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***Impacts des systèmes de cultures sur l'efficacité d'utilisation
de l'azote chez le blé et le maïs :
Influence du travail du sol, des couverts végétaux
d'interculture et de l'historique de fertilisation azotée***

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*You can not swim for new horizons until
you have courage to lose sight of the shore....*

William Faulkner

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LES ABRÉVIATIONS

CA	Conservation agriculture ; Agriculture de conservation des sols (CA
APAD	Association pour la Promotion de l'Agriculture Durable
SDCV	Semis direct sous couverture végétale
CIRAD	La recherche agronomique pour le développement
IRD	Institut de recherche pour le développement
TCS	Techniques culturales simplifiées
MO	Soil organic matter ; Matière organique du sol
SOC	Soil organic carbone ; Carbone organique du sol
SON	Soil organic nitrogen ; Azote organique du sol
Nr	Reactive nitrogen; Azote réactif
χ_s	Stomatal compensation point; Point de compensation stomatique
GS	Glutamine synthétase
PGPR	Planet growth-promoting rhizobacteria; Rhizobactéries stimulant la croissance des plantes.
HATS	High affinity transport system; Système de transport à haute affinité
LATS	Low affinity transport system; Système de transport à basse affinité
GDH	Glutamate déshydrogénase
GOGAT	Glutamine-2-oxoglutarate aminotransférase
RuBP	Ribulose-1,5-bisphosphate
NUE	Nitrogen use efficiency ; Efficience d'utilisation de l'azote
NU_pE	Nitrogen uptake efficiency ; Efficience d'absorption de l'azote
NU_tE	Nitrogen utilisation efficiency,
PFPN	Nitrogen partial factor productivity ; Facteur de productivité partielle de l'azote
AEN	Nitrogen agronomic efficiency ; Efficience agronomique d'utilisation de l'azote
CAU	Coefficient apparent d'utilisation de l'azote
NAR	Nitrogen apparent recovery fraction ; Efficience apparent de récupération de l'azote
PEN	Physiological nitrogen use efficiency ; Efficience physiologique de l'utilisation de l'azote

Gy	Grain yield ; Rendement grain
Nt	Total nitrogen uptake ; Azote total de plante
X	Nitrogen fertilizer rate ; Dose d'engrais azoté à apporter
NRem	Nitrogen remobilization ; Remobilisation de l'azote
NRE	Nitrogen remobilization efficiency ; Efficience de remobilisation de l'azote
NHI	Nitrogen harvest index ; Indice de récolte d'azote
SWC.c	Soil water content at sowing ; Teneur en eau du sol au semis
SWC.h	Soil water content at harvest ; Teneur en eau du sol à la récolte
NNI	Nitrogen nutrition index ; Indice de nutrition azotée
Nc	Critical plant nitrogen concentration ; Teneur critique en azote de la plante
PNUE	Photosynthetic nitrogen use efficiency ; Efficience photosynthétique d'utilisation de l'azote
WUE	Water use efficiency ; Efficience d'utilisation de l'eau
WUEint	Intrinsic water-use efficiency ; Efficience intrinsèque d'utilisation de l'eau
WUEins	Instantaneous water use efficiency ; Efficience instantanée d'utilisation de l'eau
SLA	Specific leaf area ; Surface foliaire spécifique
Asat	Photosynthesis rate ; Taux de photosynthèse
gs	Stomatal conductance ; Conductance stomatique
E	Transpiration rate ; Taux de transpiration,
LAR	Leaf area ratio ; Rapport surface foliaire
CCI	Chlorophyll content index ; Indice de teneur en chlorophylle

RÉSUMÉ

De tous les éléments nutritifs, l'azote (N) est le plus important pour la croissance des cultures et l'expression du rendement potentiel. La disponibilité en azote minéral constitue un des principaux facteurs limitant de la production quantitative et qualitative des plantes non légumineuses en général et des céréales en particulier. Le maintien des hauts niveaux de production actuels nécessitant de maintenir un état de nutrition azotée optimal des cultures ; L'amélioration de l'efficacité ou l'efficience des apports d'engrais azotés de synthèse devient ainsi une priorité de gestion des systèmes de productions intensifs au niveau national.

Améliorer l'efficience d'utilisation de l'azote constitue un sujet de préoccupation depuis de nombreuses années. Diverses approches ont été développées mondialement pour atteindre cet objectif. Parmi celles-ci, figure, la modification des pratiques culturales de type agroécologiques, mises en œuvre dans le cadre de l'agriculture de conservation des sols permettant simultanément de maintenir les niveaux de fertilité naturelle des sols cultivés en luttant principalement contre les phénomènes d'érosion des sols.

L'objectif principal de la thèse est d'analyser les nouveaux systèmes de cultures économiquement et écologiquement performants basés sur les techniques d'agriculture de conservation de sols au plan de la nutrition azotée. Nous avons choisi d'étudier l'influence des modifications des pratiques culturales (type de travail du sol, couverts végétaux, niveaux de fertilisation azotée) sur les divers paramètres affectant la productivité végétale et la nutrition azotée de deux céréales (Blé et Maïs). Dans ce cadre, trois expérimentations ont été mises en place au champ pour mieux comprendre les phénomènes agronomiques et processus écologiques s'opérant lors des changements de pratiques culturales.

L'objectif finalisé étant de trouver le ou les systèmes de cultures le(s) plus adapté(s) pour obtenir de bons rendements financiers tout en diminuant les apports d'azote minéraux.

La première expérimentation réalisée sur le maïs a permis de mettre en évidence, après 4 années de non travail du sol, une augmentation significative de l'efficience d'utilisation de l'azote NUE, de l'indice de récolte d'azote ainsi que de l'efficience de remobilisation de l'azote, valable pour les systèmes de culture développés avec et sans fertilisation azotée.

En ce qui concerne la deuxième expérimentation réalisée sur le blé, les résultats obtenus au cours de deux années successives ont montré les mêmes tendances que sur le maïs, avec une augmentation de l'efficience d'utilisation de l'azote, l'efficience agronomique d'utilisation de l'azote, le facteur de productivité partielle de l'azote, le coefficient apparent d'utilisation de l'azote et l'efficience de remobilisation de l'azote. De même secondairement, on a étudié l'indice de nutrition azotée (NNI) pour évaluer le statut de la nutrition azotée du blé entre les deux systèmes de travail du sol. Le travail du sol affecte de façon significative le NNI qui était plus élevé dans les systèmes de non-labour par rapport au système du labour classique.

Dans la troisième partie de ce travail réalisée sur le blé, nous avons tenté de préciser les mécanismes physiologiques à l'origine de l'amélioration des divers paramètres de l'utilisation de l'azote constatée en analysant par système de culture, l'efficience photosynthétique d'utilisation de l'azote et de l'eau chez le blé au travers divers paramètres tels que : caractéristiques physiologiques des feuilles, taux de photosynthèse, conductance stomatique, taux de transpiration, indice de teneur en chlorophylle et surface spécifique foliaire. Nous avons

ainsi constaté que le blé utilise l'eau et l'azote plus efficacement dans le système sans labour par rapport au système labour classique. Au même stade de développement du blé, le taux de photosynthèse et la surface spécifique foliaire étaient plus élevés en modalités de semis direct qu'en modalités du labour classique due principalement à la conservation de l'eau du sol dans le système non-labour.

Dans nos conditions, on peut conclure que dans nos conditions d'expérimentation, le système d'agriculture de conservation est une stratégie efficace pour améliorer l'efficacité azotée tout en préservant le sol et limitant la pollution des ressources naturelles.

Mots clés : *azote ; efficacité ; pratiques culturales ; travail du sol ; couverture végétale ; blé ; maïs*

ABSTRACT

Of all the essential nutrients, nitrogen (N) is the most important for crop growth and yield potential. The mineral N availability is a major limiting factor of quantitative and qualitative production of crops in general and cereals in particular. Maintaining the current high production levels requires managing crops to provide an optimum nitrogen nutrition. Therefore, the agricultural management practices must be at forefront of measures to sustain crop productivity and N efficiency in the intensive production systems at national level.

In the last three decades, improving N use efficiency in crops (NUE) has been one of the most important challenge in modern agriculture. Therefore, various scientific, technological and agronomic approaches have been developed in parallel to improve our knowledge on the genetic and physiological basis of NUE for further breeding and agronomic applications. In particular, it has been emphasized that agricultural management practices on both short- and long-term perspectives must be at forefront of measures to develop sustainable crop productivity with regards to NUE improvement.

The main objective of this work is to examine the new cropping systems, economically and environmentally efficient, based on conservation agriculture techniques. In other words, to investigate the influence of modification in agricultural practices (tillage system, cover crops) on the parameters affecting plant productivity and nitrogen nutrition of two cereals (wheat and maize). In this context, three field experiments were conducted to understand agricultural phenomena taking place during changes in farming practices, in order to examine the most sustainable agricultural system for maintaining crop productivity while rationalization of N fertilizer usage.

A two-year experiment was conducted in the field to measure the combined impact of tilling and N fertilization on various agronomic traits related to NUE and to grain yield in maize cultivated in the presence of a cover crop. Four years after conversion to no-till, a significant increase in N use efficiency N harvest index, N remobilization and N remobilization efficiency was observed both under no and high N fertilization conditions.

The second field study was conducted during two consecutive years to evaluate the combined effect of tilling, cover crops and N fertilization on various agronomic traits related to nitrogen use efficiency and to N nutrition in wheat. Five years after conversion to no-till, a significant increase in N use efficiency, N utilization efficiency, N agronomic efficiency, N partial factor productivity, N apparent recovery fraction and N remobilization was observed under three levels of N fertilization. Moreover, we observed that grain yield and grain N content were similar under tillage and no-till conditions. The N nutrition index was higher under no-till conditions at the three rates of N fertilization. Moreover, N use efficiency related traits and N nutrition were increased in the presence of cover crops both under no-till and conventional tilling conditions. Thus, agronomic practices based on continuous no-till in the presence of cover crops appear to be a promising strategy to increase N use efficiency and N nutrition in wheat while reducing both the use and the loss of N-based fertilizers.

In the third study, wheat plants were grown under tillage and no- till conditions, with and without cover crops under no and high nitrogen fertilization conditions, to evaluate the combined effect of tilling and N fertilization on photosynthetic nitrogen use efficiency and

photosynthetic water use efficiency through its impact on leaf physiological traits, such as photosynthesis rate, stomatal conductance, transpiration rate, leaf area ratio and specific leaf area. Six years after conversion to no-till, in the presence and in the absence of cover crops, a significant increase in water use efficiency and soil water content was observed both under no and high N fertilization conditions. Moreover, we observed that photosynthetic nitrogen use efficiency, photosynthesis rate and specific leaf area were higher under no-till conditions compared to conventional tillage.

Thus, agronomic practices based on continuous no-till appear to be a promising for increasing NUE related traits in wheat and maize, whilst preserving the soil and limiting the pollution of natural resources. This is the principle of sustainable agriculture which is a step towards a sustainable development.

Key words : nitrogen ; efficiency ; agricultural practices ; tillage ; cover crops ; wheat ; maize

INTRODUCTION GÉNÉRALE

La gestion durable des sols agricoles implique une évaluation continue de leur état au travers de la mesure d'indicateurs de la fertilité. Cette dernière est perçue comme l'aptitude du sol à assurer, de façon durable, la production de biomasse végétale (Jones and Tirado-Corbalá, 2013). La fertilité des sols résulte de facteurs physiques, chimiques et biologiques qui dépendent des conditions du milieu mais aussi et surtout de la conduite des activités humaines, en particulier des pratiques agricoles. Il existe cependant une multitude de façon de définir la fertilité. Selon la définition proposée par Schvartz et al. (2005), « La fertilité est un jugement global de valeur sur la qualité d'un milieu nécessaire à la satisfaction de la production végétale ». Le terme d'aptitude culturale peut même remplacer le mot fertilité selon Boiffin et Sébillotte (1982).

L'évaluation de la fertilité des sols peut ainsi être faite directement via l'évaluation de la productivité végétale et/ou indirectement au travers de la mesure de divers paramètres physiques, chimiques et biologiques édaphiques connus pour être corrélés positivement avec la productivité végétale. Les travaux qui seront présentés dans le cadre de cette thèse sont consacrés à l'évaluation de la fertilité des sols via l'évaluation de la productivité végétale chez le blé et le maïs en fonction de pratiques culturales différenciées dans le temps, définissant ainsi des systèmes de cultures différents (Sebillotte M., 1990).

Historiquement, ce sont les travaux de Liebig à partir de 1840 en chimie minérale et portant sur la nutrition minérale des cultures qui sont à l'origine de l'utilisation des engrais minéraux comme moyen principal de gestion de la productivité végétale ; le sol étant alors juste considéré comme le support des cultures. Cependant selon Liebig, toutes les réactions se produisant dans la nature, ne pouvaient être que de nature chimique ; il refusait d'admettre l'origine biologique de certaines réactions chimiques, démontrée pourtant simultanément par Pasteur pour la fermentation alcoolique (Dumas, 1965). De même, Liebig refusait également d'admettre d'autres éléments de Science relevant de la physiologie végétale et s'opposait à deux importants botanistes qu'étaient Schleiden and von Mohl (Werner and Holmes, 2002).

Par suite, c'est principalement Albert Howard qui édicta au royaume uni, les principes selon lesquels il était possible de gérer la fertilité des sols et la productivité végétale autrement, grâce à l'humus. Selon lui, les plantes étaient capables de se nourrir des molécules organiques contenues dans l'humus ; théorie de l'humus de Howard à la base du concept d'agriculture organique. Howard édictait ainsi les bases d'une autre forme d'agriculture, l'agriculture biologique actuelle (Howard, 1943). Récemment, au cours des dernières crises agricoles, il a pu être montré que les systèmes biologiques permettaient d'augmenter les marges financières

des exploitations comparativement aux exploitations classiques, notamment en élevage porcin et laitier.

Howard ne s'y trompait pas et était en désaccord avec Liebig car ce dernier indiquait que les plantes ne pouvaient se nourrir qu'à partir de substances minérales (Van der Ploeg et al., 1999). Or, nous savons désormais que la théorie de Liebig était inexacte puisque la nutrition azotée des plantes à partir de molécules organiques a été démontrée depuis chez de nombreuses espèces, dont le blé tendre (Näsholm et al., 2001) ou encore chez le trèfle hybride (Näsholm et al., 2000). De même, plus récemment, il a pu être mis en évidence que les acides humiques de faible poids moléculaires générés lors du transit intestinal à partir d'acides humiques de plus hauts poids moléculaires et extraits de turrículos de vers de terre, permettaient l'activation de gènes codant des protéines impliquées dans l'absorption et le transport du nitrate chez le maïs (Quaggiotti et al., 2004). Ainsi, pour la première fois, un rôle physiologique des molécules humiques « stricto sensu » dans la nutrition azotée était démontré. On comprenait, dès lors, que la vitesse d'absorption et le transport des éléments minéraux par la plante représentaient d'autres facteurs limitant la production végétale non encore identifiés jusque-là. Ainsi ce n'était plus forcément la quantité d'azote biodisponible qui représentait le seul facteur limitant la productivité des plantes non légumineuses, mais la vitesse de transport des photoassimilats. Il en fut de même vis-à-vis de la découverte d'autres processus écologiques intervenant dans la production de phytohormones par le complexe microbien rhizosphérique (PGPR) augmentant ainsi la croissance et la production végétale (Bhattacharyya and Jha, 2012), et ceci sans pour autant avoir recours à des apports de fertilisants minéraux supplémentaires dont les impacts négatifs sur l'environnement ne sont plus à démontrer. Cependant, dans le cadre de l'agriculture classique basée sur l'apport de quantités d'engrais minéraux importantes, notamment azotés, afin d'éviter les pertes d'intrants vers les milieux aquatiques, les pratiques culturales doivent viser uniquement à satisfaire les besoins des végétaux, tout en veillant à ne pas dépasser la capacité des sols à retenir les éléments nutritifs (Zebbarth et al., 2009).

Globalement, en agriculture classique, la mécanisation agricole, l'irrigation, la sélection variétale, la protection phytosanitaire, l'utilisation massive d'engrais azotés ont été les éléments clés qui ont contribué à l'amélioration significative des rendements (Cassman 1999). Ainsi dans ces systèmes de production, la croissance et le rendement des cultures non légumineuses sont en effet, fortement dépendants de la nutrition et de la fertilisation azotée (Santi et al. 2013). Après fertilisation, en fonction de la qualité, des doses et du fractionnement des engrais appliqués, mais aussi en fonction des variables climatiques et édaphiques, l'azote est directement absorbé par les plantes et/ou converti en d'autres formes au travers le cycle

biogéochimique de l'azote (Mariotti, 1997). Au plan environnemental, l'azote non utilisé par la plante est perdu par trois processus majeurs que sont le lessivage des nitrates, la volatilisation de l'ammoniac NH_3 , et la dénitrification sous forme de N_2 et/ou N_2O (Brady et al. 2008; Liu et al. 2014).

Le biodisponibilité en azote pour la plante augmente après un apport d'engrais azoté minéral, mais si les quantités d'azote apportées sont trop importantes, les pertes d'azote augmentent et menacent l'environnement (Sharifi et al., 2011). Il a été démontré qu'il existe des relations étroites entre les apports excessifs d'engrais azotés et les problèmes environnementaux tels que l'eutrophisation, l'effet de serre, et les pluies acides (Gastal and Lemaire, 2002). A partir de la révolution industrielle, à la fin du 19^e siècle, l'utilisation massive de combustibles fossiles pour la production d'énergie, les transports, l'industrie et les activités domestiques ont également augmenté fortement les quantités d'azote présentes dans l'environnement (Galloway et al. 2003). La production d'azote réactif par le procédé Haber-Bosch « faisant la synthèse d'ammoniac à partir de diazote et de dihydrogène (H_2) » a ainsi dépassé quantitativement la fixation symbiotique depuis la fin du 20^e siècle à l'échelle globale (Erisman et al., 2008).

La diminution de la fertilité initiale des sols se répercute inévitablement par une baisse de leur productivité augmentant, en réponse, le besoin en fertilisation azotée synthétique pour maintenir constants les niveaux de la productivité actuelle (Tilman et al., 2002). Les travaux de Mulvaney et al. (2009) ont montré que les apports d'engrais minéraux répétés depuis 50 années pouvaient être responsables de l'appauvrissement des sols en azote organique. Néanmoins ces travaux ont donné lieu à divers échanges controversés de la part de la communauté scientifique considérant que les conclusions des travaux de Mulvaney comportaient une incertitude à propos du rôle joué par les amendements organiques dans les expérimentations. L'origine exclusive des engrais minéraux azotés dans les mécanismes incriminés ne pouvait être validée.

Le nouveau paradigme de « l'intensification durable de la production » élaborés à la FAO (2011) reconnaît la nécessité d'une agriculture productive et rémunératrice tout en conservant et améliorant les ressources naturelles de l'environnement, et en contribuant positivement à rendre des services écosystémiques.

C'est donc dans ce contexte d'interface, entre l'agriculture classique utilisant massivement les intrants d'origine chimique, notamment azotés et l'agriculture biologique interdisant tout usage d'intrants d'origine chimique, que s'est fondée notre réflexion. N'existerait-il pas un type d'agriculture intermédiaire entre l'agriculture intensive et l'agriculture biologique qui permettrait de mieux gérer les productions végétales et la fertilité des sols ?

C'est pourquoi, en connaissance des données scientifiques mondiales, nous avons émis en première hypothèse que l'agriculture de conservation des sols pouvait globalement représenter ce moyen intermédiaire de gestion de la fertilité des sols et des niveaux de productivité végétale actuels.

L'agriculture de conservation des sols avait pour objectif initial de limiter la dégradation des sols par érosion, due essentiellement à une baisse des taux de matière organique, en instaurant l'arrêt du travail du sol, la couverture du sol en interculture ainsi que la diversification et l'allongement des rotations culturales. En France, le nombre d'agriculteurs réellement impliqués en agriculture de conservation serait de l'ordre de 4000 en 2013 (Laurent, 2015). Ce qui est extrêmement bas comparativement aux 600 000 chefs d'exploitations recensés (Agreste, 2010), soit moins de 1% des agriculteurs et uniquement 2% de la surface cultivée (APAD, 2016). Au plan fondamental, il est également nécessaire de revenir sur le paradigme de la nutrition minérale des plantes, dans la mesure où de nombreux travaux ont mis en évidence que les plantes étaient capables de prélever des éléments minéraux à partir des réseaux microcristallins en provenance de poudre de granite (Coroneos et al. 1995), sous l'action des différents mécanismes d'exploration du sol, générés par la plante au niveau de la rhizosphère. Le mécanisme du « priming effect rhizosphérique » étant un autre exemple de stratégie d'exploration du milieu édaphique capable de multiplier par 2 ou 3, la quantité de matière organique minéralisée annuellement dans le sol rhizosphérique (Kuzyakov, 2002) comparativement à du sol non rhizosphérique.

C'est pourquoi, nous avons émis en deuxième hypothèse de travail, que les couverts végétaux utilisés en agriculture de conservation pouvaient augmenter les fonctions d'exploration du sol, représentant ainsi ce que l'on pourrait appeler « la loi des avances en élément minéraux » assurée par le couvert végétal précédant chaque culture principale suivante. En effet, même si cette capacité des couverts végétaux à extraire des éléments minéraux du réseau microcristallin peut être insuffisante pour assurer la totalité des besoins de la culture suivante, les seules quantités extraites à partir de la prospection racinaire des couverts végétaux permettraient de diminuer d'autant les quantités d'éléments minéraux solubles à apporter, en provenance des engrais minéraux de synthèse chimique.

En résumé, selon une approche écologique et non plus agronomique, ce sont toutes les fonctions d'exploration du biotope ou milieu édaphique par le système racinaire des plantes qui assurent secondairement les fonctions de nutrition. Et dans la mesure où la fonction d'exploration des plantes trouve sa source d'énergie à partir de l'énergie solaire via la photosynthèse, cela permettrait, à priori, de nous engager dans une démarche d'agriculture et

de développement durable. Par extension, il faut comprendre que la graine en agriculture de conservation représente l'intrant principal de ces nouveaux systèmes de production couverts en permanence. La graine intrant étant elle-même fabriquée à partir de la photosynthèse et de l'intelligence des sélectionneurs qui ne coûte rien au plan énergétique. Ainsi, la boucle serait bouclée.

Au plan des objectifs de la thèse, nous avons finalement considéré que l'évaluation et la comparaison de l'efficacité d'utilisation de l'azote en fonction de systèmes de cultures différenciés dans le temps par leurs pratiques, constituait à la fois un indicateur de performance agronomique autant qu'un indicateur de performance environnementale. En effet, l'efficacité moyenne d'utilisation de l'azote des engrais minéraux est voisine de 50%, à l'origine des nombreuses fuites d'azote dans les écosystèmes adjacents (Smil, 1999; Hirel et al., 2011).

I. SYNTHÈSE BIBLIOGRAPHIQUE

1. Agriculture de conservation des sols (CA) : concept et effets

1.1. Historique et adoption de l'agriculture de conservation

Dans les années 1930, le travail répété des sols a conduit à des énormes pertes de terre cultivées dans les grandes plaines aux Etats Unis (Friedrich et al. 2012) (**Figure 1**). Avec le temps, le concept de la protection des sols, en réduisant le travail du sol et en gardant le sol couvert, a gagné la popularité des agriculteurs. D'ailleurs, l'augmentation du prix des combustibles fossiles au cours des années 1970 a incité les agriculteurs à s'orienter vers des systèmes agricoles économe en ressources (Haggblade and Tembo, 2003). Dans ce contexte, les communautés agricoles ont adopté l'agriculture de conservation des sols pour lutter contre la sécheresse induite par l'érosion des sols. Cette expérience aux Etats Unis s'est ensuite répandue hors de ses frontières. La technique a été adoptée par le Canada mais surtout par le Brésil qui a subi dès la fin des années 1960 d'énormes problèmes d'érosion hydrique (Lal, 1976).



Figure 1. Les Grandes Plaines et le Dust Bowl entre 1935-1938 (sources : Lavin et al. 2011)

Aujourd'hui, on peut constater que le semis direct est un système de non travail du sol qui a considérablement augmenté et gagné du terrain au cours des dernières décennies (Dimassi et al. 2013). Au niveau mondial, l'agriculture de conservation occupait une surface de 106 Mha en 2009 (Kassam et al. 2009; Derpsch and Friedrich 2009), elle a ensuite progressé pour atteindre 155 Mha en 2013 (Kassam et al. 2014). L'adoption de l'agriculture de conservation a fortement augmentée dans les pays européens, environ 2.585.000 ha étaient conduits en agriculture de conservation en 2013 (FAO-AQUASTAT 2015) (Tableau 1). Cependant, en France, il reste un concept relativement nouveau (1% des surfaces, Cf. Tableau 1) et le développement est encore très lent par rapport à d'autres régions du monde comme les États-Unis ou l'Asie (Friedrich et al., 2012). En France, 21% des cultures annuelles auraient été conduites en semis direct avant 2006, ce chiffre a évolué pour atteindre 34% en 2011 (ECAAF 2011). L'APAD « Association pour la Promotion d'Agriculture Durable » a estimé que les semis sans labour sont pratiqués par plus d'un agriculteur sur trois en France et 200000 ha sont conduits en semis direct. Néanmoins il convient de faire très attention au terme « non labour » qui ne veut pas dire que les techniques développées entraînent la non perturbation du sol comme le permet seulement le semis direct. D'autre part, il ne faut pas oublier que c'est l'adoption des trois principes de l'agriculture de conservation (non perturbation du sol, couverture végétale du sol la plus permanente possible et diversification/allongement de la rotation culturale) qui permet de dire si une exploitation agricole est en agriculture de conservation au sens strict.

