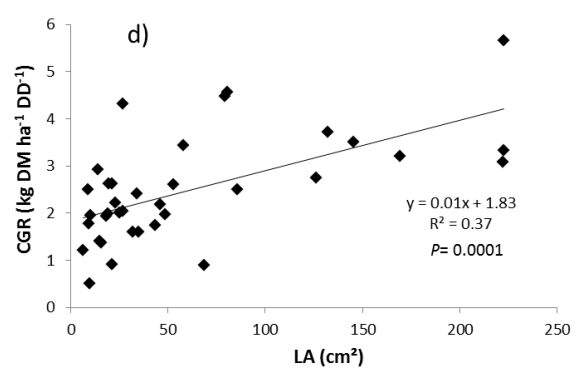
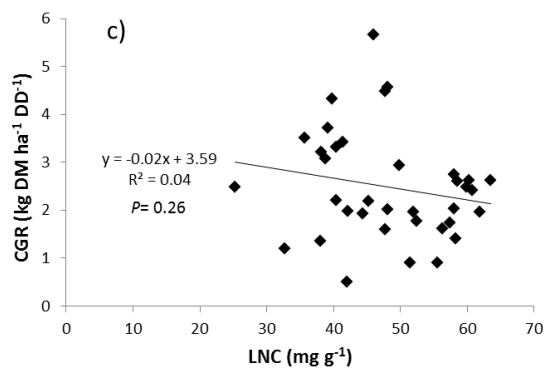
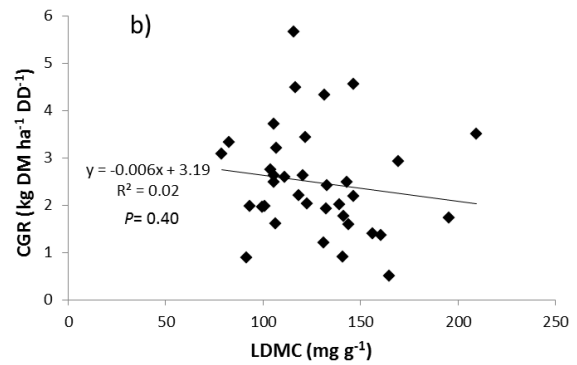
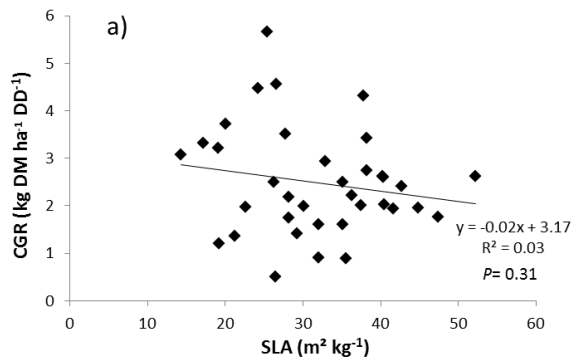
**Annexe A3.** List of original references via the global TRY plant trait database.

Data from the TRY database used in this study come from original studies cited below:

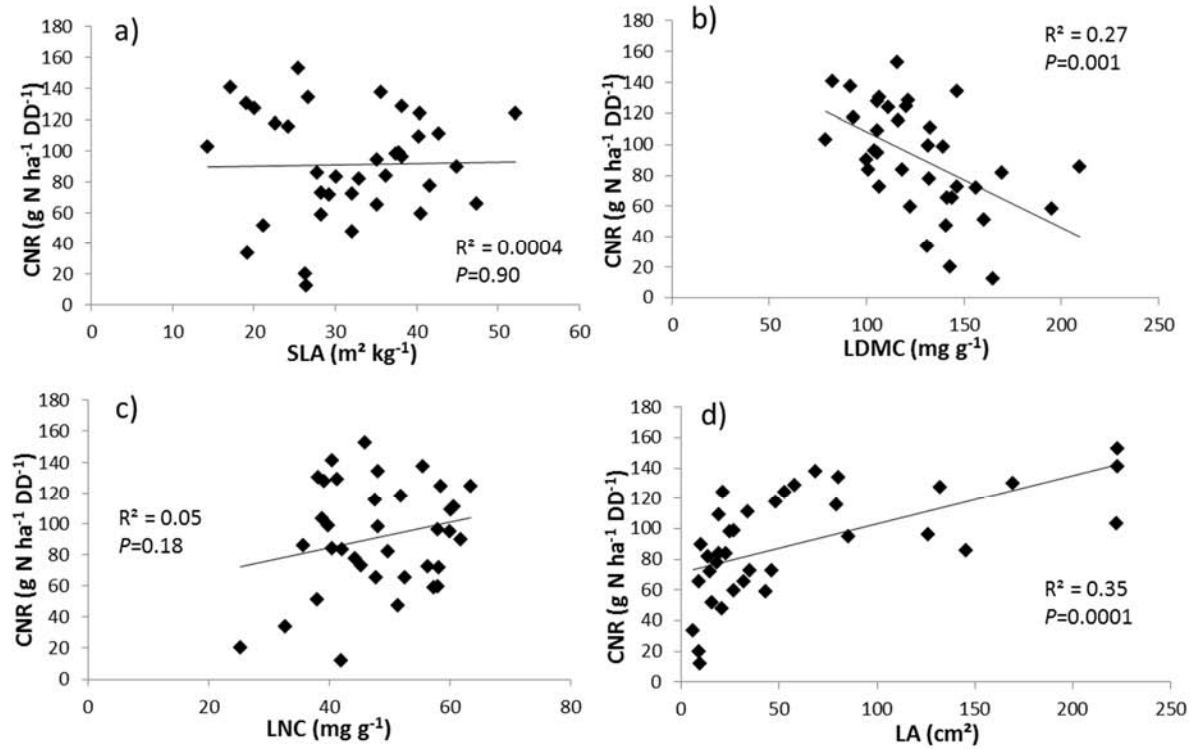
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**Annexe A4.** Relationships between crop growth rate (CGR) and leaf functional trait values: (a) specific leaf area (SLA), (b) leaf dry matter content (LDMC), (c) leaf nitrogen content (LNC), (d) leaf area (LA), (e) estimated leaf thickness (LT). The significance of differences was assessed by Student's t-tests. Values of traits and CGRs are means of both experimental sites.



**Annexe A5.** Relationships between crop N acquisition rate (CNR) and leaf functional trait values: (a) specific leaf area (SLA), (b) leaf dry matter content (LDMC), (c) leaf nitrogen content (LNC), (d) leaf area (LA)). The significance of differences was assessed by Student's t-tests. Values of traits and CNRs are means of both experimental sites.



**Annexe A6.** Mixtures performances to provide catch crop and green manure effects for the three experimental sites. The catch crop effect is presented as the achievement level by mixtures in reference to the average of non-legume sole crops based on the amount of N leached after cover crop destruction. The achievement level of the green manure effect was estimated for the mixture in reference to the average of the legume sole crops based on the amount of N mineralized from cover crop residues.

Mixtures	Auzeville		Bignan		Lyon	
	catch crop effect	green manure effect	catch crop effect	green manure effect	catch crop effect	green manure effect
bristle oat_faba bean	87%	67%	74%	111%	76%	47%
bristle oat_wild lentil	93%	70%	85%	39%	70%	39%
bristle oat_forage pea	81%	67%	90%	56%	23%	115%
bristle oat_crimson clover	79%	60%	73%	20%	48%	87%
bristle oat_purplevetch	83%	77%	113%	45%	33%	88%
foxtail millet_faba bean	63%	39%	74%	84%	79%	-1%
foxtail millet_wild lentil	79%	45%	80%	36%	155%	-27%
foxtail millet_forage pea	74%	42%	92%	48%	61%	-3%
foxtail millet_crimson clover	85%	45%	114%	42%	74%	-9%
foxtail millet_purplevetch	93%	51%	118%	66%	78%	-12%
turnip rape_faba bean	260%	33%	78%	135%	40%	96%
turnip rape_wild lentil	168%	49%	128%	54%	85%	15%
turnip rape_forage pea	218%	34%	71%	67%	31%	70%
turnip rape_crimson clover	258%	9%	111%	41%	62%	68%
turnip rape_purplevetch	154%	56%	87%	69%	58%	56%
phacelia_faba bean	72%	87%	97%	80%	85%	58%
phacelia_wild lentil	71%	73%	78%	32%	85%	6%
phacelia_forage pea	52%	104%	83%	68%	31%	71%
phacelia_crimson clover	84%	89%	88%	39%	130%	7%
phacelia_purplevetch	74%	108%	80%	58%	48%	92%
Italian ryegrass_faba bean	51%	86%	84%	92%	49%	96%
Italian ryegrass_wild lentil	56%	61%	104%	62%	70%	56%
Italian ryegrass_forage pea	57%	84%	96%	78%	26%	143%
Italian ryegrass_crimson clover	64%	65%	103%	52%	54%	91%
Italian ryegrass_purplevetch	81%	87%	79%	68%	48%	75%