Tableau 1. L'agriculture de conservation dans certains pays européens indiqué par FAO-AQUASTAT, 2015.

Country	Conservation agriculture area ('1000ha)	Conservation agriculture area as % of arable land area
Belgium	0.268	
Finland	200	7.114
France	200	1.093
Germany	200	
Greece	24	
Hungary	5	0.1742
Ireland	0.2	0.0094
Italy	380	1.116
Netherlands	5	0.0495
Portugal	32	2.936
Republic of Moldova	40	2.205
Russian Federation	4500	3.758
Slovakia	35	0.7262
Spain	792	5.242
Switzerland	17	4.037
Ukraine	700	1.845
United Kingdom	150	2.415

1.2. Le concept de l'agriculture de conservation

L'agriculture de conservation (CA) est une approche de la gestion des agroécosystèmes pour une productivité améliorée et durable, qui repose sur un ensemble de techniques culturales favorables aux cultures, à l'environnement, et au sol. Ce nouveau concept d'agriculture repose sur trois axes principaux (**Figure 2**) (Hobbs et al. 2008; Friedrich et al. 2012 ; FAO 2010) :

- 1) La réduction voire la suppression du travail du sol, la technique de semis ne devant pas perturber plus de 20-25% de la surface du sol.
- 2) La couverture permanente ou semi-permanente du sol par les résidus de culture et/ou la couverture végétale vivante. Les objectifs visés étant la rétention de résidus suffisante sur le sol pour le protéger contre l'érosion hydrique et éolienne, assurant simultanément une réduction du ruissellement et de l'évaporation de l'eau du sol. L'amélioration de l'efficacité d'utilisation de

l'eau par les cultures et des propriétés physiques, chimiques et biologiques du sol associées à une productivité durable de long terme font également partie intégrantes des objectifs de l'AC.

3) La diversification des espèces cultivées à travers la succession ou rotation culturale judicieusement choisies, associant cultures principales et des cultures de couverture dites d'interculture. L'objectif est d'utiliser des rotations de cultures diversifiées pour aider à atténuer les problèmes de protection des cultures (adventices, maladies phytopathogènes et ravageurs des cultures).

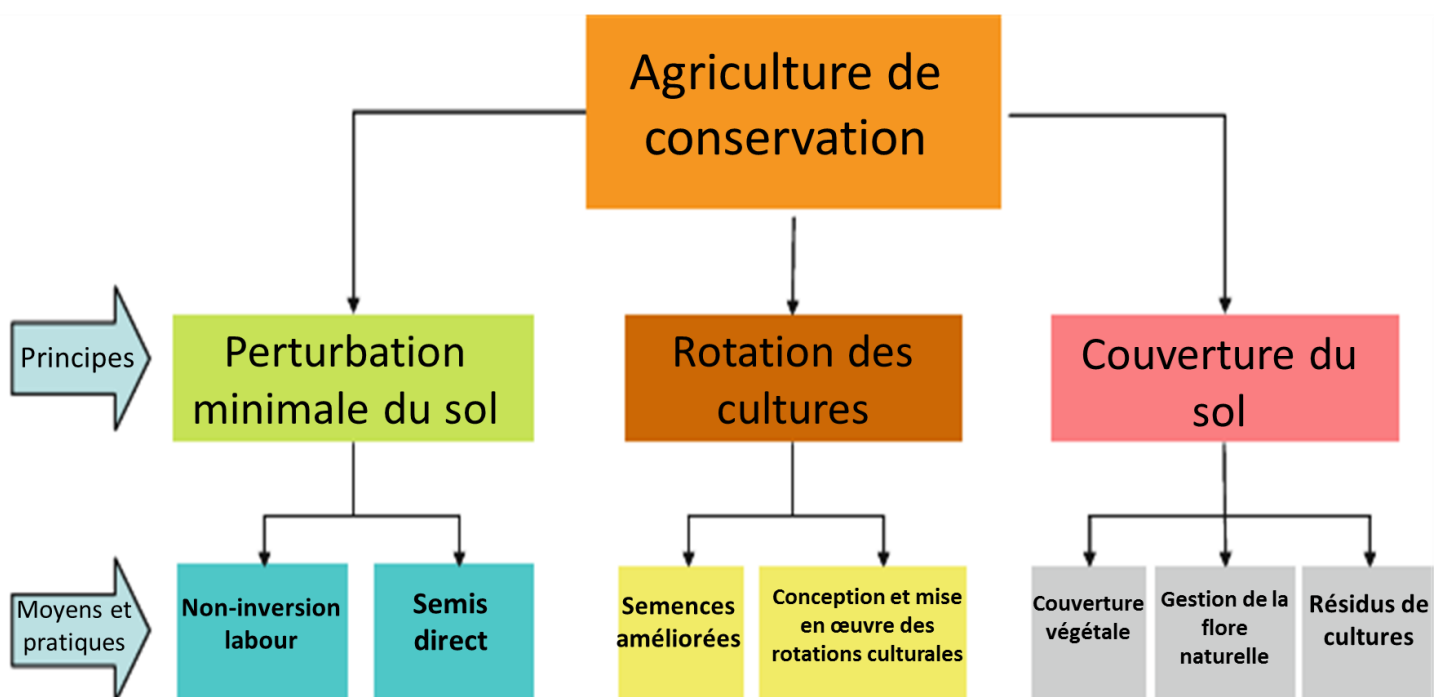


Figure 2. Les trois principes de l'agriculture de conservation et les principales pratiques et moyens associés (d'après Stagnari et al., 2009)

Le semis direct est ainsi une pratique de conservation des sols à part entière qui permet d'augmenter la teneur en matière organique des sols, de réduire l'érosion et l'utilisation de combustibles fossiles. En effet cette technique laisse en surface du sol les résidus des cultures précédentes et permet de répondre dans la majorité des situations culturales, au deuxième principe visant la couverture du sol.

1.3. Le semis direct sous couverture végétale (SDCV)

En agriculture de conservation, les résidus de culture restent à la surface du sol pour protéger l'horizon de la surface enrichi en matière organique, de l'érosion (Meijer et al. 2013; Wang et al. 2015). Toutefois, la mise en œuvre de ce principe est extrêmement diversifiée à travers le monde en fonction des modalités de travail et de couverture du sol (Lahmar et al. 2006) (Figure 3).

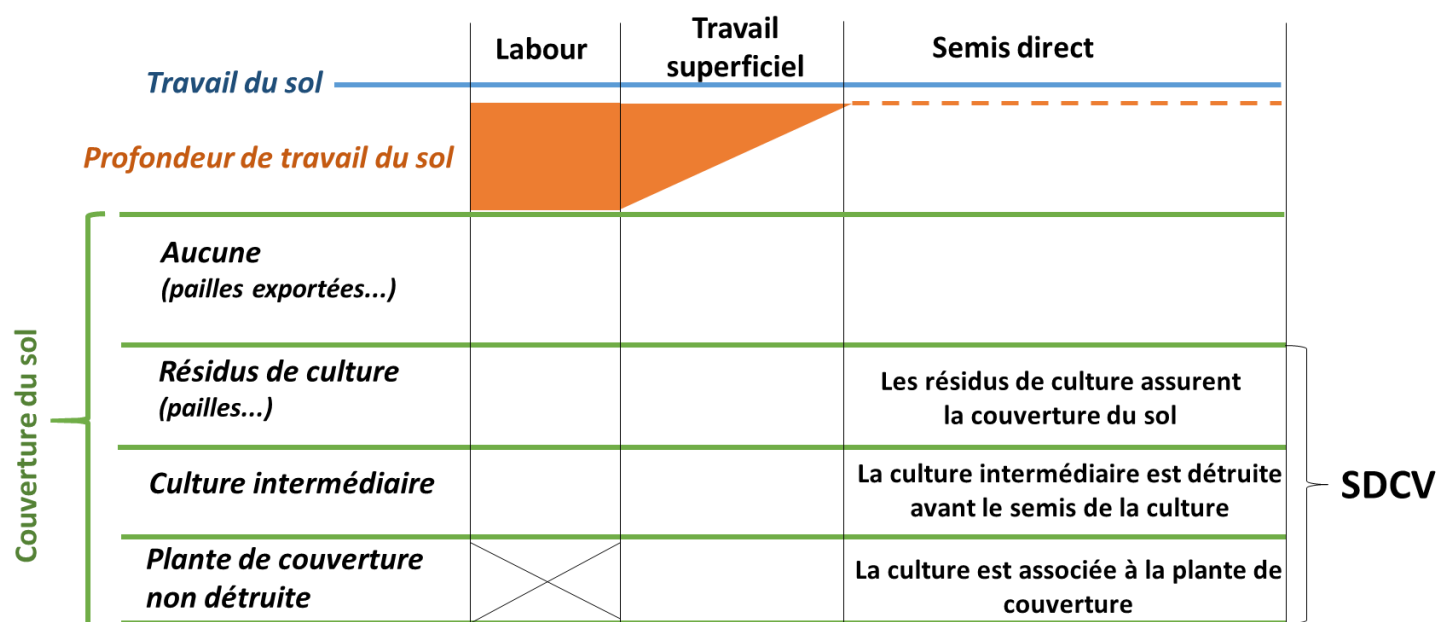


Figure 3. Description des principales pratiques culturales ; place des SDCVs parmi ces techniques (d'après Lahmar et al. 2006).

La recherche en agronomie/agroécologie sur le thème de la couverture de sols n'est pas récente, notamment grâce aux travaux initiaux du CIRAD et de l'IRD, et de nombreux autres travaux ont montré l'importance des résidus de culture sur la capacité de rétention en eau du sol et la réduction de l'érosion éolienne et hydrique (Van Doran and Allmaras 1978 ; Unger et al., 1988). Bissett and O'Leary (1996) ont montré que l'infiltration de l'eau sur le long terme, après 8 à 10 années d'agriculture de conservation était plus élevée par rapport au labour traditionnel qui ne laisse aucun résidu sur la surface. Kumar and Goh (1999) ont examiné l'effet des résidus de culture sur la qualité des sols, la dynamique de l'azote du sol et le rendement. L'étude a conclu que les résidus de culture ont une influence considérable sur la productivité agricole au travers leurs effets sur les propriétés physiques, chimiques et biologiques de sol. L'agriculture de conservation entraîne également des conséquences environnementales positives par

l'utilisation systématique des plantes d'interculture qui servent de pompe à nitrates. Lorsque le délai depuis la conversion en agriculture de conservation est important, l'adoption de l'ensemble des techniques permet la réduction des apports en éléments fertilisants et la mise en place de véritables filtres environnementaux entre la parcelle et le « milieu extérieur » (Miguel et al., 2011). En effet, le semis direct sous couvert végétal, permet de piéger l'azote non consommé par la culture principale en place et donc de diminuer l'apport en azote de synthèse sur les cultures et les pertes de nitrate par lixiviation (Farooq and Nawaz 2014). De plus, l'agriculture de conservation permet un stockage de carbone plus important qu'en labour (Roldán et al., 2003; Diekow et al., 2005) compatible avec les nouveaux objectifs de la COP 21 fixé pour la France à une augmentation de 4‰. L'agriculture de conservation est donc efficace pour stopper l'érosion des sols en ayant de nombreux impacts positifs sur la fertilité des sols au travers l'amélioration des propriétés physiques, chimiques et biologiques ainsi qu'au plan environnemental.

Parallèlement, au plan de la dynamique des cycles biogéochimiques dans les systèmes de culture couverts en permanence, c'est surtout la mise en place de processus écologiques nouveaux, inconnus des systèmes labourés et émiettés à la herse rotative, qui mobilise à l'heure actuelle la communauté scientifique internationale. Ainsi diverses associations symbiotiques n'existant pas ou peu dans les systèmes labourés ont pu être mise en évidence, telles que la mycorhization des cultures (Duan et al., 2010; Verzeaux et al., 2016b), le développement d'un mutualisme tripartite chez différentes cultures légumineuses associant mycorhize/rhizobium sur une même plante (Coventry and Hirth, 1992; Dogan et al., 2011), la fixation d'azote par la colonisation d'endophytes racinaires diazotrophes (Peters et al., 2003; Duan et al., 2010), ou encore la mise en place de complexes microbiens rhizosphériques permettant d'augmenter la croissance des plantes tel que les PGPR (plant growth promoting rhizobacteria) (Bhattacharyya and Jha, 2012).

Le terme de « soil health » en référence à la qualité du sol se définit comme la capacité des sols à fonctionner comme un écosystème vivant et indispensable pour soutenir la vie des plantes, des animaux et des Hommes (Carlisle, 2016). De même, il a pu être montré que l'état sanitaire des cultures dans les systèmes de cultures de semis direct sous couverture végétale stabilisés était améliorée de façon significative (Finley, 2016). Le terme de « complexe probiotique » a même été utilisé pour regrouper l'ensemble des relations écologiques plante/microorganisme ayant des conséquences favorables sur la santé des cultures, à l'image des effets des complexes probiotiques sur la santé des Hommes (Maheshwari, 2012).

Au plan de la dynamique de minéralisation des MO, il a pu être montré que l'incorporation de résidus végétaux frais, tels que des couverts végétaux chlorophylliens, à haute teneur en composés organiques, pouvait générer un « priming effect » qui stimule initialement, l'activité microbienne minéralisatrice des composés organiques anciens stabilisés dans le sol (Cardinael et al., 2015). La structure des communautés pouvant être affectées, principalement en fonction des conditions pédoclimatiques (Eglin et al., 2010), de l'intensité de la vie biologique initiale et du type de résidus restitués (Zhu et al., 2014b), incorporés plus ou moins profondément (labour et diverses TCS ou techniques culturales simplifiées) ou laissés à la surface du sol (semis direct sous couverture végétale). Cependant, si cette incorporation de matière fraîche tend à accroître la teneur en carbone organique du sol, elle pourrait aussi simultanément engendrer un déstockage du carbone organique préexistant (Guenet et al., 2010).

Le priming effect rhizosphérique PER peut ainsi engendrer une augmentation de la minéralisation de la matière organique du sol dans la zone rhizosphérique de 300 à 500 % mais aussi diminuer de 10 à 30% selon le type de plante considérée (Kuzyakov, 2002). Au cours de la vie de la plante, les microorganismes rhizosphériques hétérotrophes au carbone bénéficient ainsi des exsudats racinaires libérés par les racines sous forme de déchets carbonés organiques riches en sucres, en acides aminés divers et en acides organiques qui vont modifier leur activité initiale. Parmi ces microorganismes hétérotrophes figurent notamment ceux responsables de l'ammonification, consistant en la première étape de la minéralisation hétérotrophe de la MO des sols et conduisant à la production d'ammonium à partir de la matière organique (humus) des sols (Cheever et al. 2012). D'autres microorganismes rhizosphériques autotrophes au carbone CO_2 réalisent la nitrification autotrophe à partir de l'ammonium produisant des nitrates en profitant également de l'enrichissement local en CO_2 provoqué par la respiration racinaire (Liu, 2016). Dijkstra et al. 2006 ont observé des fortes augmentations dans la décomposition de la matière organique de sol causée par les effets rhizosphériques dans le sol. Ils ont montré que l'intensité du priming effet rhizosphérique sur la décomposition de la matière organique de sol est proportionnelle à la quantité de biomasse végétale produite, en particulier dans les sols ayant initialement une fertilité élevée, capable d'entretenir une productivité végétale soutenue.

Dès lors, il est aisé de comprendre que l'augmentation des entrées de matière organique via la couverture permanente des sols cultivés générant la somme des "priming effect" "engrais vert" et "rhizosphérique" doit être mieux considérée dans les modèles actuels. Leur bonne gestion pouvant permettre le maintien de la fertilité des sols conduits de cette manière.

1.4. Effet de l'agriculture de conservation sur les propriétés physiques des sols au travers leurs rôles sur la MO stable des sols.

La structure est un facteur clé dans le fonctionnement du sol et dans l'évaluation de la durabilité des systèmes de production agricole. En intervenant sur les modes d'incorporation des résidus dans le sol ainsi que sur les techniques de travail du sol, l'agriculteur peut agir sur les dynamiques de la décomposition de la matière organique. Le système de travail du sol détermine les modalités d'incorporation puis de décomposition de matières organiques fraîches retournant au sol en modifiant l'ensemble des propriétés physiques, chimiques et biologiques du sol. Le semis direct avec couverture végétale améliore la distribution de la taille des pores qui facilite la pénétration de l'air et de l'eau par rapport au labour classique (Govaerts et al., 2009). La perturbation physique de la structure du sol causée par le travail du sol se traduit par une répartition directe des agrégats du sol (Six et al., 2000) et des fragments de racines et hyphes mycorhiziens, qui sont les principaux agents de liaison de macro agrégats (Bronick and Lal, 2005).

Puisque la matière organique est un facteur clé dans l'agrégation du sol (Verchot et al., 2011), la gestion des résidus de récoltes est aussi une clé pour le développement structurel du sol et de la stabilité. Les résidus frais constituent le centre de nucléation pour la formation de nouveaux agrégats en créant des « hotspots » de l'activité microbienne où les nouveaux agrégats du sol vont pouvoir se développer (De Gryze et al., 2005; Kuzyakov and Blagodatskaya, 2015). Dans les systèmes avec travail du sol, l'incorporation des résidus est artificielle, d'origine physique et de fait de grandes quantités de résidus sont rapidement mis en contact intime avec le sol et la colonisation microbienne survient rapidement. Dans les systèmes sans labour, les résidus de culture ne sont pas incorporés artificiellement mais par l'ensemble des organismes incorporeurs de MO et les processus physico-chimiques, à l'origine de la modification profonde de l'édaphon du sol au plan taxonomique et fonctionnel. La durée d'incorporation des résidus est donc plus longue et ces derniers sont graduellement mis en contact avec le sol (Frey et al., 2000).

Au plan des propriétés physiques, la conductivité hydraulique du sol est également plus élevée dans les systèmes non travaillés mécaniquement, en raison de la plus grande proportion de macropores faisant suite à l'augmentation du nombre de biopores (McGarry et al., 2000; Eynard et al. 2004). L'agriculture de conservation peut ainsi augmenter l'infiltration et réduire le ruissellement et l'évaporation par rapport au labour conventionnel en condition de saturation des pores en eau (précipitations abondantes) ou au contraire maintenir l'humidité du sol et

accroître sa réserve utile en eau en condition de pore non saturés (précipitations faibles) est conservée et plus d'eau sera disponible pour les cultures (Soon et al., 2008; Ruiz-Colmenero et al., 2013).

Au plan de l'évolution des stocks de carbone, de nombreuses expérimentations de long terme (20 ans) ont montré des écarts de perte en carbone de l'ordre de 400 kg/ha/an entre système labour et de semis direct (Heenan et al., 2004). Enfin, Yoo et al. (2006) ont précisé que le zéro travail du sol renforçait la protection physique de carbone organique du sol (SOC) avec une augmentation de la densité apparente du sol.

1.5. Effet de l'agriculture de conservation sur la nutrition azotée des cultures

Le non travail du sol, la gestion des résidus et la rotation des cultures ont un impact significatif sur la distribution des nutriments et de ses transformations dans les sols (Galantini et al., 2000), au travers les modifications quantitative et qualitative du contenu en SOC des sols. Une augmentation de la stratification des éléments nutritifs est généralement observée, avec une meilleure disponibilité près de la surface du sol due au placement surfacique des résidus de récolte en système sans travail du sol comparativement aux systèmes labour où les résidus sont incorporés plus profondément (Duiker and Beegle 2006; Ismail et al. 1994). Le semis direct est généralement associé à une faible disponibilité d'azote en raison de son immobilisation par les résidus laissés sur la surface du sol (Bradford and Peterson 2000). Certaines études ont suggéré que la phase d'immobilisation de l'azote est transitoire, et que dans le long terme, cette immobilisation temporaire, dans les systèmes de conservation réduit la possibilité de lessivage et de dénitrification pertes de l'azote minéral (Follett and Schimel, 1989; Schoenau and Campbell, 1996).

Il a été bien documenté que la mise en œuvre des pratiques d'agriculture de conservation permettait d'augmenter le stock de carbone et d'azote organique des sols sur des essais à long terme (>10 ans) (Franzluebbers et al. 1994; Halvorson et al. 2002) ; de même vis-à-vis de la rotation des cultures (Robinson et al. 1996). Plusieurs études ont suggéré que l'augmentation en SOC en CA est suivie par une augmentation en SON (Janzen et al. 1997; Campbell et al. 1997; Maltas et al. 2007; Al-Kaisi et al. 2005), permettant d'assurer une croissance optimale des cultures. Ces résultats indiquent que le non-labour fourni plus d'azote à terme pour soutenir la croissance des cultures durant la saison de croissance (Lafond et al., 2011). Šíp et al. (2009) ont suggéré que le travail minimum du sol fournissait à la fois une amélioration de la disponibilité

de l'eau du sol pendant les phases critiques du développement et de meilleures conditions pour l'absorption d'azote. Ainsi, un meilleur rendement de blé a été obtenue en SD par rapport au "labour classique"

De même, le travail du sol intense est à l'origine de la disparition progressive des champignons mycorrhizogènes contrairement à ce qui se produit dans les sols conduits en semis direct sous couverture végétale permanente où les mycelia des champignons mycorrhizogènes non perturbés se maintiennent et assurent la mycorhization de nombreuses plantes cultivées telle le maïs et le blé (Brito et al. 2012). De telles associations symbiotiques améliorent de façon significative la capacité d'absorption en éléments nutritifs de type azote (Bücking and Kafle, 2015; Verzeaux et al., 2016b).

2. L'azote en agriculture : élément de contexte

De tous les éléments nutritifs, l'azote (N) est le plus important pour la croissance des cultures et le rendement potentiel. La disponibilité en azote minéral constitue un des principaux facteurs limitant la croissance des plantes en général et des céréales en particulier, que ce soit aux plans qualitatif et quantitatif. Il faut distinguer l'azote non-réactif sous forme de diazote (N_2) et l'azote réactif regroupant toutes les formes azotées biologiquement, chimiquement ou radiativement actives dans l'atmosphère et la biosphère terrestre et aquatique (Fowler et al., 2013). L'azote réactif inclut donc les formes de l'azote réduites (NH_3 et NH_4^+) ou oxydées (les oxydes d'azote $[NO_x]$, l'acide nitrique $[HNO_3]$ et l'acide nitreux $[HNO_2]$, le protoxyde d'azote $[N_2O]$, le nitrate $[NO_3^-]$, les nitrites $[NO_2^-]$) et les formes organiques de l'azote (l'azote organique dissous [DON], l'urée, les amines, les protéines et les acides nucléiques) (Galloway et al. 1995).

Les engrais azotés représentent 33% de la création annuelle totale d'azote réactif (Nr) ou 63% de toutes les sources anthropiques de Nr (Boyer et al. 2004). Selon Galloway et al. (2004), en 1990, les engrais azotés de synthèse représentaient plus du tiers de l'azote réactif créé au niveau continental (**Figure 4**). À cette date, l'homme était responsable de la création de 58% de l'azote réactif continental. Ainsi une fois le diazote converti en azote réactif, il est rapidement transformé d'une forme à une autre dans les différents compartiments terrestres entraînant des effets non négligeables sur la santé humaine et sur les écosystèmes.

Smil (1999) a estimé que seulement environ la moitié de tous les apports d'engrais azoté de synthèse sont prélevées par les cultures et leurs résidus (Hirel et al., 2011), le reste contribue de manière significative à l'enrichissement en Nr des eaux atmosphère, souterraines et de surface.

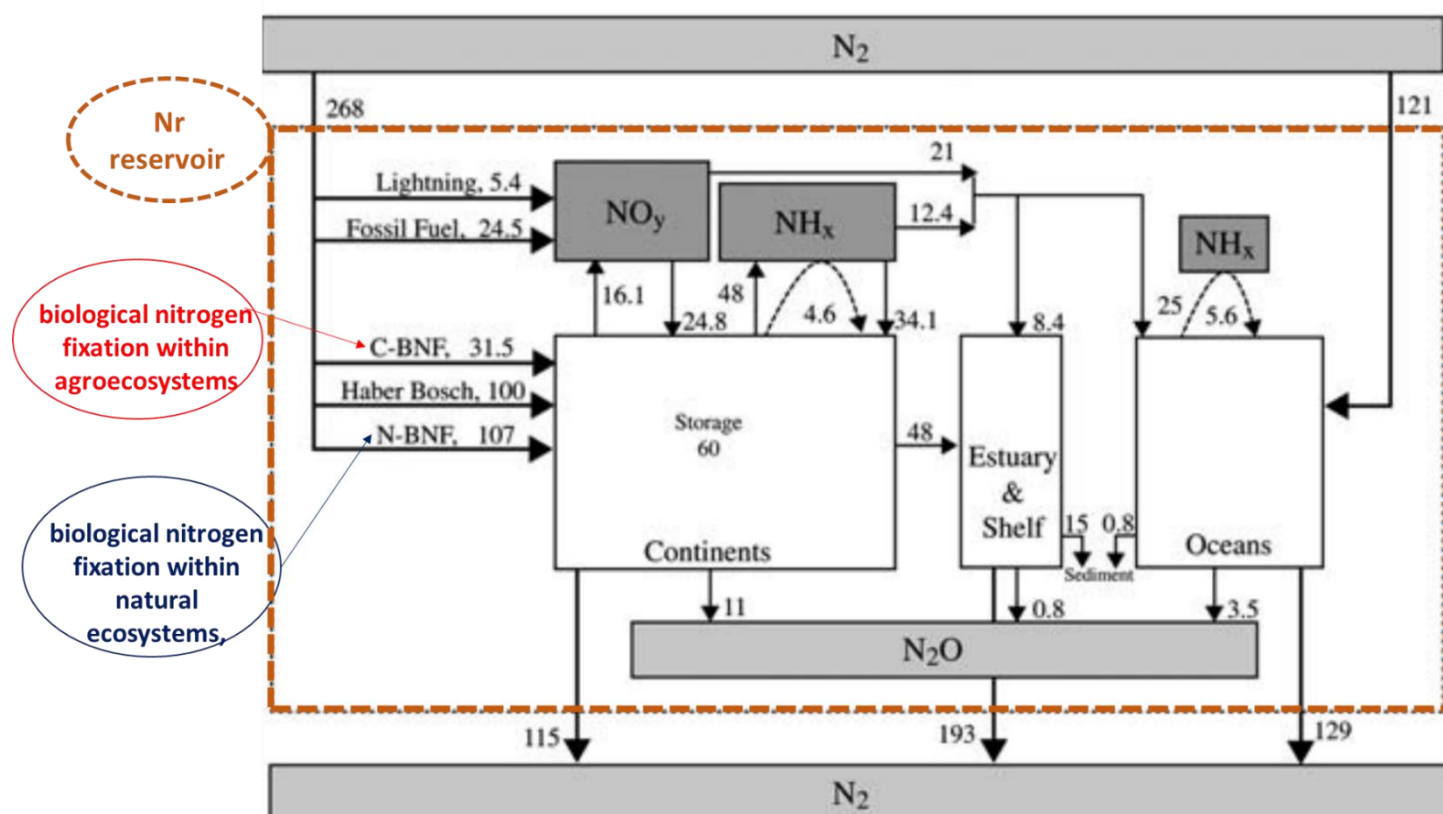


Figure 4. Représentation du cycle biogéochimique de l'azote en 1990 en $Tg\ N\ an^{-1}$ (d'après Galloway et al., 2004).

Une approche ciblée sur une forme d'azote ne peut pas être efficace, vu les transformations qui peut subir l'azote réactif. Pour cela, Oenema et al. (2009) a proposé le principe du tuyau percé (**Figure 5**) : lorsqu'on bouche un trou, on reporte la fuite vers un autre trou. Par exemple, limiter les pertes d'ammoniac a de fortes chances d'aboutir à une plus forte émission d'oxydes d'azote ou de nitrate. Les solutions les plus efficaces pour résoudre les problèmes liés à l'azote sont donc des approches systémiques, basées sur la réduction des intrants azotés à production constante ou croissante, ou sur l'augmentation de la production à intrants constants ou décroissants

Bodirsky et al. (2014) estiment que les pertes azotées liées à la production alimentaire mondiale évolueront de $185\ Tg\ N\ an^{-1}$ en 2010 à $232\ Tg\ N\ an^{-1}$ en 2050. L'amélioration de l'efficacité d'utilisation de l'azote de la production végétale permettrait de réduire les pertes azotées de $58\ Tg\ N\ an^{-1}$ (Sutton et al., 2011), et c'est la piste la plus prometteuse au niveau mondial puisqu'elle représente près de la moitié du potentiel de réduction des pertes (productions animale et végétale cumulées).

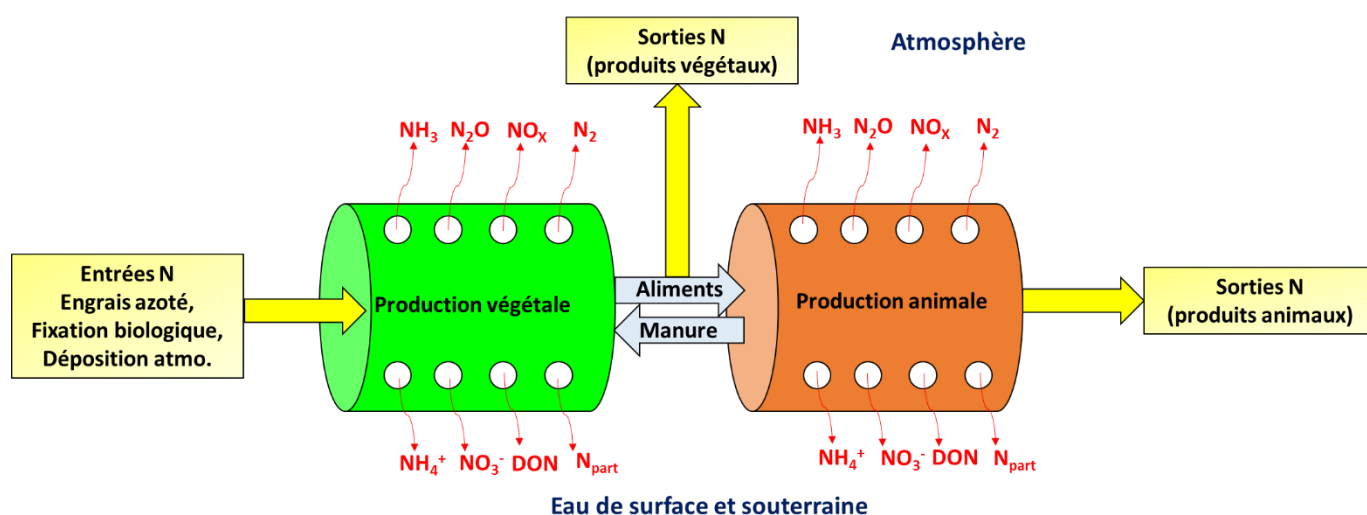


Figure 5. Le principe du tuyau percé pour les flux d'azote en production agricoles (d'après Oenema et al., 2009). N entrées, N sorties et N émissions dans l'environnement dépendent les uns des autres.

2.1. Les pertes d'azote par le système foliaire des cultures

Les transformations de composés azotés entre le sol et l'atmosphère ont été longuement analysées. Toutefois, la perte d'azote sous forme volatile par les parties aériennes des plantes reste un problème peu connu et très peu évalué et quantifié (Francis et al., 1993; Damin et al., 2010; Wang et al., 2013). Ainsi il apparaît que des pertes d'azote de l'ordre de 30 unités /ha pourraient se produire entre l'épiaison et la maturité du blé, notamment lorsque la température est forte. Ces nouvelles données sont importantes car jusque-là, seul l'azote volatilisé à partir du sol sous forme de NH_3 et/ou de $\text{N}_2/\text{N}_2\text{O}$ était considéré au niveau des bilans azotés des cultures. Cela revêt également un aspect important au plan physiologique, puisque si l'on intègre ces données à la réflexion, cela pourrait indiquer que les quantités d'azote réellement absorbées par les cultures déterminées à maturité, pourraient être plus importantes que celles trouvées, remettant partiellement en cause les bilans azotés des cultures tels qu'établis jusqu'actuellement.

D'autres formes de pertes d'azote par le système foliaire des cultures peuvent également être distinguées, telles que la translocation vers les racines et l'excrétion dans le sol (Reining et al., 1995) ; et la perte d'azote par l'élimination des parties de la plante comme le pollen, les fleurs, les feuilles et les fruits (Wetselaar and Farquhar, 1980).

La plante peut être un puit ou une source de NH_3 pour l'atmosphère. La direction et l'intensité des échanges stomatique d' NH_3 dépendent de la différence de concentration entre

l'atmosphère libre et l'intérieur des cavités sous stomatiques (Kruit et al., 2010) ; c'est cette concentration à l'intérieur des cavités sous stomatiques qui est appelée point de compensation stomatique (χ_s) (Farquhar et al., 1980) ou concentration de NH_3 pour laquelle aucun flux net ne se produit à travers les stomates. Ainsi, les plantes émettent du NH_3 quand la pression atmosphérique de NH_3 est inférieure à (χ_s) et absorbent du NH_3 par les stomates quand la concentration de NH_3 est supérieur à (χ_s) (Husted et al., 1996).

Les céréales peuvent libérer l'azote à partir de tissus végétaux principalement sous forme de NH_3 après la floraison (Francis et al., 1993). Rroço and Mengel, (2000) ont montré que l'émission maximum de NH_3 par le blé du printemps se produit lors de l'épiaison à la période de remplissage des grains. Bologna et al. (2006) ont conclu que les pertes d'azote les plus élevées ont été trouvées en post-floraison et à la maturité du blé avec 8,4% et 6,8% respectivement, de l'azote total appliqué. Kanampiu et al. (1997) ont estimé les pertes d'azote par la culture du blé à environ 27.9 kg ha⁻¹. La plupart des pertes d'azote ont eu lieu entre la floraison et 14 jours après la floraison. Ainsi il apparaît que des pertes d'azote de l'ordre de 30 unités /ha pourraient se produire entre l'épiaison et la maturité du blé, notamment lorsque la température est forte. Ces nouvelles données sont importantes car jusque-là, seul l'azote volatilisé à partir du sol sous forme de NH_3 et/ou de $\text{N}_2/\text{N}_2\text{O}$ était considéré au niveau des bilans azotés des cultures. Cela revêt également un aspect important au plan physiologique, puisque si l'on intègre ces données à la réflexion, cela pourrait indiquer que les quantités d'azote réellement absorbées par les cultures et déterminées à maturité, pourraient être plus importantes que celles trouvées, remettant partiellement en cause les bilans azotés des cultures tels qu'établis jusqu'actuellement.

La sénescence foliaire est l'un des stades de développement des plantes ayant également un potentiel élevé d'émissions de NH_3 (Vallis et Keating, 1994; Wang and Schjoerring, 2012; Cameron et al., 2013). A ce stade, il y a une augmentation de l'hydrolyse des protéines et une réduction des activités de la glutamine synthétase (GS) et de la glutamate synthase (GOGAT), enzymes responsables de l'assimilation de NH_4^+ dans les composés organiques. En outre, l'activité de la glutamate déshydrogénase (GDH), une enzyme qui agit sur la libération de NH_4^+ à partir du glutamate, augmente au cours de la sénescence (Lauriere and Daussant, 1983). Ces altérations physiologiques se traduisent par une plus grande concentration de l'ammonium dans la plante (Mattsson et al., 1998) et l'excès de NH_4^+ , qui est toxique pour la plante, peut être libéré par les stomates sous forme d'ammoniac (NH_3) (Mattsson et al., 2008). Les échanges avec la végétation jouent ainsi un rôle important dans la régulation des concentrations

atmosphériques et dans la détermination du transport à grande échelle de ces formes d'azote gazeuses (Sutton et al., 1994).

2.2. Efficience d'utilisation de l'azote

2.2.1. Définitions et indicateurs principaux de l'efficience d'utilisation de l'azote par les cultures

Selon Moll et al. (1982), la NUE correspond à la capacité de la plante à utiliser l'azote disponible déjà présente dans le sol pour produire un supplément de rendement. Divers indicateurs sont couramment utilisés dans les recherches agronomiques pour évaluer l'efficacité de l'azote appliqué (**Tableau 2**) principalement lorsque les objectifs d'études se concentrent sur la réponse des cultures à l'azote appliqué (Cassman et al. 2002). Ces indicateurs ou indices d'efficience azotée sont calculés, soit en se basant sur les différences de rendement et d'absorption totale de l'azote entre des parcelles fertilisées et non fertilisées « ou méthode de différence », soit en utilisant des engrais marqués ^{15}N pour estimer la récupération de l'azote par la culture (López-Bellido et al. 2005). Cependant, les valeurs obtenues avec la méthode à l'azote marqué sont souvent légèrement inférieures à ceux estimés par la méthode de différence (Ladha et al. 2005). Cela signifie que dans l'évaluation de l'efficience azotée, la différence entre la quantité d'azote exportée sur la parcelle fertilisée et la parcelle témoin est surestimée, en raison de certains effets confondants comme la substitution d'une proportion de l'azote minéral apporté par l'engrais au pool minéral initial du sol. L'engrais subirait le processus d'organisation, en échange d'une quantité équivalente d'azote natif pour la plante (Ladha et al. 2005). Egalement, un autre biais important existe au niveau de la méthode par différence, car l'architecture et le volume racinaire ont un développement différent en présence ou non de fertilisation azotée (Svoboda and Haberle, 2006).

Tableau 2. Indices Agronomiques de l'efficacité d'utilisation de l'azote

	NUE indice (kg kg⁻¹)	Calcul	Interprétation	Références
NUE	L'efficacité d'usage de l'azote	Rdt_x ou Rdt_0/N disponible	Plus important dans les conditions de disponibilité élevée de l'azote	(Moll et al. 1982; Huggins and Pan 1993)
NUE	L'efficacité d'utilisation de l'azote	Rdt_x ou Rdt_0/Nt	Important quand l'azote disponible dans le sol est faible	(Ma and Dwyer, 1998)
PFPN	Le facteur de productivité partielle de l'azote	Rdt_x/X	Un indice d'intégration qui quantifie la production totale en utilisant les sources de N engrais dans le system	(Yadav, 2003)
AEN	L'efficacité agronomique d'utilisation de l'azote	$(Rdt_x - Rdt_0) / X$	AEN est le produit de l'efficacité de la récupération de l'N appliquée, l'efficacité avec laquelle la plante utilise chaque unité supplémentaire de l'N appliqué	(Vanlauwe et al., 2011)
CAU/REN	Le coefficient apparent d'utilisation de l'azote ou l'efficacité de récupération de l'azote	$(Nt_x - Nt_0) / X$	Efficience dépend de la congruence entre la demande en N de la plante et la quantité d'azote libérée d'engrais azoté en fonction de la méthode fractionnement de l'azote retenue	(López-Bellido et al. 2005; Yan et al. 2014)
PEN	L'efficacité physiologique de l'utilisation de l'azote	$(Rdt_x - Rdt_0) / (Nt_x - Nt_0)$	La capacité de la plante à transformer l'azote provenant des engrais au rendement grain	(Jamaati-e-somarin et al. 2010)

Rdt_x : rendement grain dans les parcelles fertilisées en (kg ha⁻¹)

Rdt_0 : rendement grain dans les parcelles non fertilisées (kg ha⁻¹)

N disponible : l'azote disponible dans le sol pour la culture (kg ha⁻¹)

Nt_x : L'azote total de plante dans les parcelles fertilisées (kg ha⁻¹)

Nt_0 : L'azote total de plante dans les parcelles non fertilisées (kg ha⁻¹)

X : la quantité de fertilisation azotée (kg ha⁻¹)

- L'efficacité d'usage de l'azote (NUE) est un indicateur de performance d'utilisation de l'azote qui tient compte de l'azote disponible du sol pour la nutrition des cultures.
- L'efficacité d'utilisation de l'azote (NUE) est un indicateur de performance des plantes à faire plus ou moins de rendement en grains pour une unité d'azote absorbée.
- Le facteur de productivité partielle de l'azote (PFPPN) est quant à lui un indicateur à long terme. C'est une expression simple de l'efficacité de production calculée en unité de rendement obtenue par unité de nutriment appliquée. Les valeurs varient selon les types de cultures dans les différents systèmes de cultures, parce que les cultures diffèrent dans leurs besoins en nutriments et en eau.
- L'efficacité agronomique d'utilisation de l'azote (AEN) est un indicateur à court terme calculée en unités d'augmentation de rendement par unité de nutriment appliquée. Elle reflète plus étroitement l'impact direct des engrais appliqués sur la production. Cet indicateur est directement lié à la rentabilité économique. Le calcul des AEN nécessite la mise en place des parcelles avec sans apport azoté.
- Le coefficient apparent d'utilisation de l'azote ou l'efficacité de récupération de l'azote (CAU, REN) est un indicateur d'efficacité des pratiques culturales qui permet de vérifier le potentiel de perte d'éléments nutritifs par système de culture. C'est l'expression de NUE préféré par les scientifiques qui étudient la réponse de la plante aux éléments nutritifs comme l'azote.
- L'efficacité physiologique de l'utilisation de l'azote (PEN) est un indicateur d'efficacité de la plante à transformer l'azote absorbée en rendement économique. Elle définit l'augmentation de rendement par rapport à l'augmentation de l'absorption de l'azote par les plantes.

2.2.2. Stratégies pour améliorer l'efficacité d'utilisation de l'azote des cultures

Différents processus physiologiques complexes sont impliqués dans le contrôle de la NUE de la plante, incluant l'absorption de l'azote du sol et l'assimilation et la translocation de l'azote (Masclaux-Daubresse et al. 2010). En raison de cette complexité, plusieurs stratégies sont possibles pour améliorer la NUE tels que : la sélection des génotypes économes en intrants azotés (Hirel et al. 2007; Gastal et al. 2015) et/ou volatilisant moins d'azote à partir des organes foliaires (Mattsson et al., 1997); l'utilisation des techniques de manipulation génétique (Hirel et al. 2011; Han et al. 2015) ; la synchronisation des apports d'azote avec la demande des plantes

(Chen et al., 2006). Les nouvelles pratiques culturales agroécologiques permettant quant à elle d'augmenter la mise en œuvre de symbioses mutualistes fixatrices d'azote minéral N_2 et/ou capables d'exploiter l'azote sous forme organique (Wang et al., 2016) sous les actions combinées du non travail du sol et de la couverture des sols en période d'interculture. Le non travail du sol et l'introduction en interculture d'associations culturales riches en légumineuses pourrait ainsi permettre de remplacer progressivement une partie des engrais azotés de synthèse dont l'efficacité d'utilisation par la plante est faible diminuant ainsi les impacts négatifs des engrais azotés sur la qualité de l'air et de l'eau (Verzeaux et al., 2016a; b).

2.2.2.1. La sélection variétale pour optimiser l'assimilation de l'azote

L'évolution de l'agriculture au plan historique, résultant majoritairement des actions combinées de l'amélioration génétique et des progrès des techniques agricoles, s'est essentiellement tournée vers l'augmentation de la quantité de biomasse issue de cultures principales obtenue par unité de surface sans se préoccuper simultanément des autres possibilités d'augmentation des quantités de biomasse produites par l'allongement des durées de cultures des sols via la couverture permanente des sols cultivés.

Au plan génétique, suite à la mise en évidence de la variabilité génétique existante chez les céréales pour la nutrition azotée (Hirel et al. 2007; Le Gouis et al. 2004), la sélection de nouveaux génotypes économes en intrants azotés a nécessité la mise au point simultanée de marqueurs physiologiques et moléculaires capables de révéler les mécanismes physiologiques régulateurs impliqués dans les rendements d'assimilation de l'azote, et adaptés à la réorientation des objectifs de production dans un contexte de développement durable.

Ce défi est particulièrement pertinent pour les céréales pour lesquelles de grandes quantités d'engrais azoté sont nécessaires pour atteindre un rendement maximal et pour lequel la NUE est estimé à moins de 50% (Raun and Johnson 1999 ; Zhu 2000; Hirel et al 2007). C'est pourquoi, il est utile de transposer au plan agronomique, la validation des premiers marqueurs physiologiques pour caractériser parmi les variétés de céréales actuelles, celles qui par leur rendement d'assimilation azoté présenteraient la meilleure efficacité de conversion photosynthétique. Des gènes clés spécifiques ont été ciblés comme des critères potentiels pour l'amélioration de NUE (McAllister et al. 2012). Les céréales en général doivent remobiliser l'azote accumulé dans les tissus végétatifs en période de pré-floraison vers les grains et l'assimiler également en période de post-floraison afin de produire une efficacité azotée plus élevée (Fageria, 2014). L'amélioration de l'assimilation de l'azote doit être recherchée par un

accroissement de l'activité des enzymes clés du métabolisme de l'azote (glutamine synthétase, glutamate synthase, ...) ou d'enzymes à l'interface entre les métabolismes carboné et azoté comme l'alanine amino-transférase (Le Gouis, 2012). Parallèlement, les marqueurs physiologiques seraient également évalués en tant qu'outil de diagnostic au champ, permettant ou non le typage de la fertilisation azotée en fonction des variétés ou groupes de variétés orientées en mode de production économe en azote (Le Gouis, 2012).

2.2.2.2. Maximiser l'absorption d'azote par le système racinaire

L'utilisation efficace de l'azote doit commencer par une capture ou absorption efficace de l'azote par le système racinaire dépendant du volume et de l'architecture racinaire (Ran et al., 1994). Le volume et l'architecture racinaire étant fortement tributaire de la qualité de la structure des sols, explique pourquoi la gestion de cette dernière devrait constituer un objectif majeur de la gestion des systèmes agricoles, bien avant la gestion de l'azote par exemple. Or, il faut bien être conscient qu'à l'heure actuelle, les agriculteurs disposent de très peu d'outils d'aide à la décision pour déterminer si des actions de type décompaction ou, fissuration des sols doivent être ou non envisagées, particulièrement dans les systèmes de culture sans labour où le risque de compaction est plus important dans les sols limoneux.

L'azote est préférentiellement absorbé sous forme de nitrates (NO_3^-) (Maathuis, 2009), bien que la plante dispose de mécanismes permettant l'absorption d'ammonium (NH_4^+) et d'acides aminés (Hawkesford, 2014).

Les plantes ont développé la faculté de moduler leur architecture racinaire en fonction de la disponibilité en nitrates du milieu, qui affecte à la fois le nombre ainsi que la position des sites d'initiation de racines latérales (Malamy and Ryan, 2001). Ainsi, lorsque le milieu ne contient pas ou peu de nitrates, l'initiation de racines latérales est inhibée et la racine croît uniquement en longueur. Lorsque la racine atteint une zone plus riche en nitrates, l'initiation de racines latérales est désinhibée et les racines prospectent alors efficacement le volume de sol environnant. Ce mécanisme adaptatif présente l'avantage de favoriser la prolifération de racines latérales dans les zones riches en nitrates, et d'ainsi optimiser par retour la quantité de carbone alloué aux racines pour répondre aux besoins de croissance du système racinaire.

L'absorption racinaire des nitrates à partir du milieu est un processus actif qui nécessite des apports énergétiques issus du métabolisme du carbone. Il repose principalement sur l'action de deux familles de transporteurs racinaires différenciées par leur affinité pour le nitrate : les transporteurs à basse affinité (« Low Affinity Transport System » ; LATs) et les transporteurs

à haute affinité (« High Low Affinity Transport System » ; HATS) (Daniel-Vedele et al. 1998; Williams and Miller 2001). Le système HATS étant capable de transporter les ions NO_3^- lorsqu'ils sont présents dans le milieu à des concentrations inférieures à 500 μM . Il présente une cinétique de saturation de type Michaelis-Menten et une sensibilité à la température et aux inhibiteurs du métabolisme (Glass et al. 1992). A l'inverse, le système LATS opère pour de fortes concentrations externes ($> 500 \mu\text{M}$) en NO_3^- . Il présente une cinétique linéaire en fonction de la concentration en NO_3^- et il est moins sensible à la température et aux inhibiteurs du métabolisme que le système HATS (Glass et al. 1992).

La plupart des études publiées ont été effectuées sur des espèces modèles telle que *Arabidopsis thaliana*, mais une récente étude réalisée sur le maïs a permis de quantifier l'expression des deux systèmes, haute et basse affinité, sur l'ensemble du cycle de vie et a démontré comment l'expression spécifique des gènes était couplé à la demande en azote (Garnett et al., 2013).

Le fonctionnement racinaire doit être optimal tout au long du cycle de culture, avec un rôle important d'absorption en période de post floraison du blé contribuant à la qualité des cultures (Kichey et al., 2007). Au Royaume-Uni, il a été montré que les variétés modernes de blé pouvaient être moins efficaces que les variétés anciennes pour prélever l'azote du sol comparativement à l'azote issu de l'engrais (Foulkes et al., 1998). Une hypothèse pour expliquer ce résultat serait l'absence de sélection directe pour le système racinaire (Le Gouis, 2012). Les idéotypes pour les systèmes racinaires efficaces comprennent la prolifération près de la surface et en profondeur sont intégrés au schémas de sélection (Foulkes et al., 2009).

Cependant, les mécanismes d'absorption très complexes et efficaces qui existent dans les plantes cultivées offrent probablement peu de possibilités de sélection pour une meilleure performance d'absorption de l'azote minéral à l'heure actuelle.