**Annexe A7.** Table of input variables for the sole crops models. CGR: crop growth rate, CNR: crop N acquisition rate, C:N SC is the ratio C:N of sole crop, SLA: specific leaf are, LDMC: leaf dry matter content, LA: leaf area, LNC: leaf nitrogen content, LCC: leaf carbon content.

Botanical family	Species latin name	Common name	CGR (kg ha <sup>-1</sup> DD <sup>-1</sup> )	CNR (g ha <sup>-1</sup> DD <sup>-1</sup> )	C:N	SLA (m <sup>2</sup> kg <sup>-1</sup> )	LDMC (mg g <sup>-1</sup> )	LA (cm <sup>2</sup> )	LNC (mg g <sup>-1</sup> )	LCC (%)	Leaf C:N	Flowering height (cm)	Seed mass (mg)	Root speed (mm DD <sup>-1</sup> )	Cover height (cm)	Soil cover (%)	Cover N (%)	Cover C (%)
Asteraceae	<i>Guizotia abyssinica</i>	Nyger	2.7	105	10.8	33.8	58	45	42.1	41.9	10.0	74	3.3	0.6	74	100	3.8	41.3
	<i>Helianthus annuus</i>	Sunflower	5.3	146	16.2	24.9	121	197	44.7	42.5	9.5	130	48	1.03	127	100	2.8	44.7
Brassicaceae	<i>Brassica carinata</i>	Abyssinian cabbage	3.4	119	12.3	20.2	107	154	42.9	43.0	10.0	60	5	0.95	67	100	3.5	42.9
	<i>Brassica juncea</i>	Green mustard	4.3	113	17.1	24.0	114	103	52.1	42.9	8.3	76	3	0.99	76	100	2.6	45.1
	<i>Brassica napus</i>	Forage seedrape	2.5	102	10.2	17.4	113	150	39.2	42.3	10.8	50	2.7	0.78	53	100	4.2	42.4
	<i>Brassica rapa</i>	Turnip rape	3.3	114	12.6	15.8	76	253	37.5	41.0	11.0	40	3.7	0.82	50	100	3.4	42.8
	<i>Camelina sativa</i>	Camelina	1.1	50	8.8	44.1	90	12	64.4	41.0	6.4	42	1.3	0.34	42	61	4.7	41.5
	<i>Eruca sativa</i>	Salad rocket	1.6	100	6.6	25.6	77	54	64.0	38.5	6.0	45	1.3	0.78	25	93	6.1	40.4
	<i>Raphanus sativus</i>	Forage radish	3.1	135	9.8	18.5	83	274	43.8	42.3	9.7	45	13	0.99	54	100	4.3	42.5
	<i>Sinapis alba</i>	White mustard	4.2	127	14.8	27.2	147	82	53.5	42.8	8.0	120	8	1.03	100	100	3	44.5
Fabaceae	<i>Lathyrus sativus</i>	Grass pea	2.5	106	9.4	41.1	108	15	62.0	46.4	7.5	35	176	0.95	35	100	4.2	39.6
	<i>Lens nigricans</i>	Wild lentil	1.8	68	11.5	43.9	159	9	52.5	46.2	8.8	40	21.5	0.6	23	100	3.8	43.2
	<i>Medicago lupulina</i>	Black medic	0.5	12	10.9	25.3	167	11	46.6	44.6	9.6	40	1.5	0.26	6	21	2.5	26.8
	<i>Melilotus officinalis</i>	Yellow melilot	1.2	62	8.5	28.1	158	11	59.1	44.2	7.5	80	2.5	0.65	15	100	5.2	44.2