2.2.2.3. Favoriser les interactions racines-microorganismes du sol

La rhizosphère des plantes est un environnement riche en composés organiques qui favorisent la croissance et l'activité microbiennes (Bais et al., 2006). Certaines bactéries rhizosphériques dites PGPR (Plant Growth-Promoting Rhizobacteria) stimulent directement la croissance des plantes lorsqu'elles sont associées aux racines (Vessey, 2003; Lugtenberg and Kamilova, 2009). Ces PGPRs sont réparties en trois catégories (i) les PGPR phytostimulatrices qui stimulent directement la croissance des plantes via une modification de leur balance hormonale en produisant des phytohormones (e.g. auxine, cytokinine) (Filippi et al., 2011; Abbasi et al., 2011) et l'amélioration de leur nutrition hydrominérale en augmentant le

prélèvement des éléments nutritifs du sol (*e.g.* fixation biologique de l'azote BNF (Fischer et al., 2007), solubilisation des phosphates (Krey et al., 2013)); (ii) les PGPRs phytoprotectrices qui protègent la plante contre les pathogènes par la production de composés antimicrobiens et/ou par l'induction de la résistance systémique de la plante (Lugtenberg and Kamilova, 2009; Glick et al., 2012; Pérez-Montañón et al., 2014); (iii) les PGPR auxiliaires qui favorisent la mise en place d'autres symbioses plantes-microorganismes, notamment avec les champignons mycorhiziens et/ou les bactéries nodulantes (de Varennes and Goss, 2007; Dardanelli et al., 2008; Fox et al., 2011).

L'utilisation de PGPRs est ainsi une stratégie efficace pour améliorer la production et l'état nutritionnel des cultures, réduisant en conséquence le besoin d'engrais chimiques sous forme soluble. Par exemple, l'inoculation de la semence de blé avec *Pseudomonas fluorescens* a permis la diminution d'utilisation d'engrais azotés, phosphatés et potassiques (Shaharoon et al., 2008). De même, une meilleure nutrition azotée a pu être observée dans le cas des associations *Azospirillum*-céréales, et *Bacillus*-céréales (Gaskins et al., 1985). Plusieurs études ont également montré une augmentation du rendement grain et d'efficacité azotée chez le blé et le maïs lorsque les plants ont été inoculés avec *Burkholderia cepacia*, *Azospirillum brasilense* et *Pseudomonas aurantiacada* (Riggs et al., 2001; Saubidet et al., 2002; Cassá N et al., 2008; Rosas et al., 2009).

2.2.2.4. Optimiser l'efficacité photosynthétique et la remobilisation de l'azote

Améliorer l'efficacité photosynthétique a le potentiel d'augmenter le rendement des cultures (Dawson et al. 2008). Les voies pour améliorer l'efficacité photosynthétique peuvent être d'exploiter la variation naturelle des processus impliqués dans la photosynthèse, ou par manipulation des voies biochimiques directement, en ciblant la régénération de la RuBP et les propriétés catalytiques de Rubisco (Parry and Reynolds, 2007; Reynolds et al., 2009). En général chez les céréales, de 40 à 90 % de l'azote des grains provient de la remobilisation d'azote absorbé avant floraison et stocké dans les parties végétatives. Cette remobilisation se traduit par la sénescence complète de l'ensemble de l'appareil végétatif et l'arrêt de la photosynthèse (Le Gouis, 2012). Les génotypes ayant une surface foliaire verte et restant active après la floraison, de type "stay-green" ont une plus grande capacité à absorber l'azote pendant le remplissage du grain, grâce à une prolongation de la période d'assimilation du carbone autorisant indirectement une absorption prolongée de l'azote du sol (Woodruff 1972). Le contrôle de la sénescence foliaire influence également le rendement et la concentration en

protéines des grains. Les variétés à sénescence retardée ont une durée de photosynthèse plus longue et donc un rendement plus élevé, si aucun stress abiotique de type thermique et/ou hydrique ne vient s'exercer en période post-floraison (Thomas and Howarth, 2000). Les mutants stay-green pour le blé dur rapportés par Spano et al. (2003), montrent une augmentation du rendement et une amélioration de leur capacité d'absorption en azote.

3. La fertilisation azotée des céréales : gestion et raisonnement

3.1. La gestion de la fertilisation azotée

La demande mondiale d'engrais azotés de synthèse a augmenté de 111 400 000 tonnes en 2013 à 113 100 000 tonnes en 2014, soit un taux de 1,5 pour cent de croissance. Il devrait être d'environ 119 400 000 tonnes en 2018 avec une croissance annuelle de 1,4 % (FAO, 2015). A ce jour, plus de la moitié de l'azote fixé chimiquement est utilisé par l'agriculture. Cette quantité correspond à plus de 80 Mt par an dans le monde entier (Hawkesford, 2014), dont environ 47 Mt appliquée aux céréales. Au niveau mondial entre les années 1961-2010, le maïs, le riz et le blé ont reçu un total de 1594 Tg d'engrais azoté (Ladha et al., 2016). Le taux d'application d'azote ($\text{kg ha}^{-1} \text{ an}^{-1}$) suit une légère tendance curviligne (Figure 6).

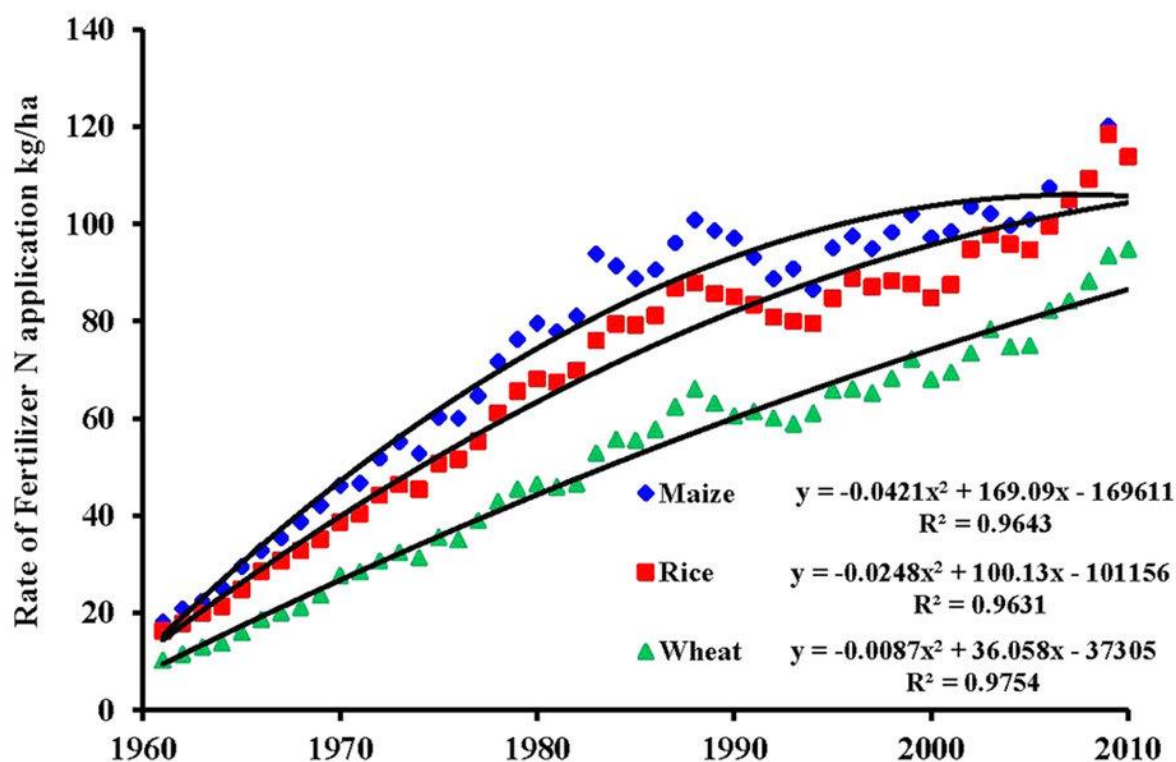


Figure 6. Evolution des apports d'engrais azoté sur le maïs, le blé et le riz entre 1960 et 2010 (moyennes mondiales en kg/ha, d'après Ladha et al., 2016)

De tous les éléments nutritifs, l'azote est le plus important pour la croissance des cultures et le rendement potentiel, hors cultures légumineuses. Une quantité optimale d'azote dans le sol stimule l'absorption des autres éléments nutritifs et favorise le développement de la plante et des racines (Haynes, 1986). Par ailleurs l'excès d'azote sur la plante entraîne un certain d'effets négatifs : excès de biomasse qui augmente la tendance à la verse des céréales, retard des plantes à la maturité, sensibilité plus grande aux maladies cryptogamiques. C'est pourquoi son emploi en agriculture est plus délicat que celui des autres éléments (Cassman et al., 2002). Une gestion adaptée de la fertilisation azotée doit permettre de satisfaire les besoins de la plante pour obtenir un bon rendement en termes de quantité ou de qualité, en évitant toute perte d'azote minéral et gazeux vers l'environnement avoisinant.

3.2. La courbe de réponse à l'azote

Lorsque que l'on teste l'effet de doses croissantes de fertilisants azotés sur le rendement d'une culture, on obtient une courbe de réponse à l'azote (Angus 1995). Ces courbes de réponse des cultures aux engrais azotés se décomposent généralement en trois phases (Figure 7) (Simon and Le corre, 1992 ; Franc 1996).

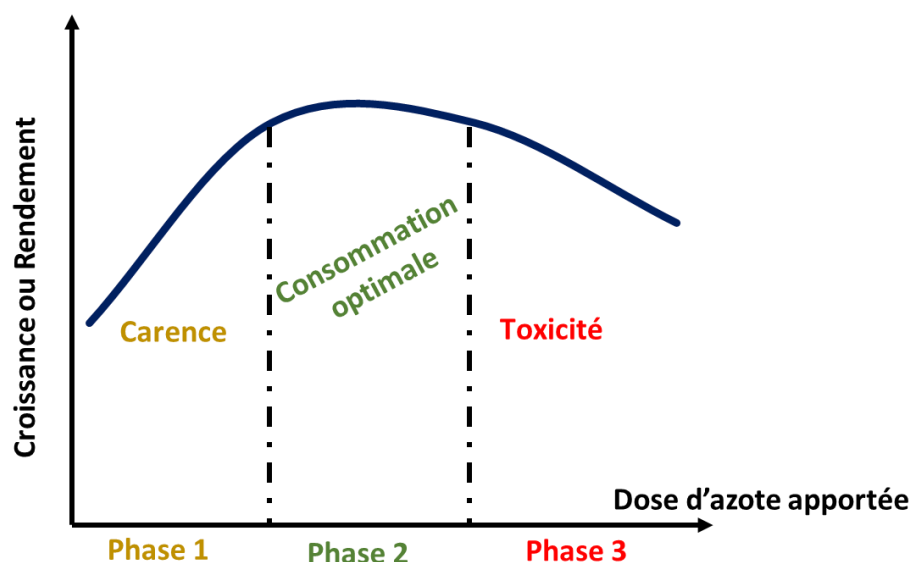


Figure 7. Courbe de réponse de la culture de céréale (ex. Blé) aux apports d'engrais azotés (d'après Franc, 1996).

Les fortes déficiences en azote se traduisent par des carences en nutriments et baisse des rendements (phase 1). Pour des doses croissantes d'azote, la production augmente jusqu'à une valeur maximale (phase 2). On estime que le potentiel génétique de la plante est atteint. A l'approche du niveau d'apport optimal, la courbe tend vers une asymptote. Les augmentations supplémentaires d'apport azotés n'augmentent plus la production, et deviennent même toxiques pour la plante et le rendement diminue (phase 3). La dose optimale d'azote est définie comme la plus petite quantité d'azote permettant d'obtenir le rendement brut maximal (Antoniadou and Wallach, 2002; Henke et al. 2007). Lorsque l'on dépasse cette dose optimale, l'azote supplémentaire n'est pas utilisé par la plante, car la production est généralement limitée par un d'autres facteurs, augmentant parallèlement les risques de pollution environnementale (Henke et al. 2007). Néanmoins, il faut souligner qu'à l'heure actuelle il n'existe pas de courbes de réponses à l'azote différencié pour les différents types de systèmes de culture, ie, pour les différents systèmes de cultures testés dans le cadre de ce travail.

3.3. Le raisonnement de la fertilisation azotée

Le raisonnement de la fertilisation azotée est défini par le COMIFER (1996) comme une méthode permettant d'ajuster les apports d'engrais minéraux ou organiques aux besoins de la culture pour atteindre un objectif de production donné en prenant en compte les autres fournitures d'azote par le sol. Néanmoins, il s'agit d'une méthode et plusieurs autres méthodes reposant sur des concepts différents sont élaborées mondialement pour le calcul de la dose totale d'engrais nécessaire aux cultures de céréales.

3.3.1. “Nmin method”

L'évaluation de l'azote minéral résiduel a suscité l'intérêt des chercheurs dans plusieurs régions du monde, notamment en Allemagne (Wehrmann and Scharpf, 1979), Canada (Soper and Huang, 1963) et Pays-Bas (Borst et Mulder, 1971). Le principe de Nmin méthode consiste à évaluer la quantité d'azote minéral présente dans le sol à la sortie de l'hiver (Wehrmann and Scharpf, 1979). La profondeur de prélèvement des échantillons de sol doit correspondre à la profondeur d'enracinement de la culture. Cette quantité d'azote minéral du sol est ensuite soustraite d'une valeur cible de fertilisation azotée afin d'établir les recommandations finales d'azote à apporter. La valeur cible de la méthode Nmin représente la quantité totale d'azote qu'il faut apporter à une culture pour obtenir un rendement optimal (Scharpf et al., 1991). Pour chaque culture, il y a une teneur cible précise en azote pour atteindre un maximum de croissance et de rendement. La valeur cible est déterminée expérimentalement et est la somme de l'azote déjà disponible dans le sol et de l'azote apporté par l'épandage d'engrais. Pour les céréales, il a été déterminé que la valeur cible variait de 140 (seigle) à 200 (blé) kg N/ha (Wehrmann and Scharpf, 1986). Néanmoins, la méthode Nmin telle que développée dans les pays nommés ci-dessus ne tient pas compte de l'incorporation de matière organique fraîche qui peut libérer une quantité additionnelle d'azote au cours de la saison. Les effets du milieu, les caractéristiques du sol et les pratiques culturales qui influent sur la minéralisation de l'azote varient considérablement d'une région à l'autre.

La détermination de l'azote minéral présent à l'entrée du bilan sur le profil racinaire fait partie intégrante de la méthode COMIFER (terme Ri ou Rh ou Re de l'équation du bilan de fertilisation azoté). Egalement, la quantité d'azote minéral trouvée lors de l'analyse de l'azote résiduel (Nmin de l'équation du bilan de fertilisation COMIFER) est considérée comme étant utilisée à 100%, ce qui n'est pas le cas. Il a en effet été montré récemment que la biomasse

microbienne du sol était capable de prélever 7 fois plus d'azote minéral que les cultures (Liu et al., 2016).

3.3.2. Les sondes PRS (*Plant root simulator*)

Pour d'autres pays tels le Canada et les Etats Unis, il existe beaucoup d'autres méthodes de calcul, notamment la méthode PRS pour « plant root simulation » qui détermine les flux d'éléments nutritifs minéralisés à partir du sol au moyen de membranes anioniques et cationiques posées directement dans le sol (Western Ag innovation, 2016). Le modèle PRS ayant été paramétré à partir des quantités d'éléments nutritifs réellement retrouvés dans la plante à différents stades de la culture par analyse ICP. Puis dans un second temps, ces quantités sont mises en corrélation avec les flux d'éléments nutritifs captés par les membranes anioniques et cationiques enfoncées dans le sol pour les mêmes stades de culture. Cette méthode présente l'avantage de capter directement les flux d'éléments minéraux présents dans le sol ne nécessite pas d'étapes d'extraction des éléments minéraux par des solvants chimiques sensés mimés l'action de la racine contrairement à ma méthode du COMIFER. Ainsi, par exemple, la méthode de référence utilisée pour doser l'azote minéral du sol utilise t'elle du KCl à 1mole/litre, ce qui ne correspond pas à la situation réelle se produisant au champ dans la solution ionique du sol.

3.3.3. Méthode d'estimation prévisionnelle des besoins en azote des céréales (*b de l'équation du bilan de fertilisation*)

Les besoins d'une culture en azote sont définis comme les quantités que celle-ci doit absorber à chaque instant (besoins instantanés) ou sur l'ensemble du cycle (besoins totaux) pour obtenir le rendement optimum et la meilleure qualité possible (Coïc 1956). En d'autres termes, c'est la quantité d'azote nécessaire permettant à une culture d'atteindre un objectif de croissance et de rendement. L'estimation des besoins en azote de céréale est fondée sur la fixation d'un objectif de rendement.

Besoins (b) = objectif

(b) est la quantité d'azote nécessaire pour produire 1 quintal de grains (kg N/q).

Pour le blé dur à 14 % de teneur en protéines, le coefficient b est fixé à 3,5 Kg N/q de grains (Gate 1995). Le coefficient b tel que déterminé par la méthode COMIFER varie cependant en fonction des variétés (**Tableau 3**).

Tableau 3. Indicatif des besoins en azote pour quelques grandes cultures (d'après COMIFER, 1996)

Culture	Besoin en azote par quintal de grain récolté kg/q
Blé tendre d'hiver	3
Orge d'hiver	2,4
Blé tendre de printemps	3
Orge de printemps	2,2
Blé dur	3,5
Colza	6,5
Maïs grain	2,3
Tournesol	4,5

Avec cette méthode, l'estimation de l'objectif de rendement peut se faire dès le semis en fonction des potentialités de la variété et les conditions pédo-climatiques, et être éventuellement révisée au cours du cycle, à la hausse ou à la baisse au cours du cycle cultural, en fonction des fractionnements de la dose X apportée (généralement 3 apports).

L'objectif du fractionnement des apports azotés étant de se calquer sur les besoins de la culture au cours du cycle de développement en apportant les quantités proportionnellement aux besoins de la culture, minimisant ainsi les pertes par volatilisation, dénitrification et lessivage.

Néanmoins, il faut tenir compte que la détermination du (b) a été réalisée principalement dans les systèmes labourés, non couverts en permanence et sans considérer l'historique des systèmes de cultures. Ainsi, la détermination du (b) fait abstraction des niveaux d'interactions différents pouvant se produire entre les différents postes du bilan, notamment valables pour les systèmes de culture non labourés et couverts en permanence, pour lesquels de nombreux processus écologiques de type mutualiste peuvent se mettre en place. Egalement, l'ensemble des paramètres du milieu tel que le mode de travail du sol, affectant la répartition et le volume racinaire, ne sont pas pris en compte dans la détermination du (b).

3.3.4. Méthode du bilan prévisionnel

Le concept de bilan d'azote minéral du sol prévisionnel développé par Hébert en 1969 puis complétée par Rémy and Hébert en 1977. Elle consiste à équilibrer les besoins prévisibles des cultures avec les fournitures d'azote du sol. L'ouverture du bilan se situe en fin d'hiver où le lessivage des ions nitrates est négligeable et sa fermeture a lieu à la récolte. L'équation est sous la forme (Meynard et al. 1996) :

$$\text{Etat final} - \text{Etat initial} = \text{Entrées} - \text{Sorties}$$

$$R_f - R_i = (M_n + X) - (P_f - P_i + L)$$

La dose d'engrais azotée à apporter s'obtient par :

$$X = \text{Besoins de la culture} - \text{Fourniture du sol}$$

$$X = (Pf - Pi) - (Mn + Ri - L - Rf)$$

Avec (en kg):

Rf = Quantité d'azote minéral dans le sol à la fermeture du bilan (récolte).

Ri = Quantité d'azote minéral dans le sol à l'ouverture du bilan (fin d'hiver pour les cultures d'hiver).

Mn = Minéralisation nette de l'azote dans le sol = Minéralisation nette de l'azote humifié du sol (Mh) + Minéralisation nette des résidus de récolte (Mhr) + Minéralisation nette de l'azote des produits organiques (Ma).

X = Quantité d'azote minéral apporté par l'engrais.

Pf = Quantité d'azote absorbé par la culture jusqu'à la fermeture du bilan.

Pi = Quantité d'azote contenu dans la culture à l'ouverture du bilan.

L = Lessivage d'azote nitrique au-delà de la profondeur « z » considérée comme accessible aux racines de la culture, entre l'ouverture et la fermeture du bilan.

La valeur de Mh est calculée à partir d'un modèle qui prévoit la valeur de K_2 (coefficient de minéralisation de la matière organique) dépendant de la teneur en argile et en CaCO_3 , de la teneur en azote du sol, de la masse de sol, d'un coefficient d'occupation du sol et d'un facteur climatique (Limaux, 1999). Cette méthode a été largement vulgarisée en France grâce à la mise au point du logiciel AZOBIL (Machet et al. 1990) et AzoFert (Machet et al., 2007). Son utilisation dans la pratique courante ne nécessite que la mesure du terme Ri .

Au niveau de la détermination du terme Mh de l'équation du bilan azoté, de grandes incertitudes peuvent aussi demeurer dans la mesure où il a été démontré récemment que la quantité d'azote organique minéralisée annuellement pouvait être multipliée par 2 ou 3 dans le sol rhizosphérique par le mécanisme du priming effect rhizosphérique (Kuzyakov, 2002; Zhu et al., 2014a), en fonction des cultures et des systèmes de cultures. Pourtant, la formule de calcul du terme Mh ne comporte actuellement aucun coefficient permettant de prendre en compte l'intensité du priming effect rhizosphérique en fonction des cultures et des systèmes de culture,

ni non plus en fonction de l'architecture et du volume racinaire pouvant se développer différemment dans les sols non labourés.

Egalement, dans cette méthode, il faut tenir compte que la quantité d'azote minéral trouvée lors de l'analyse de l'azote résiduel ou « reliquats azotés » à l'entrée du bilan azoté (soit N_{min} de l'équation du bilan prévisionnel de fertilisation COMIFER) est considérée comme étant utilisée à 100% par la plante, ce qui n'est pas le cas : l'efficacité moyenne d'utilisation de l'azote minéral issu des engrais n'étant en moyenne que de 50%. Cependant, il a été montré récemment que la biomasse microbienne du sol était capable de prélever jusqu'à 7 fois plus d'azote minéral que la plante en système prairial (Liu et al., 2016). Ces observations récentes permettraient d'expliquer en partie le différentiel entre la dose d'engrais X appliquée/ha et la proportion de l'apport de l'engrais réellement retrouvé dans la plante au travers le calcul du CRU, minimisant ainsi les quantités d'azote réellement perdues en provenance de l'engrais par dénitrification, volatilisation et lessivage. Ainsi, même si le CRU de l'azote est estimé à 50%, cela ne veut pas dire pour autant que 50 % d'azote est perdu, mais que la somme des autres postes de consommation d'azote par l'édaphon du sol et/ou de pertes réelles en azote minérale représente 50%. A l'heure actuelle, c'est notamment les compartiments microbien et biologique des sols qui focalisent toutes les attentions car les quantités d'azote transférées entre la biomasse microbienne et le sol, ainsi que la dynamique de transfert demeurent pour l'instant encore énigmatique.

Enfin, plus globalement, il faut tenir compte que le paramétrage historique et récent des modèles AZOBIL et AZOFERT de grandes cultures a surtout été établi pour les sols labourés, rien n'indique si ces modèles fonctionnent aussi dans les systèmes de cultures non labourés que nous souhaitons étudiés. Egalement, nous ne savons pas dans quelle mesure intervient l'ancienneté de l'arrêt du labour et/ou de la présence des couverts végétaux.

II. OBJECTIFS D'ÉTUDE

Ma thèse fait partie d'un programme de recherche plus vaste, financé par les ETS Bonduelle et la société SYNGENTA. Ce programme de recherche fondamentale et appliquée vise à définir l'impact des pratiques culturales sur la productivité végétale et la fertilité des sols. L'objectif finalisé étant de proposer aux agriculteurs des pistes écologiquement viables compatibles avec un développement durable de l'agriculture, au travers une démarche de progression de type agroécologique.

L'origine de ce projet est liée aux interrogations suscitées par les différentes pratiques agricoles actuelles, qui depuis une vingtaine d'années, nécessitent pour maintenir les niveaux de production actuelle, l'utilisation grandissante de quantités d'intrants supplémentaires à l'origine de la dégradation des écosystèmes adjacents et fléchés du doigt en tant que tel par le consommateur. Au surplus, le fait de devoir « augmenter » sans cesse les facteurs de production ou quantités d'intrants pour maintenir un état de production donné, peut constituer pour les agriculteurs, un signe avant-coureur de l'affaiblissement du potentiel de la fertilité de leurs sols ; Par extension, à terme, la diminution de la fertilité des sols pouvant affecter leur outil de travail, le sol étant alors considéré comme un capital foncier.

Au plan économique également, l'augmentation des quantités de facteurs de production génère une augmentation des coûts de production par rapport à la vente de la production diminuant ainsi les marges économiques ; ce qui fragilise les exploitations agricoles. Cependant, sur cet objectif global, il faut bien entendu tenir compte du fait que les modifications de pratiques culturales requièrent généralement un niveau de compétences élevé en agronomie ainsi qu'une haute technicité de la part des agriculteurs. Peu de données sont actuellement disponibles pour l'applicabilité des techniques agroécologiques, qui doivent être adaptées à la diversité des climats et des sols de France, particulièrement pour des cultures exigeantes comme les cultures légumières (pois de conserve, haricot), la betterave sucrière ou le maïs qui intéressent les financeurs privés.

A l'heure actuelle, le challenge de l'agriculture est donc de produire autant tout en consommant moins d'intrants et en visant la réduction des impacts environnementaux de l'agriculture. Ceci passe par des recherches expérimentales au champ pour valider ces nouvelles techniques de cultures agroécologiques et les adapter aux différents systèmes d'exploitation des agriculteurs

En d'autre terme, notre hypothèse générique de recherche est :
Est-ce que le système de culture influence la nutrition azotée des cultures et comment ?

En ce sens, nous avons arbitrairement choisi d'étudier l'influence des modifications des pratiques culturales (type de travail du sol, couverts végétaux, niveaux de fertilisation azotée)

sur les divers paramètres affectant la productivité végétale et la nutrition azotée de deux céréales (Blé et Maïs) intégrées dans au sein d'une même rotation culturale. Dans notre étude, la rotation culturale est une constante des systèmes de culture étudiés et ce sont les pratiques culturales testées (travail du sol, couverture du sol et niveaux d'intensification azotée) qui définissent les différents systèmes de cultures étudiés.

Dans ce cadre, trois expérimentations ont été mises en place au champ pour mieux comprendre les phénomènes agronomiques et processus écologiques s'opérant lors des changements de pratiques culturales. Les conséquences de la mise en place de couverts végétaux d'interculture et l'arrêt du labour sur la nutrition azotée des cultures seront comparées aux systèmes de culture traditionnels de type labourés et comportant ou non des couverts végétaux d'interculture.

Parallèlement aux deux variables d'entrée « travail du sol x couverture végétale » étudiées, nous avons ajouté une troisième variable d'entrée définissant le niveau d'intensification azoté des systèmes, en prenant comme référence, la dose d'engrais azoté X (N2) telle que définie historiquement par la méthode du bilan prévisionnel de fertilisation azoté pour les systèmes de culture labourés classiques. Une dose Nx-25% (N1), dite bas-intrants, a également été définie, permettant de comparer les différentiels de production et de qualité de la nutrition azotée pour deux systèmes de cultures de niveaux d'intensification en azote différents. Enfin, en supplément, nous avons défini un troisième niveau d'intensification azoté dit système non intensifié ou système zéro azote N0. Ce système étant par ailleurs indispensable pour déterminer de nombreux paramètres d'utilisation de l'azote par les cultures selon la bibliographie internationale.

A noter que le système N0 sert également de témoins pour d'autres objectifs non développés dans le cadre de cette thèse, notamment pour comprendre les effets de la fertilisation azotée à moyen et long terme sur l'activité biologique des sols et le fonctionnement des cycles biogéochimiques (notamment évolution des teneurs en C et N organique, activités enzymatiques des sols, biomasse microbienne des sols...).

En ce sens, nous étudierons l'influence de l'historique de fertilisation azotée sur les différents paramètres affectant la nutrition azotée en définissant 3 niveaux d'intensification azoté des systèmes de culture étudiés et représentant la troisième variable étudiée.

Au plan pratique, nous avons choisi d'analyser les trois variables d'entrée considérées sur le maïs et le blé (cultures à priori exigeantes en azote minéral).

L'objectif finalisé étant de trouver le ou les systèmes de cultures le(s) plus adapté(s) pour obtenir de bons rendements financiers tout en diminuant les apports d'azote minéraux.

Cet objectif est traité au travers trois groupes de questions de recherche définissant les 3 chapitres de la thèse au niveau de la partie « Résultats et Discussion »

Question1 (Chapitre 1) : Le semis sans labour sous couverture végétale diminue t'il ou améliore t'il l'efficacité de l'utilisation de l'azote chez le maïs ? La diminution ou l'augmentation putative de la NUE procède t'elle en diminuant ou en améliorant l'efficacité de remobilisation de l'azote par rapport au labour classique ? Est-ce que cette amélioration ou diminution hypothétique de la NUE dépend ou non des niveaux d'intensification azotés des systèmes de culture étudiés (historique de fertilisation des systèmes de culture selon doses annuelles N1 et N0) ?

Question 2 (Chapitre 2) : Le semis sans labour avec ou sans couverture végétale diminue t'il ou améliore t'il l'efficacité de l'utilisation de l'azote chez le blé ? La diminution ou l'augmentation putative de la NUE procède t'elle en diminuant ou en améliorant l'efficacité de remobilisation de l'azote par rapport au labour classique ? Est-ce que cette amélioration ou diminution hypothétique de la NUE dépend ou non de présence de couverts végétaux et des niveaux d'intensification azotés des systèmes de culture étudiés (historique de fertilisation des systèmes de culture selon doses annuelles N2, N1 et N0).

Question 3 (Chapitre 3) : En supposant que la nature du système de culture modifierait l'utilisation de l'azote chez le blé, le semis sans labour avec ou sans couverture végétale améliore t'il l'efficacité de l'utilisation de l'eau et/ou l'efficacité photosynthétique d'utilisation de l'azote chez le blé au travers différents processus physiologiques ?

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III. RESULTATS ET DISCUSSION

Chapitre 1: Conversion to no-till improves maize nitrogen use efficiency in a continuous cover cropping system



Chapitre 1. Conversion to no-till improves maize nitrogen use efficiency in a continuous cover cropping system

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RESEARCH ARTICLE

Conversion to No-Till Improves Maize Nitrogen Use Efficiency in a Continuous Cover Cropping System

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Abstract

A two-year experiment was conducted in the field to measure the combined impact of tilling and N fertilization on various agronomic traits related to nitrogen (N) use efficiency and to grain yield in maize cultivated in the presence of a cover crop. Four years after conversion to no-till, a significant increase in N use efficiency N harvest index, N remobilization and N remobilization efficiency was observed both under no and high N fertilization conditions. Moreover, we observed that grain yield and grain N content were higher under no-till conditions only when N fertilizers were applied. Thus, agronomic practices based on continuous no-till appear to be a promising for increasing N use efficiency in maize.

1. Introduction

Maize (*Zea mays* L.), also known as corn, is an essential dual-use food and energy crop, both in terms of cultivated area and production of harvestable material. The yearly increase in maize productivity worldwide has been much higher compared to other cereals, being on average 60 kg per ha every year since 1960 [1]. The total world production of maize reached a record of 877×10^9 kg in the 2011–2012 fiscal year [2]. Maize requires large amounts of nitrogen (N) inputs for optimum grain and silage production, due mainly to the ability of the crop to produce large quantities of dry matter [3–5]. However, in several studies it has been shown that increasing N fertilization above a certain threshold, neither leads to an increase in plant uptake nor in grain production [6–8]. N use efficiency (NUE), originally defined by Moll et al. [9] as the grain yield or biomass production yield obtained per unit of N available in the soil (already present and originating from fertilizer application), is inversely proportional to the amount of N fertilizer applied [10]. When the rate of N fertilization is too high, nitrate leaching occurs, leading to multiple damaging effects on the diversity and functioning of non-agricultural bacterial, animal and plant ecosystems [11,12]. In addition, fertilizer-derived N oxide emissions into the atmosphere contribute to the depletion of the ozone layer [13], whilst volatilized ammonia

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is returned as wet or dry deposition, which can cause acidification and eutrophication. Moreover it has been reported that an excessive application of N fertilizers can even lead to a decrease in grain yield [14,15]. Thus, improving NUE is particularly relevant for maize, for which global NUE has been estimated to be less than 50% [16,17].

Both from a physiological and agronomic point of view, NUE is the result of two main biological processes: N uptake efficiency (NUpE) which corresponds to the amount of N taken up per unit of available N, and N utilization efficiency (NUE) which corresponds to the increase in biomass or yield per unit of N taken up [18,19]. During the plant developmental cycle, a number of complex physiological processes are involved in the control of plant NUE notably N uptake, N assimilation and N translocation [9,18–20]. Cereals in general and maize in particular, need to remobilize the N accumulated in proteins in vegetative tissues and at the same time take up and assimilate N after anthesis, in order to ensure storage protein deposition in the grain. In maize, both N uptake and N remobilization processes contribute equally to NUE [21], measurement of these two components was a major part of this study, which aimed at optimizing tillage practices for optimal soil N recovery.

It is well known that on top of mineral N fertilization, intensive mechanical cultivation practices such as tillage generally alters soil biological activity [22–24]. These intensive cultivation practices create compaction zones in the soil [25–27], expose the soil surface to wind and water erosion [28,29] and alter the soil organic matter (SOM) decomposition rate. Reicosky and Archer [30] reported that larger amounts of CO₂ were released into the atmosphere as the result of tillage, which, in turn reduced the soil carbon (C) content. In contrast, conservation tillage practices under continuous cropping systems are known to improve SOM content [31–36], notably by enhancing C accumulation in soil aggregates. Hence, compared to conventional tillage, agricultural practices based on the use of conservation tillage are in many cases beneficial in terms of crop yield improvement [37–39].

Moreover, it has been shown that both under tilling and no-till cultivation conditions, the use of cover crops captures the excess mineral N remaining in the soil during winter and early spring periods, thus limiting the amount of mineral N that can leach into ground water [40–42]. Furthermore, several studies have demonstrated that due to their ability to fix atmospheric N, legume cover crops have a beneficial impact on crop production [43–46] by increasing soil fertility, notably by increasing the N content [47].

A large number of studies have focused on improving N fertilizer management practices in order to increase both NUE and yield in many crops, including maize [48–51]. Among these management practices no-till has been increasingly used. However, its impact remains to be thoroughly characterized both in terms of plant NUE and plant productivity.

In the present study, maize plants were grown over four years in the presence of cover crops under tillage and no-till conditions to evaluate the combined effect of tilling and N fertilization on NUE and NUE-related traits. In the absence of mineral N fertilization, an increase in NUE and NUE-related traits including nitrogen remobilization (NRem), nitrogen remobilization efficiency (NRE) and nitrogen harvest index (NHI) under no-till conditions was observed. Thus, no-till appears to be a promising strategy for maintaining maize productivity without additional N fertilizers inputs.

2. Materials and Methods

2.1. Site Description and Experimental Design

The field experiment was conducted at the experimental site of La Woestyne, in North France (50°44'N, 2°22'E, 40 m a.s.l.). The owner of the land "Bonduelle company" gave permission to conduct the study on this site. The field studies did not involve endangered or protected

species. Physical and chemical soil characteristics are presented in [Table 1](#). Weather-related parameters for this area are as follows: average annual rainfall 675 mm, average annual temperature 10.5°C.

The field was managed under a chisel plough and rotary power system until 2010, when the experiment was initiated. The field experiment was split into four treatments with three replicated plots placed randomly for each of the four treatments including: no-till with (NTN1) or without (NTN0) N fertilization; conventional tillage with (CTN1) or without (CTN0) N fertilization. The individual plot size was 7m×8m for each treatment. Since the beginning of the experiment in 2010, the conventional tillage in CT plots was performed using the moldboard plowing technique followed by the passing of a rotating harrow (Kuhn, France) for shallow tillage (30 cm tillage depth). In 2013 (3 years after the beginning of the field experiment) and in 2014 (4 years after the beginning of the field experiment), maize samples were collected in each plot in 2013 and in 2014. The crop rotation preceding maize cultivation in 2013 consisted of green bean (*Phaseolus vulgaris* L.) in 2010, followed by wheat (*Triticum aestivum*) in 2011, pea (*Pisum sativum*) in 2012. In the maize culture performed in 2014, the crop rotation consisted of wheat (*Triticum aestivum*) in 2010, followed by green bean (*Phaseolus vulgaris* L.) in 2011, wheat (*Triticum aestivum*) in 2012, pea (*Pisum sativum*) in 2013 ([S1 Fig](#)).

Before sowing the main crop, cover crop residues were buried in CT plots and left on the soil surface in NT plots. This cover crop consisted of a mixture of legume and non-legume species which were sown as follow: 12 kg ha⁻¹ of Egyptian clover (*Trifolium alexandrinum* L.), 100 kg ha⁻¹ of faba bean (*Vicia faba* L.), 20 kg ha⁻¹ of vetch (*Vicia sativa* L.), 5 kg ha⁻¹ of flax (*Linum usitatissimum* L.), 4 kg ha⁻¹ of phacelia (*Phacelia tanacetifolia* Benth.), 10 kg ha⁻¹ of oats (*Avena sativa* L.). To evaluate N inputs from cover crops residues, in each of the four plots,

Table 1. Characteristics of the soil used for evaluating the impact of no-till and N fertilization on maize NUE at the beginning of the experiment in 2010.

Parameters (units)	Depth range (cm)	Value
Clay <2 μm (g kg ⁻¹)	0–30	211.6
Silt 2–20 μm (g kg ⁻¹)	0–30	232.3
Silt 20–50 μm (g kg ⁻¹)	0–30	436.3
Fine sand 50–200 μm (g kg ⁻¹)	0–30	95.2
Coarse sand (200–2000 μm) (g kg ⁻¹)	0–30	24.6
pH in H ₂ O	0–15	6.9
CEC ^a (cmol ⁺ kg ⁻¹)	0–15	12
P ^b (mg kg ⁻¹)	0–15	24
Organic Carbon (g kg ⁻¹)	0–15	11.6
Exchangeable cations (cmol ⁺ kg ⁻¹)		
Ca ²⁺	0–15	17.5
Mg ²⁺	0–15	0.83
Na ⁺	0–15	<0.43
K ⁺	0–15	0.77
Penetration Resistance (MPa)		
With a soil moisture of 33%	0–15	0.7
	15–30	1.1
	30–45	1.7
	45–60	2.2

^a Cation-exchange capacity (Metson method)

^b Available phosphorus (Olsen method)

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3 × 1 m² of cover crops were sampled each year. Samples were dried in an oven at 65°C for three days and then weighed. The total aboveground biomass of cover crops was ground into powder prior total N measurements. From the beginning of the experiment in 2010, means of N input originating from the cover crops residues in each of the four treatments were (123 kg ha⁻¹, 127 kg ha⁻¹, 125 kg ha⁻¹ and 128 kg ha⁻¹) under NTN0, NTN1, CTN0 and CTN1 conditions respectively.

The amount of N fertilizer applied under N1 conditions was determined according to the N budget method for maize [52], based on the predictive balance-sheet method (Software Azobil, INRA, Laon, France) using the following formula:

$$B + R_f = (R_i - L) + M_n + X$$

Where B is the N requirement of the crop, R_f is the residual soil mineral nitrogen content at harvest, R_i is the readily available soil mineral nitrogen in a determined depth of soil before maize planting, L is the soil mineral nitrogen potential loss during the period from analysis of soil N to N-fertilizer application, X is N the fertilizer rate and M_n is the net supply of soil mineral nitrogen during the growing season. M_n results from the sum of the net mineralization from SOM, the mineral N supply from previous crop residues and the mineral N supply from organic manures. All the terms are expressed in kg N ha⁻¹.

The final amounts of N fertilizer applied under N1 conditions were 97 kg N ha⁻¹ in 2013 and 80 kg N ha⁻¹ in 2014. The N fertilizer was composed of 50% urea, 25% ammonium, 25% nitrate applied in a liquid form on the soil surface through broadcast applications at daybreak or at nightfall. Under these conditions of application, it was assumed that N volatilization was negligible.

2.2. Soil Sampling and Chemical Analyses

In March 2013 and 2014, six 30-cm deep soil cores were randomly collected using a 2-cm diameter auger in each of the three replicated plots for the four treatments (NTN1, NTN0, CTN1 and CTN0). Six soil cores from each replicate plot were collected and pooled, thus forming a single sample in each of the three replicates. Soils were then sieved using a 2 mm mesh and divided into two parts, for soil total N and soil residual N analysis. For soil total N measurements, the sieved soil was dried in an oven at 45°C for 48 h and ball milled ground (MM 400, Retsch, Germany). Soil total N (expressed as % of dry soil) was quantified using the combustion method of Dumas [53] using a Flash EA 1112 elemental analyzer, Thermo Electron, Germany.

Residual N (expressed in kg N ha⁻¹) corresponds to the N originating from nitrate and ammonium present in the soil. Nitrate and ammonium were extracted using 20 g of fresh soil mixed with 100 mL of 1 M KCl. After shaking for 1 h, the soil extracts were centrifuged for 10 min at 4,000 g and the supernatant was analyzed using a continuous flow analytical system (San⁺⁺ system, Skalar, Holland). The measured amounts of total N and residual N present in the soil before maize sowing in April 2013 and 2014 are shown in Table 2.

Soil water content (%) at sowing in April (SWC.s) and at crop harvest (SWC.h) in October were determined by using a moisture meter connected to a Penetrologger (Eijkelkamp, The Netherlands).

2.3. Crop Sampling and Plant Analysis

Maize (*Zea mays*, var. SY Cookie, Syngenta, Switzerland) was sown in 75 cm spaced rows using a Kuhn Maxima drill (Kuhn, France). At anthesis and at crop maturity when both stover and grains were dried [54], 6 rows of 1m length were sampled in 2013 and 2014 in each of the four

Table 2. Soil total N (%) and Soil residual N (kg ha⁻¹) under two tillage systems and N fertilizer rates in the two studied years.

Year		2013		2014	
N fertilizer	Tillage	Soil total N (%)	Soil residual N (kg ha ⁻¹)	Soil total N (%)	Soil residual N (kg ha ⁻¹)
N0	NT	0.26	50.07	0.26	44.66
	CT	0.25	64.40	0.24	64.29
N1	NT	0.27	79.55	0.26	52.98
	CT	0.28	63.80	0.27	48.21

NT = No-till with cover crops, CT = Conventional tillage with cover crops, N0 = no fertilization, N1 = N fertilization

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treatments (NTN1, NTN0, CTN1 and CTN0). The shoots were clipped at ground level and threshed to separate the grain for yield per m² measurements. Shoots and grain were dried in an oven at 60°C for 3 days, weighed and finally ground in a Retsch mill (Retsch zm200, Haan, Germany) to obtain a fine powder (0.75 mm particles). Grain and stover N contents were quantified using the same elemental analyzer as that used for soil N content analysis.

Traits related to NUE were calculated according to Moll et al. [9], Huggins and Pan [55] and López-Bellido et al. [49] using the following equations:-

$$\text{NUE (kg kg}^{-1}\text{)} = \text{Gy}/\text{Nsupply} \quad (1)$$

$$\text{NUtE (kg kg}^{-1}\text{)} = \text{Gy}/\text{Nt} \quad (2)$$

$$\text{NHI (\%)} = (\text{Ng}/\text{Nt}) \times 100 \quad (3)$$

where, Gy corresponds to grain yield (kg ha⁻¹), Nt to total plant N at maturity (kg ha⁻¹), Ng is the grain N (kg ha⁻¹) and N supply, the soil N available to the crop (expressed in kg kg⁻¹). The available N corresponds to the sum of applied N fertilizer and of total plant N uptake in non-fertilized plots in the tilled and no-tilled cultivation systems [6]. To measure the amount of N remobilized from vegetative to reproductive organs after anthesis (NRem), the following equations were used according to the method described by Cox et al. [56], Beheshti and Behboodi [57] and Masoni et al. [58]:

$$\begin{aligned} \text{NRem (g plant}^{-1}\text{)} \\ = \text{N content of the whole plant at anthesis} - \text{N content of leaves, stem and chaff at maturity.} \end{aligned} \quad (4)$$

$$\text{NRE (\%)} = (\text{NRem}/\text{N content of the whole plant at anthesis}) \times 100. \quad (5)$$

2.4. Statistical Analyses

All statistical analyses were performed in R Statistical Software version 3.2.3 [59]. Data were subjected to variance analysis (Two-way ANOVA), using tillage practices (CT, NT) as the main parameters and the level of N application (N0, N1) as the second parameters. All explanatory variables were examined for normality using the Shapiro-Wilk test [60] and for homogeneity of variances with the Bartlett test [61]. Means of each of the four treatments (NTN1, NTN0, CTN1 and CTN0) were compared using Duncan's new multiple range test at a 95% family-wise confidence level (Agricolae package) [62]. Correlations between agronomical variables (grain yield, plant N, soil N, soil water content) and NUE-related traits (NUE, NUtE, NRem, NRE and NHI) were computed using a Pearson product-moment correlation

coefficient at $P < 0.05$ (Hmisc package) [63]. Principal component analysis (PCA) (ade4 package) [64] was also carried out to visualize relationships existing between NUE-related traits (NUE, NUtE, NRem, NRE and NHI) and agronomic traits (Grain yield, soil N total, plant N, SWC.s and SWC.h).

3. Results

3.1. Effect of Tillage on Agronomic and NUE-Related Traits

Grain yield over the two years of experimentation ranged from 8060.00 to 12757.33 kg ha⁻¹ (Table 3). In 2013 and in 2014, grain yield was not significantly different between tillage and no-till conditions, whereas N fertilization significantly increased grain production both under NT and CT conditions ($P < 0.001$, $P < 0.05$ in 2013 and 2014 respectively).

Total biomass production was not significantly modified under CT or NT conditions over the two years of experimentation. In 2013, N application increased the total biomass production significantly, irrespective of the tilling conditions (Table 3).

Both in 2013 and 2014, total N uptake was not significantly modified under CT and NT conditions. However, N uptake was higher when N fertilizers were applied (N1 treatment) both in the tilling and no-till system (Table 3).

Tillage did not modify grain N content both in 2013 and in 2014. In contrast, when N fertilizers were applied, a significant increase ($P < 0.001$) in the grain N content was observed, only in 2013 (Table 3).

The ANOVA statistical analysis indicated that SWC.s and SWC.h were not significantly different between N0 and N1 (Table 4). In contrast, tillage had a significant effect on SWC both at

Table 3. Impact of tilling and nitrogen fertilization on maize agronomic traits (mean $\pm$ standard error).

Source of variance			Agronomic trait			
Year	N fertilizer	Tillage	Total biomass (kg ha ⁻¹)	Total plant N uptake (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	N grain (kg ha ⁻¹)
2013	N0	NT	17817 $\pm$ 1534 <i>b</i>	166.53 $\pm$ 22.94 <i>b</i>	8060.0 $\pm$ 1064.7 <i>b</i>	108.02 $\pm$ 17.98 <i>b</i>
		CT	19833 $\pm$ 748 <i>b</i>	212.49 $\pm$ 6.08 <i>b</i>	9458.0 $\pm$ 517.4 <i>b</i>	118.44 $\pm$ 6.40 <i>b</i>
	N1	NT	24513 $\pm$ 1059 <i>a</i>	298.76 $\pm$ 29.86 <i>a</i>	12757 $\pm$ 895 <i>a</i>	186.06 $\pm$ 19.16 <i>a</i>
		CT	23919 $\pm$ 480 <i>a</i>	301.26 $\pm$ 11.74 <i>a</i>	11846 $\pm$ 315 <i>a</i>	159.59 $\pm$ 3.10 <i>a</i>
2014	N0	NT	24578 $\pm$ 1891	252.22 $\pm$ 37.90 <i>c</i>	9676.4 $\pm$ 972.0 <i>b</i>	107.51 $\pm$ 14.68
		CT	30822 $\pm$ 1791	305.53 $\pm$ 22.34 <i>bc</i>	10408 $\pm$ 282 <i>b</i>	112.32 $\pm$ 5.43
	N1	NT	29400 $\pm$ 2002	366.39 $\pm$ 37.74 <i>ab</i>	10074 $\pm$ 592 <i>a</i>	122.03 $\pm$ 7.71
		CT	32200 $\pm$ 1225	431.49 $\pm$ 17.67 <i>a</i>	10829 $\pm$ 582 <i>a</i>	130.82 $\pm$ 11.00
Analyse of variance			$P > F$ (n = 6)			
2013	Tillage		ns	ns	ns	ns
	N fertilizer		<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
	Tillage $\times$ N fertilizer		ns	ns	ns	ns
2014	Tillage		ns	ns	ns	ns
	N fertilizer		ns	<0.001 ***	<0.05*	ns
	Tillage $\times$ N fertilizer		ns	ns	ns	ns

NT = No-till with cover crops, CT = Conventional tillage with cover crops, N0 = no fertilization, N1 = N fertilization. Data for each parameter were subjected to variance analysis (Two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different. (*, *** = significant at 0.05 and 0.001 probability level, respectively). ns = not significant.

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Table 4. Impact of tilling and nitrogen fertilization on soil water content (%) at sowing and at crop harvest (mean $\pm$ standard error).

Source of variance		2013		2014	
N fertilizer	Tillage	SWC.s (%)	SWC.h (%)	SWC.s (%)	SWC.h (%)
N0	NT	38.83 $\pm$ 0.47 a	40.83 $\pm$ 0.94 a	39.33 $\pm$ 0.33 a	41.50 $\pm$ 0.76 a
	CT	37.00 $\pm$ 0.57 b	37.66 $\pm$ 0.49 b	34.50 $\pm$ 0.99 c	37.31 $\pm$ 0.66 b
N1	NT	38.16 $\pm$ 0.47 ab	39.66 $\pm$ 0.66 ab	38.16 $\pm$ 0.47 ab	39.33 $\pm$ 0.80 b
	CT	36.66 $\pm$ 0.21 b	38.00 $\pm$ 0.36 b	36.66 $\pm$ 0.21 b	38.00 $\pm$ 0.36 b
Analyse of variance		$P > F$ (n = 6)			
Tillage		<0.01**	<0.01**	<0.001***	<0.001***
N fertilizer		ns	ns	ns	ns
Tillage $\times$ N fertilizer		ns	ns	<0.01**	<0.05*

NT = No-till with cover crops, CT = Conventional tillage with cover crops, N0 = no fertilization, N1 = N fertilization, SWC.s = soil water content at sowing, SWC.h = soil water content at harvest. Data for each parameter were subjected to variance analysis (Two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different. (*, **, *** = significant at 0.05, 0.01, 0.001 probability level, respectively). ns = not significant.