	<i>Onobrychis viciifolia</i>	Sainfoin	1.0	38	12.0	21.1	167	15	38.3	46.2	12.1	80	23	0.6	15	88	3.9	46.3
	<i>Pisum sativum var. ASSAS</i>	Forage pea ASSAS	2.0	96	9.5	39.7	120	44	56.4	46.5	8.3	70	168.8	0.86	30	95	4.9	46.3
	<i>Pisum sativum var. PFX</i>	Forage pea PFX	1.1	50	9.4	32.4	115	22	54.5	47.3	8.7	90	214.5	0.6	27	78	4.6	43.3
	<i>Trifolium alexandrinum</i>	Egyptian clover	2.6	74	16.0	33.8	163	13	49.2	45.4	9.2	50	3	0.95	50	100	2.9	45.7
	<i>Trifolium incarnatum</i>	Crimson clover	1.7	74	10.6	28.5	105	17	46.1	44.1	9.6	55	4.7	0.69	19	100	4.3	45.6
	<i>Vicia benghalensis</i>	Purple vetch	2.4	111	10.0	41.0	147	34	60.6	45.6	7.5	45	41.4	0.82	45	100	4.7	47.1
	<i>Vicia faba var. LAURA</i>	Faba bean LAURA	2.4	92	12.0	34.3	113	77	62.4	45.9	7.4	60	442.8	0.69	52	100	3.9	46.6
	<i>Vicia faba var. SSNS</i>	Faba bean SSNS	2.5	90	13.1	39.6	107	112	58.8	46.7	8.0	90	359.6	0.65	72	100	3.6	47
<i>Vicia sativa</i>	Common vetch	1.6	49	12.9	40.8	133	25	61.9	46.0	7.4	50	53.8	0.43	31	100	3	38.7	
<i>Vicia villosa</i>	Hairy vetch	2.3	109	9.0	51.3	132	20	61.9	44.9	7.2	50	26.7	0.6	51	100	4.8	43.4	
Hydrophyllaceae	<i>Phacelia tanacetifolia</i>	Phacelia	1.7	92	7.1	39.5	84	86	61.1	38.3	6.3	80	1.8	0.52	28	98	5.5	39.1
Poaceae	<i>Avena sativa</i>	Spring oat	2.8	94	14.0	27.5	156	44	42.8	44.8	10.5	90	39.4	0.56	60	100	3.4	47.2
	<i>Avena strigosa</i>	Bristle oat	2.2	91	10.9	31.6	155	36	49.1	45.1	9.2	100	16.1	0.52	46	100	4.1	45.3
	<i>Lolium hybridum</i>	Hybrid ryegrass	2.6	99	11.5	36.6	120	25	48.6	42.3	8.7	75	3.4	0.52	24	100	3.8	44.2
	<i>Lolium multiflorum</i>	Italian ryegrass	1.7	69	10.8	38.6	137	17	48.9	43.3	8.9	75	2.7	0.34	29	100	4.1	44.1
	<i>Secale cereale</i>	Winter rye	1.7	88	8.7	33.4	142	33	54.2	44.7	8.3	130	32.3	0.34	33	100	5.2	45.5
	<i>Secale multicaule</i>	Rye forage	1.6	82	9.2	39.3	138	23	55.7	45.5	8.2	180	18.8	0.65	27	100	5	46.2
	<i>Setaria italica</i>	Foxtail millet	1.3	46	13.2	29.3	206	23	39.8	44.3	11.2	40	2.2	0.82	40	100	3.5	45.4
	<i>Sorghum sudanense</i>	Forage sorghum	4.5	112	19.0	26.1	232	162	37.0	46.3	12.5	150	13.8	1.03	117	100	2.5	47.1
Polygonaceae	<i>Polygonum fagopyrum</i>	Buck wheat	4.3	99	18.7	38.3	126	25	45.0	40.6	9.0	50	25	0.34	63	99	2.3	43.6