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sowing and at harvest both in 2013 and in 2014. A significant increase in SWC.s and SWC.h was also observed in NT compared to CT, only under N1 conditions.

In 2013 and in 2014, tillage had a significant and negative impact on NRE compared to the NT conditions ($P < 0.001$ in both years) (Fig 1A). The application of N fertilizer increased NRE under NT conditions. However, N application did not increase NRE under CT conditions.

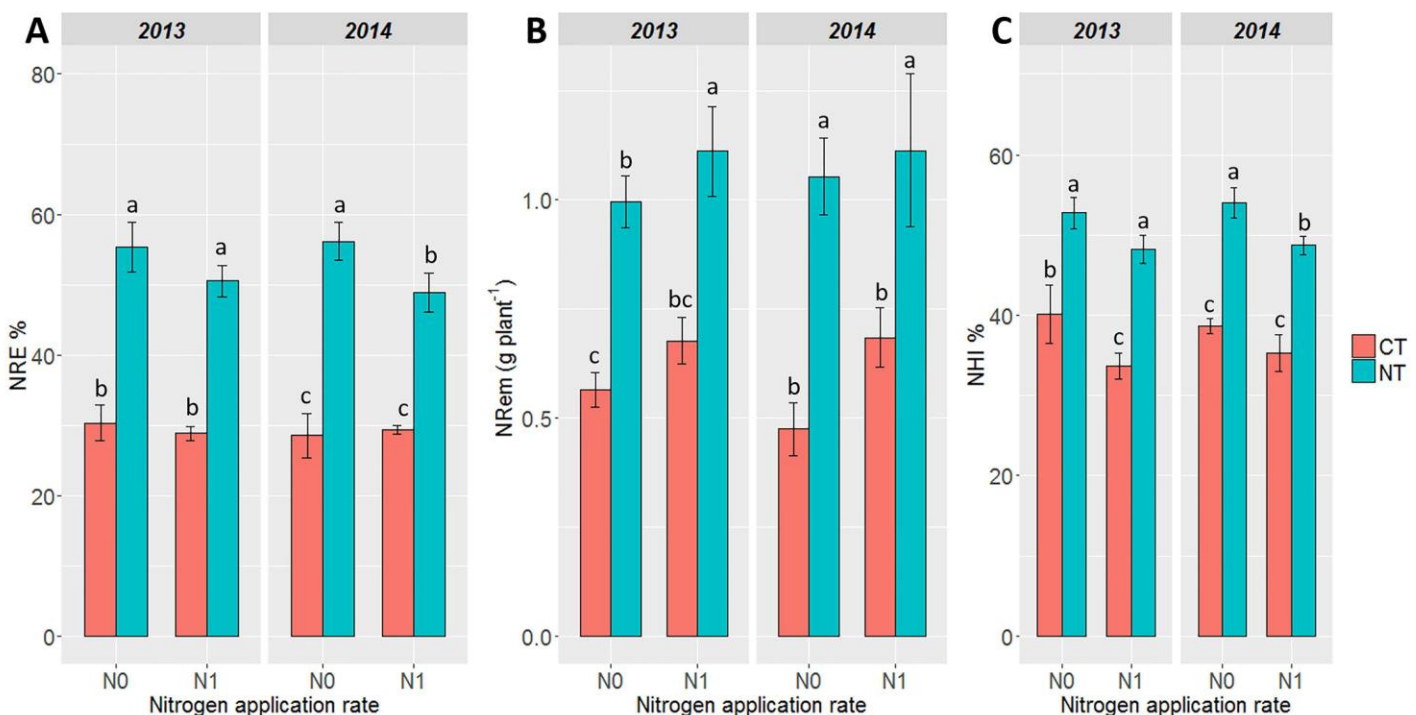


Fig 1. Impact of tillage practice and N application on (A) NRE; (B) NRem and (C) NHI, according to the soil tillage treatment in 2013 and 2014. (NT) No-till with cover crops, (CT) Conventional tillage with cover crops. N0 = no fertilization, N1 = N fertilization. Data for each parameter were subjected to variance analysis (two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

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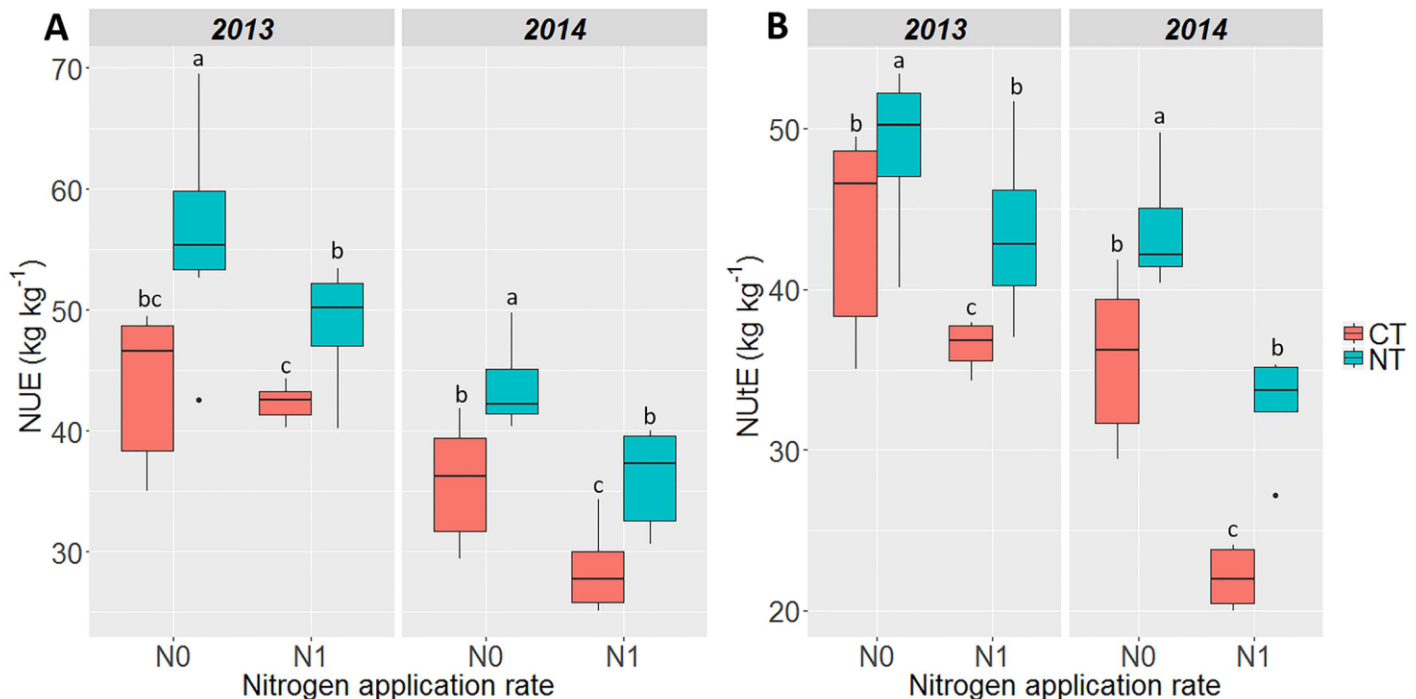


Fig 2. Impact of tillage practice and N application on (A) NUE of maize grain (kg kg^{-1}) and (B) NUtE of maize grain (kg kg^{-1}) according to the soil tillage treatment in 2013 and 2014. (NT) No-till with cover crops, (CT) Conventional tillage with cover crops. N0 = no fertilization, N1 = N fertilization. Box plots are represented with the median and the 25th-75th percentile with the minimum and the maximum. Data for each parameter were subjected to analysis of variance (two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

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The tillage system had a significant effect on NRem. This positive effect was significantly higher ($P < 0.001$) under NT compared to CT (Fig 1B) in both years, whereas Nrem was not modified whatever the N the fertilization conditions.

In 2013, the N fertilization did not significantly modify NHI, either under NT or CT conditions. However, both in N0 or N1, tillage had a negative effect on NHI compared to the NT cultivation system over the two years of experimentation ($P < 0.001$, $P < 0.05$ in 2013 and 2014 respectively) (Fig 1C). The N fertilization significantly modified NHI under CT conditions in 2013 and under NT conditions in 2014.

Both in 2013 and 2014, CT and N application had a significant negative impact on NUE and its component NUtE ($P < 0.001$). Under N0 and N1, both NUE and NUtE were significantly higher in NT compared to CT conditions (Fig 2). A significant decrease in NUE and NUtE was also observed when N fertilizers were applied both under NT and CT conditions.

3.2. Correlation Analyses

Pearson correlations between NUE, NUtE, yield, soil N, total plant N, NRem, NRE, SWC.s, SWC.h and NHI over the two years of experimentation are presented in Fig 3. NUE and NUtE were significantly and positively correlated with NRem, NRE, NHI, soil N, SWC.s and SWC.h. Similarly, NRE, NRem and NHI were significantly correlated with the soil N content, SWC.s and SWC.h. Conversely, NUE, NUtE, NRE and NHI were significantly and negatively correlated with the plant N content. A PCA analysis was then performed to obtain a visual

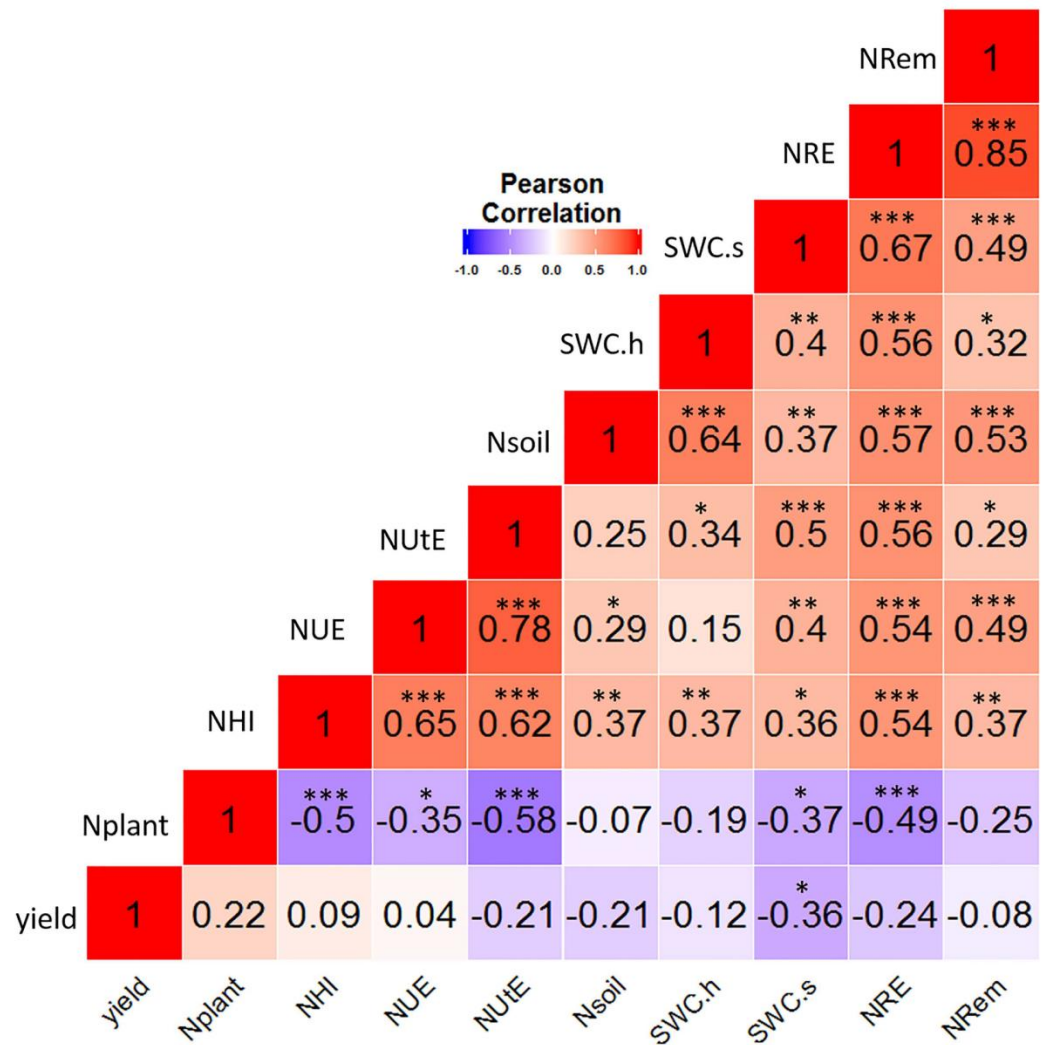


Fig 3. Pearson correlation coefficient r between NUE and NUE-related traits. (*, **, *** = significant at 0.05, 0.01, 0.001 probability level, respectively.).

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representation of the correlations between agronomic and NUE-related traits, according to the tillage system and the level of N fertilization (Fig 4). The first two axes of a PCA using NUE traits explained 61.59% of the variation in the data set. The variables were separated into four groups corresponding to tillage system and fertilizer application rate. Axis.1 (46.94% of variance explained) was positively correlated with plant N and yield, and negatively correlated with soil N, SWC.s, SWC.h, NRem, NRE, NHI, NUE and NUtE, which matches the Pearson correlation test. NRem, NRE, soil N, SWC.s and SWC.h were strongly correlated and positively grouped along Axis.2 (14.65% of variance explained). Similarly, NUE, NUtE and NHI were strongly correlated and negatively grouped along Axis.2. The first axis clearly separated the CT treatment from the NT treatment. The N0 and N1 fertilization conditions were separated along the second axis. NUE and NUtE related traits were markedly higher under NT conditions compared to the CT treatment.

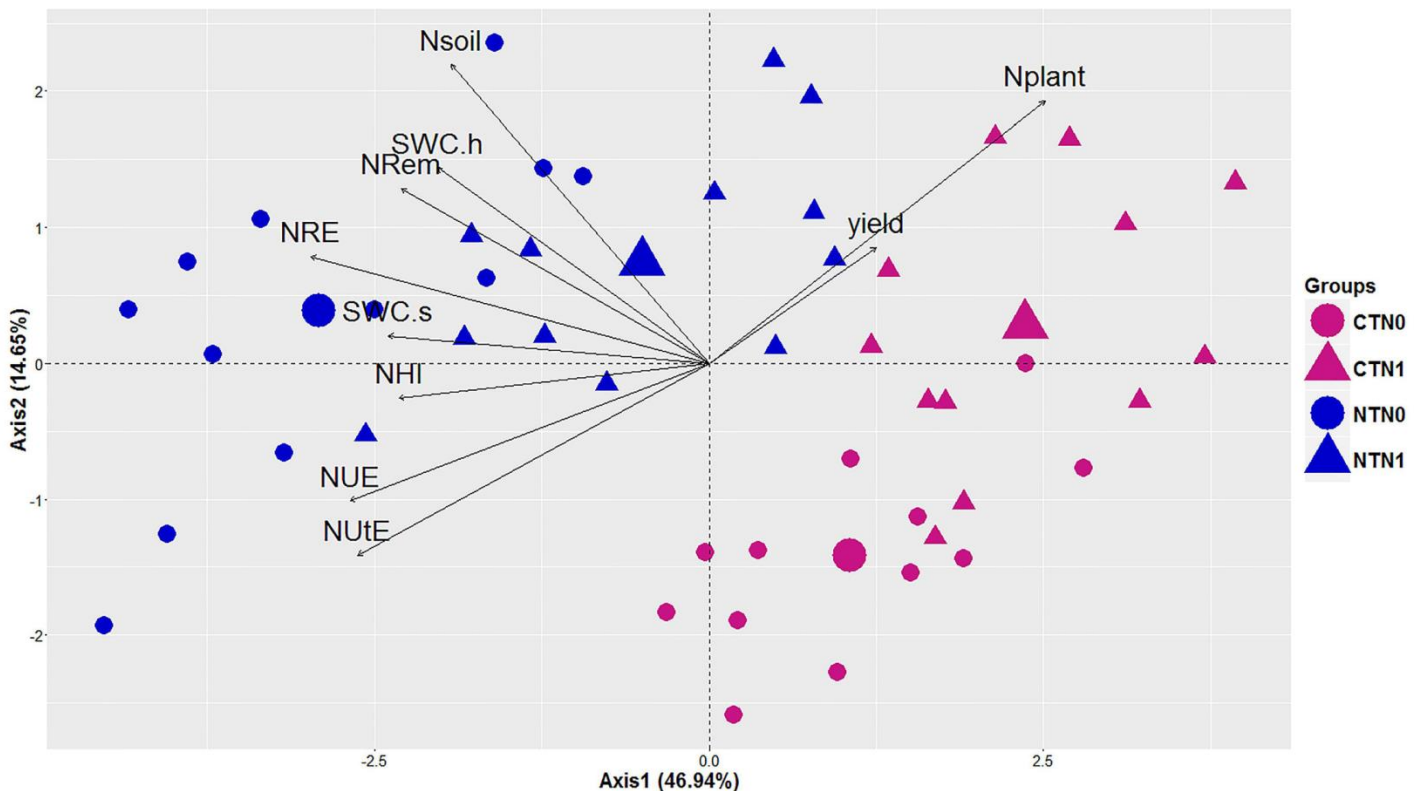


Fig 4. PCA analysis showing the correlations between tilling conditions, N fertilization and NUE-related traits. Diagrams were defined by the first two axes of the PCA of the variables ($n = 12$). Axis.1 (46.94% of variance explained) and Axis.2 (14.65% of variance explained). NTN0 = No-till without N fertilization, CTN0 = Conventional tillage without N fertilization, NTN1 = No-till with N fertilization, CTN1 = Conventional tillage with N fertilization.

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4. Discussion

The field experiment performed over two consecutive years showed that conversion to no-till under a continuous cover cropping system significantly increased maize NUE and NUtE, in comparison to a cultivation system based on continuous till. Moreover, such an increase occurred both under low and high N fertilization conditions (Fig 2). These results are in agreement with those obtained with wheat by Soon et al. [65], who showed that NUE was increased under no-till conditions. In contrast, Brennan et al. [6] and López-Bellido and López-Bellido [7] found that in wheat, NUE was lower under reduced or no-till conditions respectively, likely because under their experimental conditions, crop N uptake was reduced. Another survey conducted by Dalal et al. [34] over 40 years of experimentation, led to the conclusion that wheat NUE remained constant, irrespective of the tilling practices employed. These contrasting results can be explained by the fact that in conservation systems, there is often an inefficient mobilization of N generated by plant residues left at the soil surface, thus leading to a decrease in NUE [66,67]. The originality of our study was to show that in maize, a crop rarely tested for its ability to valorize N under continuous till conditions, NUE is higher when the soil is not plowed, irrespective of the N fertilization regime.

In agreement with Burgess et al. [66] and Torbert et al. [68], grain yield, remained similar either under low or high N fertilization, regardless of the tilling conditions. In other studies, it has been reported that maize yields decrease slightly when no-till is used instead of conventional tilling, likely because the soil N availability is lower leading to a reduction in crop productivity

[69,70]. In spring cereals such as barley, oats and wheat, it was generally observed that under no-till conditions, grain yield was substantially reduced [71–73]. Under the experimental conditions employed in these studies, the combined effect of tillage and of the level of N fertilization did not markedly modify N uptake, as slightly more N was taken up by the maize plants under CT conditions. Moreover, in agreement with Al-Kaisi and Kwaw-Mensah [74], we observed that such a small increase in N uptake did not lead to an increase in the grain N content (Table 3).

NUE is a complex agronomic traits depending on soil N availability, resulting from the efficiency of N uptake by the roots and N utilization and N remobilization by the plant [75]. Among the various traits representative of NUE, NHI was higher under NT conditions compared to CT, irrespective of the level of N fertilization. Similarly, other NUE-related traits such as NRem and NRE were significantly higher under NT compared to CT, whether or not N fertilizers were applied (Fig 1A and 1B). In addition, NHI and NRE were positively and significantly correlated with NUE and NUtE (Fig 3). Although the leaf N content at maturity was higher in CT than in NT, the grain N content was similar irrespective of the tillage practice (Table 3).

In this study, tillage had a negative impact on the amount of water stored in the soil. In particular, without additional N fertilization (N0), SWC.s and SWC.h were significantly lower following CT under N0 conditions in comparison to NT over the two years of experimentation (Table 4). The absence of tillage is known to preserve soil moisture [65,76] by maintaining total soil pore space while keeping the exchanges between the macro- and micro-pores in the soil [77]. It has been shown that soil water retention under NT conditions is beneficial to the crop, notably during the grain filling period after anthesis [78]. During this period, N remobilization largely depends on soil water availability [79–81]. In line with these observations, it has been reported that in wheat both N uptake and N remobilization and thus NUE were reduced when there was a shortage of water [82,83]. It is likely that in the NT system, soil water retention was one of the components that favored post-anthesis N uptake and thus NUE.

PCA analysis allowed a refinement of the correlations observed between NUE, and the various NUE-related traits such as NUtE, NRE and NHI and their relationship with the tillage system according to the level of N fertilization (Fig 4). The first axis clearly separated CT plots from NT plots. The second axis mainly separated N0 from N1 plots. Remarkably, NUE and NUtE were the two traits that contributed the most to the increase in NUE under NT conditions. Such an analysis thus confirmed that the no-till system had a positive impact both on NUE and NUE-related traits.

5. Conclusion

In the present study, a field experiment was conducted over a 4-year period to ensure that the impact of the conversion to a no-till system on NUE and NUE-related traits was rapidly and accurately monitored. Both NUE and NUE-related traits, which could not have been accurately measured using longer-term experiments, were used as markers in order to investigate the benefit of the no-till cultivation system. As in a number of previous studies [74,84–86], measurements of these traits were performed using short-term experiments in order to detect the effect of no-till at any time during the entire field experiment. Over two years of experimentation, the results showed that the use of a continuous no-till system with a cover crop is a promising way to increase the NUE of maize, and consequently to reduce both the use and the loss of N fertilizers without any yield penalty.

Supporting Information

S1 Fig. Chronological representation of crop rotation over the 4-year experiment. (NT) no-till, (CT) conventional tillage, (N0) no fertilization, (N1) N fertilization, (Ø) no cover crops. (TIF)

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Methodology: HH TT.

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III. RESULTATS ET DISCUSSION

Chapitre 2: No-till with or without cover crops improves nitrogen nutrition and nitrogen use efficiency of winter wheat



Chapitre 2: Investigating the combined effect of tillage, cover crops and nitrogen fertilization on nitrogen use efficiency in winter wheat.

Ce chapitre correspond à un article soumis dans la revue *PlosOne*

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Abstract

A field study was conducted in Northern France over two consecutive years to evaluate the combined effect of tilling, cover crops and nitrogen (N) fertilization on various agronomic traits related to N use efficiency in wheat. Five years after conversion to no-till, a significant increase in both N use efficiency and N utilization efficiency was observed under two N fertilization regimes (no fertilization and 215 kg ha⁻¹) compared to conventional tillage over a two period of experimentation. At 161 kg N ha⁻¹, such an increase was observed in only the second year. N agronomic efficiency, N partial factor productivity and N apparent recovery fraction were increased under no-till conditions at 215 kg N ha⁻¹ over two years and at 161 kg N ha⁻¹ in only the second year of experimentation. Compared to conventional tillage, higher amounts of N were remobilized under no-till conditions in the presence of a cover crop at 161 kg N ha⁻¹, over two consecutive years. N remobilization was also higher under no-till conditions with and without cover crops at 215 kg N ha⁻¹, but only in the second year. Although a decrease in grain yield and grain N content occurred when N fertilization was lowered, both agronomic traits remained similar under conventional tillage and no-till conditions. The N nutrition index was higher under no-till conditions compared to conventional tillage at the three rates of N fertilization over two years. Moreover, a positive effect of the cover crops on NUE-related traits and N nutrition index was observed both under no-till and conventional tilling conditions. Despite the fact that the positive impact of cover crops and no-till conditions on wheat N use efficiency was variable between years, both agricultural practices appear to be promising ways of reducing the application rate of N-based fertilizers.

Keywords: Nitrogen use efficiency; Tillage system; Cover crops; Nitrogen application; Grain Yield; Winter wheat

1. Introduction

Over the last 50 years, N fertilizers have been used extensively to increase wheat production [1]. However, the imbalance between the amount of N applied and the ability of the crop to utilize a large part of the added N fertilizers can lead to either N accumulation or N deficiency in a number of areas of the world [2–4]. It has been estimated that only half of all anthropogenic N inputs are taken up and recovered in harvested crops and their residues [5]. The N remaining in the soil is lost by volatilization or leaching [6], which can have a major

detrimental impact on the diversity and functioning of non-agricultural bacterial, animal and plant ecosystems [7,8].