**Auteur** : H  l  ne Tribouillois

**Titre**: Caract  risation fonctionnelle d'esp  ces utilis  es en cultures interm  diaires et analyse de leurs performances en m  langes bisp  cifiques pour produire des services   cosyst  miques de gestion de l'azote.

**Directeurs de th  se** :   ric Justes et Pablo Cruz

**Lieu et date de soutenance** : Toulouse, le 04 d  cembre 2014

### **R  sum  **

Les cultures interm  diaires d  livrent des services   cosyst  miques de r  duction de la lixiviation de nitrate et de production de l'effet engrais vert pour la culture principale suivante. L'objectif de ce travail   tait de caract  riser un grand nombre d'esp  ces et d'analyser les performances de m  langes bisp  cifiques de type l  gumineuse/non-l  gumineuse pour produire simultan  ment ces services   cosyst  miques de gestion de l'azote.

Les traits fonctionnels foliaires mesur  s sur 36 esp  ces sont robustes mais sont peu pr  cis pour diff  rencier les strat  gies des esp  ces. Les temp  ratures cardinales de germination mesur  es au laboratoire indiquent que la majorit   des esp  ces est adapt  e    des conditions de semis d'  t  . Une mod  le conceptuel impl  ment   en GLM permet de pr  dire le comportement des deux esp  ces en m  lange. Les mesures r  alis  es en exp  rimentations au champ coupl  es avec des simulations avec le mod  le STICS ont permis d'  valuer les performances de certains m  langes bisp  cifiques pour produire simultan  ment les services   cosyst  miques. L'efficacit   d  pend du choix des esp  ces associ  es, qui doit   tre adapt  e en fonction des conditions p  doclimatiques et de la date de destruction des couverts.

**Mots cl  s** : azote, compl  mentarit  , culture interm  diaire, engrais vert, germination, interactions intersp  cifiques, interculture, lixiviation du nitrate, m  lange, traits fonctionnels.

**Discipline** : Agrosyst  mes, Ecosyst  mes et Environnement

**Unit   de recherche** : UMR AGIR 1248 INRA/INPT-ENSAT, Toulouse

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**Title**: Functional characterization of species used as cover crops and analysis of their performances in bispecific mixtures to produce ecosystem services of nitrogen management.

**PhD directors** : Eric Justes et Pablo Cruz

**Place and date of defense** : Toulouse, December 4<sup>th</sup> 2014

### **Summary**

Cover crops produce ecosystem services for nitrogen management during fallow period such as decreasing nitrate leaching and producing green N manure effect for the next cash crop. The aim of this work was to characterize a large number of species using a functional analysis and to analyze the performances of bispecific legume/non-legume mixtures to simultaneously produce both ecosystem services.

Leaf functional traits measured on 36 cover crops were found robust but lacked of precision in differentiating species strategies. The cardinal temperatures for germination measured in laboratory have shown that the majority of species is adapted to summer sowing conditions. A conceptual model was designed and implemented in GLM to predict the behavior of species in mixtures. The measurements carried out in field experiments coupled with STICS model simulations confirmed the potential efficiency of some bispecific mixtures to simultaneously produce ecosystem services of nitrogen management. This efficiency depends on the choice of the two species to associate which must be adapted according to the pedoclimatic conditions as well as the date of cover crop destruction.

**Key-words** : nitrogen, complementarity, cover crop, green manure, germination, species interactions, fallow period, nitrate leaching, mixture, functional traits.

**Discipline** : Agrosyst  mes, Ecosyst  mes et Environnement

**Laboratory** : UMR AGIR 1248 INRA/INPT-ENSAT, Toulouse