In the last three decades, improving N use efficiency (NUE), defined as the yield obtained per unit of available N supplied by the soil and by added N fertilizer [9], has been one of the most important challenges in modern agriculture. Therefore, various technological and agronomic approaches have been developed in parallel with improving our understanding of the genetic and physiological basis of NUE for further breeding and agronomic applications [10]. In particular, it has been emphasized that agricultural management practices viewed from both short- and long-term perspectives must be at the forefront of measures to develop sustainable crop productivity with regards to NUE improvement. A number of these agricultural practices are based on the development of soil conservation, a technique known to improve the uptake of soil N by the plant [11,12] and to increase the content of soil organic matter (SOM). In turn, such an accumulation of SOM leads to an accumulation of C inside the soil aggregates [13–18]. Due to the presence of cover crops, soil conservation systems are also able to reduce or even prevent the runoff of nitrate and xenobiotics, that can lead to groundwater pollution [19,20]. During conventional tillage, degradation of the soil physico-chemical properties [21,22], alteration of soil biological activity [23] and exposure of the soil surface to wind and water erosion generally occurs [24,25]. In contrast, both under tilling and no-till cultivation conditions, cover crops can prevent soil erosion by covering the ground with living vegetation, thus allowing their roots to maintain the soil structure [26]. Lastly, C input and storage in the soil can be significantly improved both in tilled and no-till soils [27].

In field studies, several indices have been used to estimate various agronomic parameters related to NUE [9,28]. Among them, N utilization efficiency (NUE), N agronomic efficiency (AEN), N partial factor productivity (PFPN) and N apparent recovery fraction (NAR) [29], have been used to monitor the performance of agricultural management practices. In addition, the N nutritional status of a crop, in particular a deficiency of N, can be monitored using the N nutrition index (NNI) [30] that can be calculated using species-specific N dilution curves [31]. Monitoring the crop nutritional status using NNI [32], allows the application of N fertilizers at a period coinciding with the N requirement of the crop [33], which in turn reduces N losses that are potentially detrimental to the environment [34].

With the objective of reducing N losses, a large number of studies have focused on improving N fertilizer management practices in order to increase both NUE and yield in wheat [11,35]. Among these management practices, no-till has been increasingly employed, as it

appears to be very promising for increasing crop NUE, without any yield penalty, even when less N-based fertilizers have been used.

In the present study, the combined effects of tilling, cover crops and N inputs on wheat NUE related traits and N nutrition were evaluated. The changes observed in NNI and NUE traits such as NUtE, AEN, PFPN and NAR allowed the development of a strategy for maintaining wheat productivity in a sustainable agricultural system, based on the rationalization of N fertilizer usage.

2. Materials and methods

2.1. Site description and experimental design

Field experiments were conducted in La Woestyne, Northern France (50°44'N, 2°22'E, 40 m above sea level). The owner of the land "Bonduelle company" gave permission to conduct the study on this site. The field studies did not involve endangered or protected species. Physical and chemical soil characteristics were essentially the same as described by Habbib et al. [12]. Weather-related parameters for this area were as follows: average annual rainfall 675 mm, average annual temperature 10.5°C. The field was managed under a chisel plough and rotary power system until 2010, when the experiment was initiated. In 2010, the experimental field was split into twelve treatments with three replicated plots placed randomly: three different N fertilization regimes [no added N (N0), 161 kg N ha⁻¹ (N1) and 215 kg N ha⁻¹ (N2)]; four tillage/cover crop systems: no-till with (NTcc) or without (NT) cover crops and conventional tillage with (CTcc) or without (CT) cover crops.

In 2014 and 2015 (4 and 5 years after the beginning of the field experiment, respectively), wheat (*Triticum aestivum* var BTH intensive EXPERT, Syngenta, Switzerland) samples were collected from each plot (see section 2.3 for details). The crop rotation preceding wheat cultivation in 2014, consisted of maize (*Zea mays* L.) in 2010, followed by wheat (*Triticum aestivum* L.) in 2011, flax (*Linum usitatissimum* L.) in 2012 and beet (*Beta vulgaris* L.) in 2013. In the wheat cultivation performed in 2015, the crop rotation consisted of pea (*Pisum sativum* L.) in 2010, followed by maize (*Zea mays* L.) in 2011, wheat (*Triticum aestivum* L.) in 2012, flax (*Linum usitatissimum* L.) in 2013 and beet (*Beta vulgaris* L.) in 2014.

The conventional tillage in CTcc and CT plots was performed using the mouldboard ploughing technique followed by the passing of a rotating harrow (Kuhn, France) for shallow tillage. Before the sowing of the main crop, cover crop residues were buried in CTcc plots and

left on the soil surface in NTcc plots. The cover crops consisted of a mixture of three legumes and three non-legume species, which were sown as follows: 400 seeds m⁻² of Egyptian clover (*Trifolium alexandrinum* L.), 30 seeds m⁻² of faba bean (*Vicia faba* L.), 50 seeds m⁻² of vetch (*Vicia sativa* L.), 80 seeds m⁻² of flax (*Linum usitatissimum* L.), 200 seeds m⁻² of phacelia (*Phacelia tanacetifolia* Benth.), 60 seeds m⁻² of oats (*Avena sativa* L.).

The amount of N fertilizer applied under N2 conditions was determined according to the N budget method for wheat [36], based on the predictive balance-sheet method (Software Azobil, INRA, Laon, France) using the following formula:

$$B + R_f = R_i + M_n + X$$

Where B is the N requirement of the crop, R_f is the residual soil mineral N content at harvest, R_i is the soil mineral N readily available at a determined depth of soil before wheat planting, M_n is the net supply of soil mineral N during the growing season and X is the N fertilizer rate. M_n results from the sum of the net mineralization from SOM, and the mineral N supply from previous crop residues. All the terms of the equation are expressed in kg N ha⁻¹. As there were no cover crops grown before the wheat experiment, it was not necessary to take into account any residual N derived from the previous crops.

The N1 treatment represents an economic N input. The amount of N fertilizer applied under N1 conditions was calculated as N2 minus 25%. The N fertilizer was composed of 50% urea, 25% ammonium, 25% nitrate applied in a liquid form on the soil surface, through broadcast applications at nightfall. Under these conditions of application, it was assumed that N volatilization was negligible.

2.2. Soil sampling and chemical analysis

Prior to the first application of N fertilizer in March 2014 and 2015, six 30 cm deep soil cores were randomly collected using a 2 cm diameter auger from each of the three replicate plots, for each treatment. The six soil cores from each replicate plot were combined together as a single sample. Soils were then sieved using a 2 mm mesh and divided into two parts for total N and residual N analysis. For soil total N measurements, the sieved soil samples were dried in an oven at 45 °C for 48 h and ground using a MM 400 mill (Retsch, Germany). Soil total N (expressed as % of dry soil), was quantified using the combustion method of Dumas [37] using a Flash EA 1112 elemental analyzer, Thermo Electron, Germany.

2.3. Crop sampling and plant analysis

Wheat seeds were sown in rows 12.5 cm apart using an AS 400 drill (Alpego, Italia). At anthesis in May and at crop maturity in July, 6 rows of 1m in length were sampled from each treatment. The shoots were clipped at ground level and threshed to separate the grains for measurements of yield per m². Shoots and grains were dried at 60 °C for 3 days, weighed and finally ground using a ZM 200 mill (Retsch, Germany), to obtain a fine powder (0.75 mm particles). Grain and straw N contents were determined using the same elemental analyzer as for soil.

The critical N dilution curve corresponds to the above ground biomass of a crop during vegetative growth [38]. NNI is defined as the ratio of the % N concentration in the plant (N_{ms}) and the critical N (N_c) of the same amount of dry matter produced during vegetative growth, as in the following [31]:

$$NNI = N_{ms} / N_c$$

Where, N_{ms} corresponds to the above-ground % N concentration at flowering, and N_c to the critical plant N concentration defined as the minimum N concentration needed for maximum growth rate, calculated as

$$N_c = a (DM)^{-b}$$

Where, DM corresponds to the above ground dry matter produced (Mg ha⁻¹) for the reference curve Wheat: a = 5.35 and b = 0.442 [31].

Traits related to NUE were calculated according to Moll et al.[9]; Dobermann, [29]; López-Bellido et al.[35] and Huggins and Pan, [39] using the following formulae:

$$NUE \text{ (kg kg}^{-1}\text{)} = Gy / N \text{ supply in fertilized plots, } Gy_0 / N \text{ supply in control plots}$$

$$NUE_t \text{ (kg kg}^{-1}\text{)} = Gy / N_t \text{ in fertilized plots, } Gy_0 / N_{t0} \text{ in control plots}$$

$$AEN \text{ (kg kg}^{-1}\text{)} = (Gy - Gy_0) / X$$

$$PFPN \text{ (kg ha}^{-1}\text{)} = Gy / X$$

$$NAR \text{ (\%)} = (N_t - N_{t0}) / X$$

Where, Gy corresponds to grain yield in fertilized plots (kg ha⁻¹), Gy₀ to grain yield in the control plots (kg ha⁻¹), N_t to total plant N at maturity in fertilized plots (kg ha⁻¹), N_{t0} to total plant N at maturity in the control plots (kg ha⁻¹), X to N application amount (kg ha⁻¹) and N supply to the soil N available to the crop (expressed in kg kg⁻¹). The available N corresponds to the sum of applied N fertilizer and total plant N uptake in non-fertilized plots for each tillage system [12].

To measure the amount of N remobilized from vegetative to reproductive organs after anthesis (NRem), the following equations were used according to the methods described by Cox et al. [40]; Beheshti and Behboodi, [41] and Masoni et al. [42]:

$$\text{NRem (g plant}^{-1}\text{)} = \text{N content of the whole plant at anthesis} - \text{N content of leaves, stem and chaff at maturity.}$$

2.4. Statistical analyses

All statistical analyses were performed using R statistical software version 3.3.0 [43]. Data were subjected to variance analysis (Two-way ANOVA), using soil management (CTcc, NTcc, CT, NT) as the main parameter and the level of N application (N0, N1, N2) as the second parameter. All explanatory variables were examined for normality using the Shapiro-Wilk test and for homogeneity of variances using the Bartlett test (Agricolae package). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level (Agricolae package). Linear regression was performed with Stats package to evaluate the slope between NNI and N fertilization application for each tillage treatment. To account for the allometry between N uptake and crop growth, a linear model was constructed between NNI and NUE for each tillage treatment. Analysis of covariance (ANCOVA) (Stats package), was applied in order to compare slopes and intercepts obtained with four tillage/cover crop conditions (NTcc, NT, CTcc and CT). Principal component analysis (PCA) (ade4 package) was also carried out to visualize relationships existing among agronomic traits (yield, N grain, biomass total, N total, N soil and NNI) and NUE traits (NUE, NUtE, AEN, PFPN, NAR and NRem).

3. Results

3.1. Effect of tillage treatment and N fertilizer rate on agronomic traits

Both in 2014 and 2015, a higher total biomass was produced under CTcc compared to NTcc in the N0 treatment. Over the two years of experimentation, N application significantly increased total biomass production, irrespective of the tilling conditions (Table 1). Only in 2015, did the previous cover rotation produce a significant increase in total plant biomass under no-till condition (NTcc) in N1 and under conventional tilling conditions (CTcc) in N2.

In 2014, grain yield was not significantly different between tillage and no-till conditions. However, in 2015, the no-till system had a significant effect on grain yield under some treatments. This positive effect of NT was significant ($P < 0.001$) under NTcc and NT compared

to CTcc and CT under N1 conditions (Table 1). The same effect was observed under N2 conditions between NT and CT. N fertilization increased grain production significantly over two years. Cover crops had a significant effect on grain yield, with a higher grain yield in CTcc than CT under N2 conditions and in NTcc than NT under N1 conditions in 2015.

Although, a significant increase ($P < 0.001$) in the grain N content was observed with increasing levels of N fertilization, tillage did not modify grain N content in either 2014 or 2015 (Table 1). Both in 2014 and 2015, total plant N uptake was not significantly modified under the four tillage/cover crops treatments. No significant effects of cover cropping on total N uptake were observed in either year.

3.2. Effect of tillage treatment and N fertilizer rate on different NUE indices

Both in 2014 and 2015, tillage had a significant negative impact on NUE under N0 and N2 fertilization regimes (Fig 1), but following N1 fertilization, this reduction was only detected in 2015. A significant decrease in NUE was also observed when N fertilizers were applied ($N0 > N1$ and $N2$ in all tillage treatments). Cover crops had a positive impact on NUE in both years under no-till and N1 fertilization. Similarly, cover crops had a positive impact on NUE in both tillage treatments in 2014 following N2 fertilization and in 2015 following no additional application of N.

The same negative impact of tillage was observed on NUtE in both years, but only when N was not applied (Fig 2) or there was N1 fertilization in 2015. A significant decrease in NUtE was also observed when N fertilizers were applied ($N2 < N1$, $N2 < N0$), following CTcc treatment over two years. The cover cropping system had a positive effect on NUtE in 2015, which was higher under NTcc compared to NT with no additional N fertilizer and higher in CTcc compared to CT following both N0 and N2 N fertilization.

Tillage had a significant and negative effect on AEN, following N2 N fertilization over two years ($P < 0.001$). Following N1 N fertilization, tillage had a significant and negative effect on AEN in 2015. However, the negative effect of tillage on AEN following N1 N fertilization was not significant in 2014. Cover crops had a positive impact on AEN following both CTcc and NTcc and N2 N fertilization in 2014 and there was a similar effect of NTcc following N1 N fertilization in 2015 (Table 2).

PFPN was significantly lower following CT tillage and N2 N fertilizer in both years (Table 2). The effect of tillage on PFPN following N1 N fertilization was not significant in

2014. However, the effect of N1 fertilizer was significant in 2015, with lower PFPN following tillage in CTcc and CT. A significant decrease in PFPN was observed when additional N fertilizer was applied ($N_2 < N_1$) in all treatments in 2014 and under no-till conditions (NTcc) in 2015. Cover crops had a positive impact on PFPN following tillage (CTcc) and N2 N fertilization in 2014 and under no-till conditions (NTcc) following N1 N fertilization in 2015 (Table 2).

Tillage (CT; CTcc) had a significant negative effect on NAR following the application of N2 N fertilizer in both 2014 and 2015 ($P < 0.001$) (Table 2). Significantly lower NAR was also observed following tillage and N1 N fertilization in 2015, but not in 2014. A significant decrease in NAR was also detected when N fertilizers were applied ($N_2 < N_1$) to the CTcc and CT tillage treatments over both years. The cover cropping system had a positive effect on NAR only in 2014, following treatment with N2 N fertilizer (Table 2).

Both in 2014 and 2015, greater amounts of N were remobilized (NRem) under no-till conditions in the presence of a cover crop (NTcc), following N1 N fertilization. Similarly, no-till with and without cover crops had a significant and positive effect on NRem following N2 fertilization in 2015, compared to conventional tillage. Following N0 N fertilization, tillage did not have a significant effect on NRem. Following no-till in the presence of a cover crop, NRem increased, notably following N1 N fertilization. Following conventional tillage, the presence of a cover crop did not markedly modify NRem, under either N1 or N0 N fertilization conditions. However, cover crops had a positive impact on NRem following tillage (CTcc) and N2 N fertilization in 2015 (Fig 3).

3.3. Effect of tillage treatment and N fertilizer rate on NNI

A linear increase of NNI was observed for both years of experimentation from low (no added N, N0), to the highest level (N2, 215 kg ha⁻¹) of N fertilization (Fig 4). An ANCOVA test demonstrated that the slopes of the lines of the four tillage/cover crop conditions (CT, CTcc, NT and NTcc) were significantly different in 2015 ($P < 0.001$), but not in 2014. Moreover, the y-intercepts for these four cultivation conditions were significantly different ($P < 0.01$, $P < 0.001$ in 2014 and 2015, respectively). The y-intercepts were always higher under NT conditions irrespective of the presence of a cover crop. Moreover, the presence of a cover crop significantly increased NNI under both CT and NT conditions.

When NNI and NUE were plotted, a negative relationship was observed between the two agronomic traits (Fig 5). The differences between the two CT and NT conditions being

similar to that observed for NNI alone. Over the two years of experimentation, the y-intercepts of the four tillage conditions were significantly different ($P < 0.001$). Moreover, the slopes of the four linear regression lines were significantly different in 2015 ($P < 0.05$), but not in 2014. The slopes representing the negative linear function between NNI and NUE were higher in the absence of a cover crop under conventional tillage (Fig 5).

3.4. Correlation analysis

PCA analysis was performed in order to obtain a visual representation of the correlations existing between agronomic traits (yield, N grain, biomass total, N total, N soil, and NNI) and NUE traits (NUE, NUtE, AEN, PFPN, NAR and NRem) (Fig 6). The first two axes of the PCA explained 80.2 % of the variation in all studied traits. Traits were separated into twelve groups namely CTccN0, CTccN1, CTccN2, CTN0, CTN1, CTN2, NTccN0, NTccN1, NTccN2, NTN0, NTN1 and NTN2 corresponding to the combined effect of the tillage system, the presence of cover crops and the amount of N fertilization. Axis 1 (71.1% of variance explained) allowed a separation of the N0 N fertilization treatment from both N1 and N2 N fertilization. No-till (NT) and conventional tillage (CT) were clearly separated along Axis 2 (9.1% of variance explained). Cover crop conditions were not clearly separated along the two axes. NUE and NUtE were markedly higher following N0 N fertilization and were highly correlated with the no-till system. NRem, NNI, AEN and PFPN were higher under the no-till system following treatment with either N1 or N2 N fertilizer.

4. Discussion

In the present study, the combined effects of tillage, cover crops and N fertilization on a number of NUE traits were evaluated. A two-year field experiment showed that for wheat, no-till irrespective of the presence of cover crops, had a positive impact on a number of key NUE-related traits such as NUE, NUtE, AEN, PFPN and NAR. Following growth under NTcc and NT conditions, NUE traits were significantly higher than when grown using conventional tillage (CT). However, the effect of no-till was not significant following N1 fertilization in 2014. These results are in agreement with those obtained by Soon et al. [44] and Chang et al. [45], who showed that for wheat, NUE was higher following no-till compared to conventional tilling conditions. However, it should be noted that on some occasions with spring wheat, different results were obtained, when NUE was higher in tilled soils due to an increase in yield [39,46]. In this study, the effect of cover crops was variable between years with positive effects

on NUE-traits in certain tilling and N fertilization conditions. In 2015, NUE was higher after growth with cover crops under NTcc, compared to NT following both N0 and N1 N fertilization. NUE was also higher after CTcc compared to CT but only after N0 N fertilization. In 2014, higher NUE was observed after growth with cover crops under NTcc and CTcc compared to NT and CT following N2 N fertilization. It has been reported previously that NUE was increased by cover crops under both NTcc and CTcc conditions, compared to NT and CT respectively. Such positive effects of the cover crops on NUE were attributed to a better N supply originating from the cover crop residues and to an improvement in the physical and chemical properties of the soil [47,48].

Interestingly, we found that total biomass production was higher under CTcc compared to NTcc following N0 N fertilization, whereas grain yield was not significantly modified. In a number of studies, it has been shown that in wheat, conservation tillage e.g. no-till or reduced tillage could in some cases lead to an increase in grain yield in comparison to conventional agricultural systems [49–52]. However, in another study, Machado et al. [53] found that over six years, yield in spring wheat was on average 21% lower in non-tilled soils.

In agreement with López-Bellido et al. [52] and Giacomini et al. [54], we found that both plant N uptake and N grain content were not significantly modified by the tillage system. In contrast Känkänen et al. [55] found that with spring wheat, N content was lower under no-till conditions. In our study, we found that there is a weak relationship between NUE-traits and several agronomic traits such as grain yield and grain N content, which were similar, irrespective of the tilling practice.

In many cases it has been shown that no-till had a negative impact on NUE, mostly because grain yield was reduced [39,46,56]. In other studies, it was found that NUE was increased under no-till conditions, apparently due to a reduction in plant N uptake. This is likely because the availability of N in the soil was lower, thus increasing the ratio of yield/N uptake [57,58]. In the present study, we found that NUE-related traits were higher under no-till conditions, irrespective of the presence of cover crops, without a reduction in N uptake. One possible explanation could be that the capacity of the plant to take up N at a high rate post-anthesis, under no-till conditions is maintained, thus enhancing NUE. Most importantly, we also observed that grain yield, total plant N and grain N content (corresponding to total plant N uptake), were similar under no-till and conventional tilling conditions. In addition, we found that remobilized N (NRem) was higher following growth under no-till (NTcc) compared to conventional till (CTcc) conditions, in the presence of cover crops after N1 N fertilization in both years and after N2 N fertilization in 2015 (Fig 3).

To our knowledge, the effect of tillage on the Nitrogen Nutrition Index (NNI) has not previously been evaluated in wheat. Thus, this key agronomic trait was used to evaluate the N status of the wheat crop under the two tillage treatments, with and without cover crops and under the three levels of N fertilization (Fig 4). The y-intercepts of the linear regression lines using the two different tillage and two cover crops systems as the four main variables were significantly different over the two years of experimentation ($P < 0.01$, $P < 0.001$ in 2014 and 2015, respectively), meaning that NNI was different under the four growth conditions. The slopes of the four linear regression lines were significantly different in 2015 ($P < 0.05$), but were not in 2014. The y-intercepts of the four tillage conditions were higher when there was no-tillage (NTcc and NT) prior to growth on the three rates of applied N fertilizer, compared to conventional tillage (CTcc and CT) (Fig 4). These results suggest that N is limiting at the early stages of plant growth following tillage, due to a faster growth rate of the shoots during this period. However, at maturity, both total plant N and grain N content were similar under no-till and conventional tilling conditions. Stanislawska-Glubiak and Korzeniowska, [59] suggested that wheat plants grown under conventional tillage conditions tend to enter into their vegetative developmental phase earlier than plants cultivated under no-till conditions. This is probably because the temperature of the upper soil layer is lower in early spring when the soil is not physically disturbed, the topmost soil layer down to 5 cm deep, being compact and moist when there is no tillage [60].

To take into account any correlation that may exist between N uptake and crop growth, we examined if the relationship between NNI and NUE for each tillage treatment was linear (Fig 5). We observed that NUE was inversely proportional to NNI and that for a similar plant N status, NUE was higher following no-till treatment (NTcc and NT) compared to conventional tilling (CTcc and CT). Such a higher NUE under the two NT conditions, could be attributed to a better plant fitness during vegetative growth. Šíp et al. [51] suggested that during plant vegetative growth, the environmental conditions were more favorable for N uptake, due to a better soil moisture availability when tillage was reduced. Moreover, Maltas et al. [61] reported that when the soils were converted from CT to NT in the presence of cover crops, N mineralization increased over the conversion period, thus increasing the availability of soil N [62].

PCA analysis allowed a visualization of the correlations between agronomic traits and NUE traits, as well as their relationship with the tillage system and the level of N fertilization (Fig 6). The first axis clearly separated the N0 plot from the N1 and N2 plots of N fertilization. The second axis mainly separated no-till from conventional tillage plots. NUE and NUtE were

considerably higher under no-till conditions, when the plants were grown following N0 N fertilization. Similarly, NRem, NNI, PFPN and AEN were higher under no-till conditions, when the plants were grown following either N1 or N2 N fertilization. Such an analysis thus confirms that the no-till system had a positive impact both on NNI and NUE traits. Moreover, tillage had no effect on grain yield and N grain in both years. Our results indicate that NUE improvement can be obtained by combining no-till and the use of cover crops.

5. Conclusion

In wheat, the positive impact of no-till on both NUE and plant productivity still needs to be assessed, if we consider that in a number of cases negative effects on these two traits have been reported (see Discussion). In the present study, we showed that five years after conversion to a no-till system, similar yields were obtained in comparison to continuous conventional tilling conditions. Under no-till conditions, NUE, NUtE and other NUE-related traits such as AEN, PFPN, NAR and NNI were increased at least in one year of experimentation. However, we observed that in some cases the increase in these traits did not occur at all three levels of N fertilization. An increase in N remobilization was also observed under no-till conditions but this increase was strongly dependent upon the presence of cover crops at a given level of N fertilization. Taken together, these results highlight that the positive effects of no-till on plant performance and NUE-related traits strongly depend on the presence of cover crops. In addition, these positive effects also depend on the level of N fertilization and are not necessarily observed from one year to the other. Thus, our findings could partly explain why contrasting results are reported in the literature. Both the design of the field trial and the environmental conditions (notably N fertilization), appear to be key parameters for obtaining reproducible results over several years. Nevertheless, our study strongly suggests that the use of a continuous no-till system with cover crops is a promising strategy for sustainable wheat production based on the reduction of N fertilizer usage, without any yield penalty.

Figures.

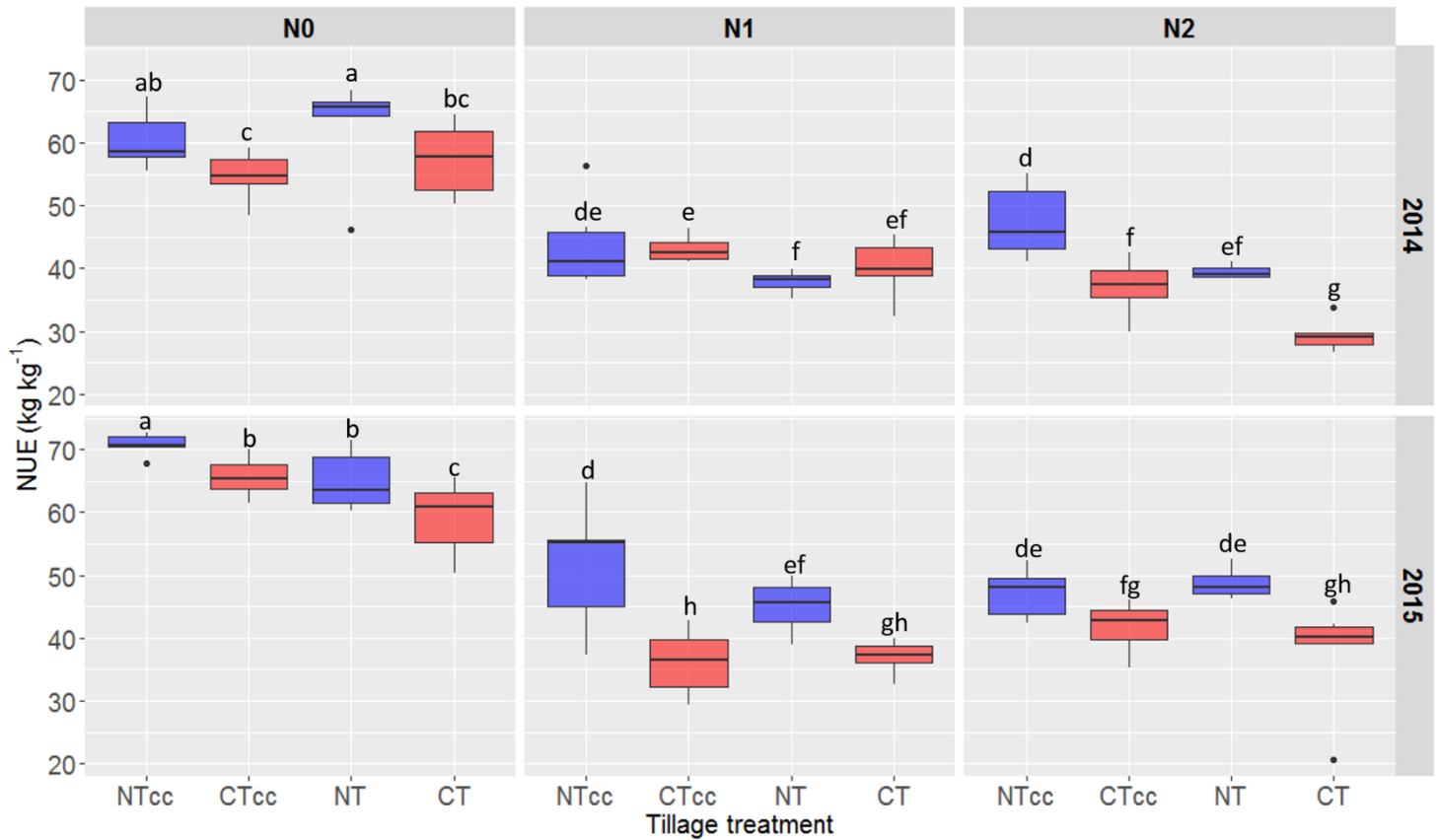


Fig 1. Impact of soil tillage treatment, cover crops and N application on NUE of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no N fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹. Data for each parameter were subjected to analysis of variance (two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

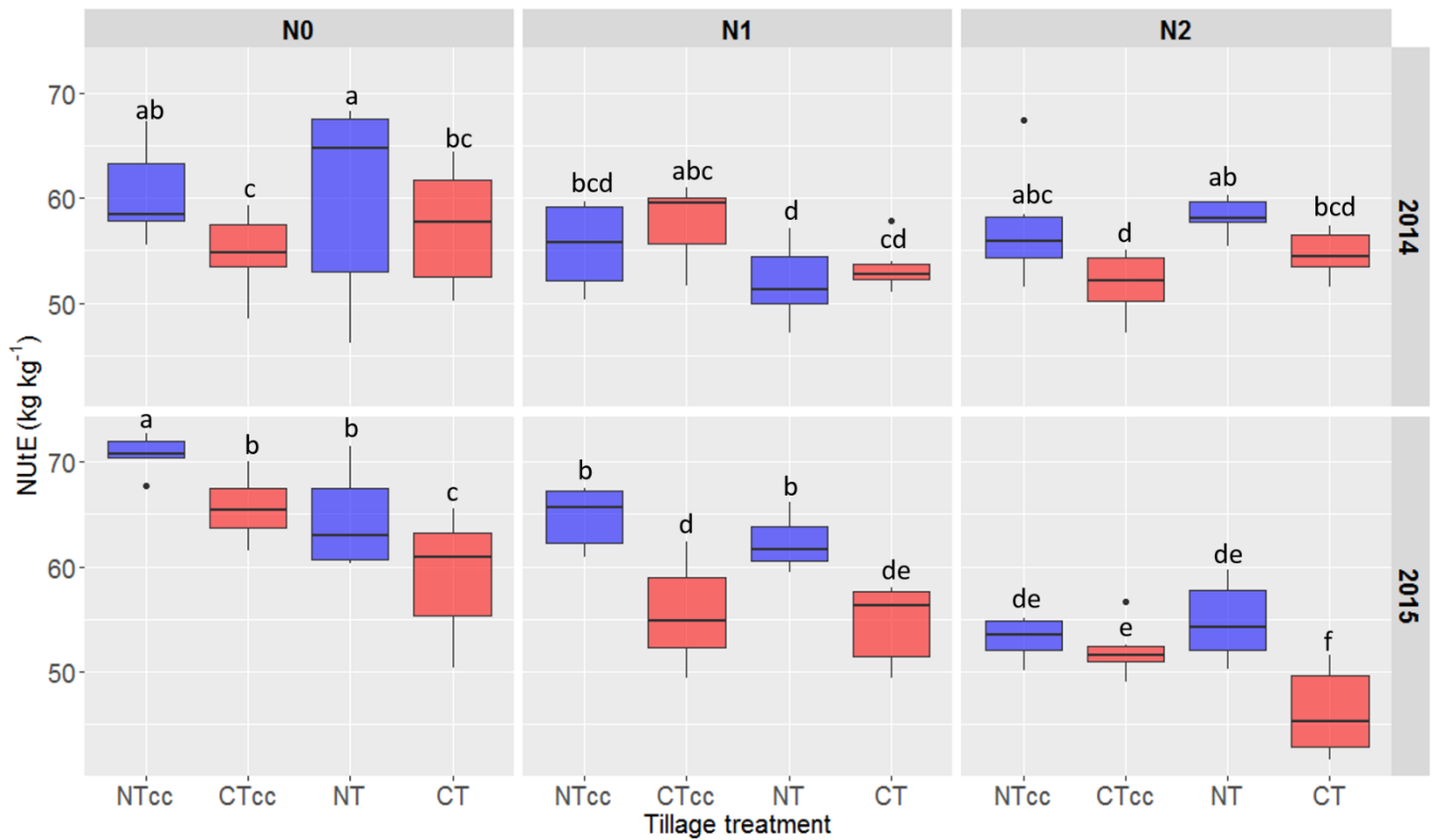


Fig 2. Impact of soil tillage treatment, cover crops and N application on NUtE of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no N fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹. Data for each parameter were subjected to analysis of variance (two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

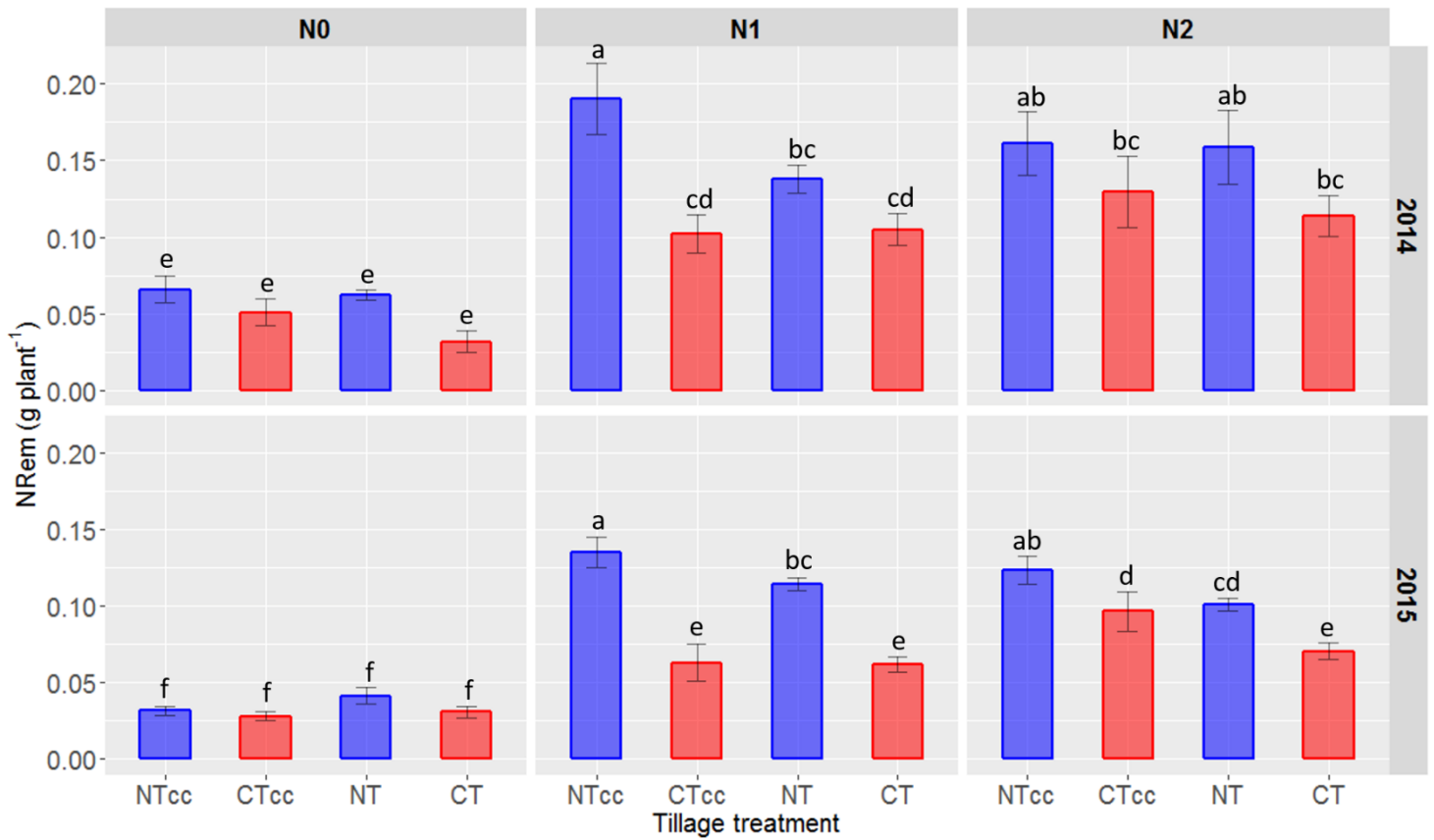


Fig 3. Impact of tillage treatment, cover crops and N application on NRem of wheat grown in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no N fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹. Data for each parameter were subjected to analysis of variance (two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

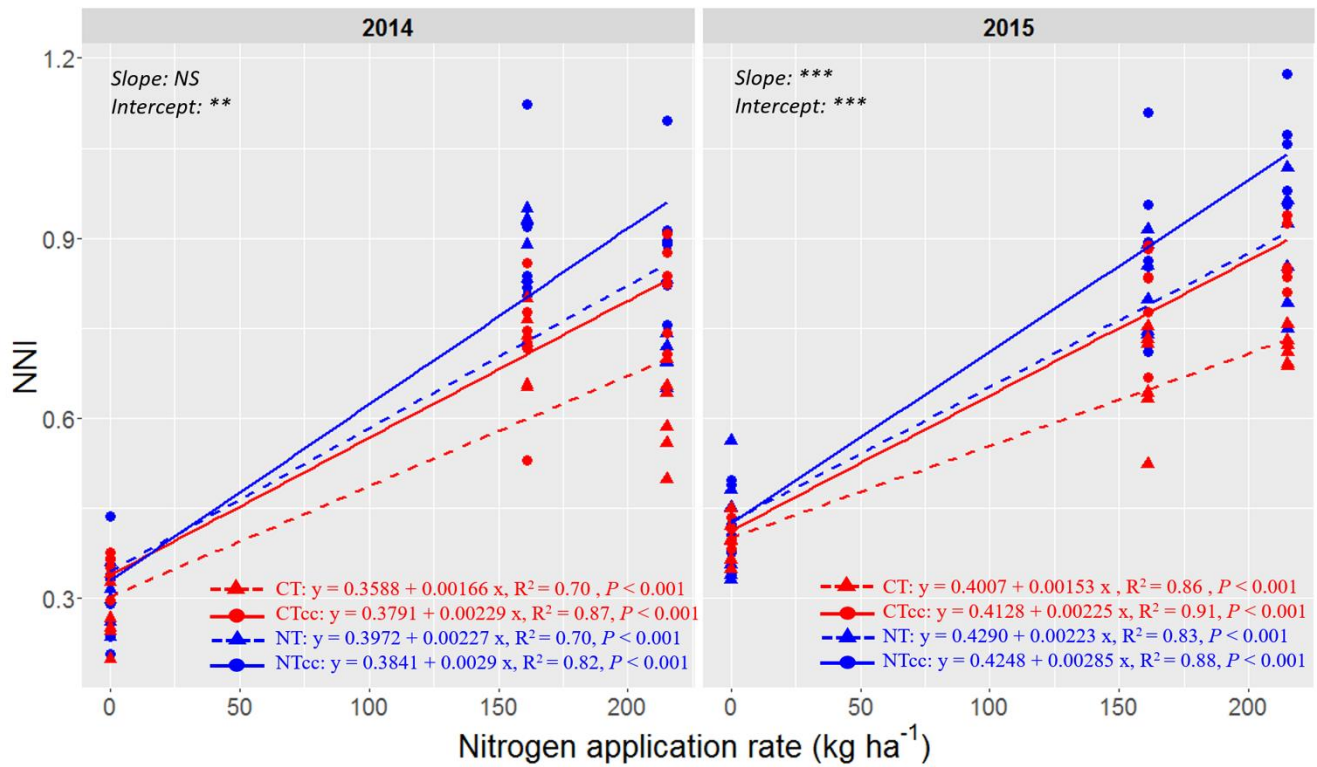


Fig 4. Nitrogen nutrition index (NNI) of wheat as a function of N application rate grown with four different tillage treatments in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. Slopes and intercepts obtained with the four tillage treatments were evaluated using analysis of covariance (ANCOVA) (**, *** = significant at 0.01, 0.001 probability level, respectively).

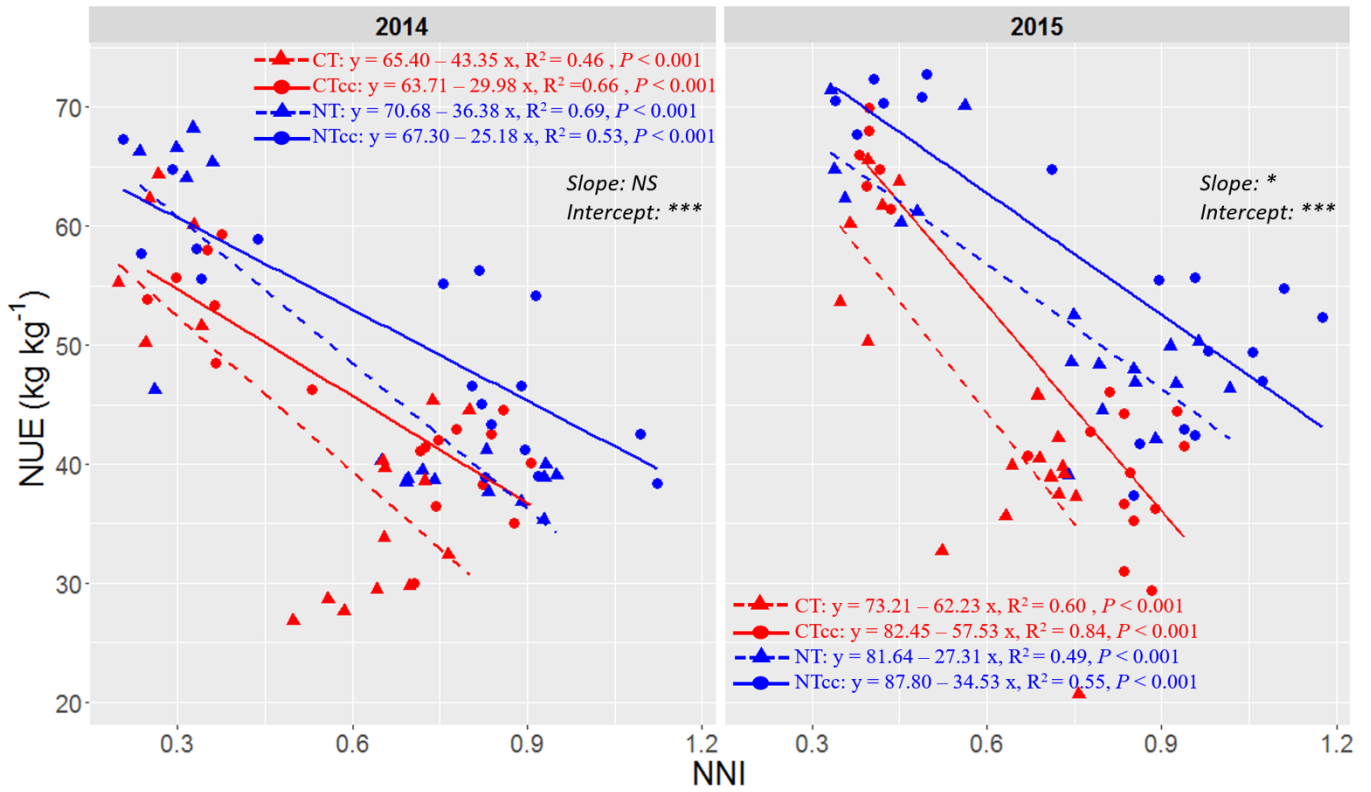


Fig 5. Nitrogen-use efficiency (NUE) of wheat as a function of N nutrition index (NNI) grown with four different tillage treatments in 2014 and 2015. NTcc: No-till with cover crops. NT: No-till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. Slopes and intercepts obtained with the four tillage treatments were evaluated using analysis of covariance (ANCOVA) (*, *** = significant at 0.05, 0.001 probability level, respectively).

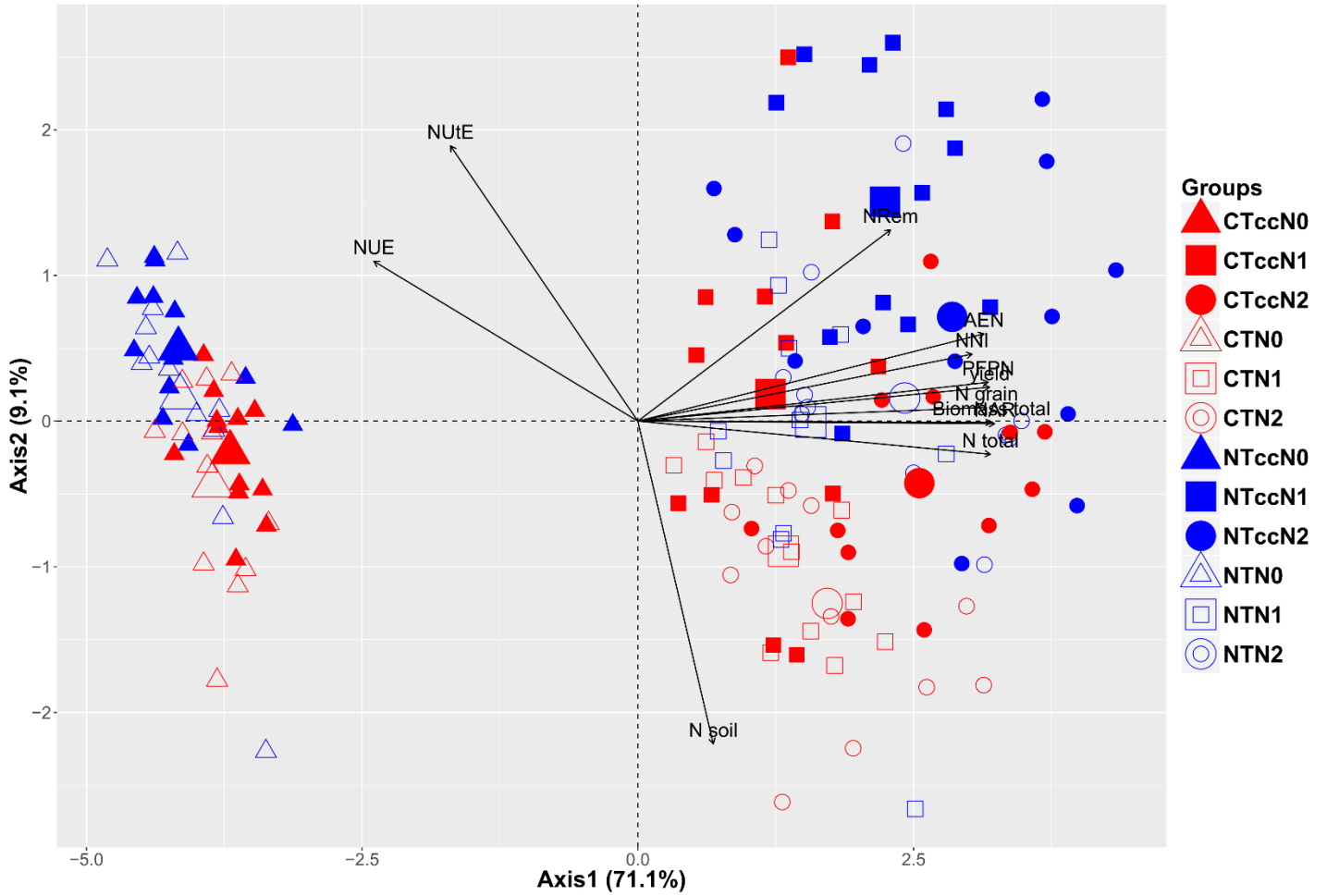


Fig 6. PCA analysis showing the correlations between agronomic traits and NUE traits according to the tilling conditions, the presence of cover crops and the level of N fertilization over two years. Diagrams are defined by the first two axes of the PCA of the different variables (n=12); Axis1 (71.1% of variance explained) and Axis2 (9.1 % of variance explained). NTcc: No till with cover crops. NT: No till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹.

Table 1. Impact of tilling, cover crops and N fertilization on wheat agronomic traits.

Source of variance		2014					2015				
		N fertilizer	Tillage	Total biomass (kg ha ⁻¹)	Total plant N uptake (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	N grain (kg ha ⁻¹)	Total biomass (kg ha ⁻¹)	Total plant N uptake (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	N grain (kg ha ⁻¹)
N0	NTcc			3499 f	40.98 c	2467 c	34.38 e	5229 g	35.21 d	2492 fg	28.69 e
	CTcc			5583 e	45.96 c	2530 c	37.05 e	7192 f	53.24 d	3495 f	38.97 e
	NT			3520 f	32.09 c	1869 c	22.76 e	4541 g	36.02 d	2298 g	26.47 e
	CT			5868 e	38.62 c	2228 c	34.92 e	5870 fg	43.06 d	2548 fg	28.88 e
N1	NTcc			15434 cd	147.6 ab	8109 ab	118.9 bc	19084 cd	155.7 c	10123 bc	129.2 bc
	CTcc			16231 bc	148.8 ab	8534 ab	119.6 bc	15891 e	138.6 c	7645 e	103.5 dc
	NT			13921 d	135.7 a	6463 a	93.48 dc	15637 e	143.0 c	8907 d	118.3 cd
	CT			17805 ab	166.8 ab	8390 ab	115.7 c	16039 e	140.4 c	7627 e	105.5 d
N2	NTcc			17196 abc	160.1 ab	9065 ab	137.4 ab	22440 a	222.3 a	11816 a	177.5 a
	CTcc			18936 a	187.0 a	10069 a	143.8 a	20453 bc	212.9 ab	11061 ab	170.4 a
	NT			18194 ab	153.9 ab	8967 ab	118.8 bc	21588 ab	211.2 ab	11560 a	171.6 ab
	CT			17409 abc	159.5 ab	8409 ab	114.2 c	18206 d	197.4 b	9175 cd	141.2 b
Analyse of variance		P > F									
Tillage				<0.01 **	ns	ns	<0.001 ***	<0.001 ***	ns	<0.001 ***	<0.001 ***
N fertilizer				<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Tillage×N fertilizer				ns	ns	ns	ns	<0.001 ***	ns	<0.001 ***	<0.001 ***

Bold: Tillage effect significant over two years. Italics: Fertilizer effect significant over two years (N0, N1 and N2). Underlined : cover crops significant effect over two years. NTcc: No till with cover crops. NT: No till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no N fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹. Data for each parameter were subjected to variance analysis (Two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different. (**, *** = significant at 0.01, 0.001 probability level, respectively). ns = not significant.

Table 2. Impact of tilling, cover crops and N fertilization on NUE indices.

Source of variance		2014				2015			
N fertilizer	Tillage	AEN (kg kg ⁻¹)	PFPN (kg kg ⁻¹)	NAR (%)		AEN (kg kg ⁻¹)	PFPN (kg kg ⁻¹)	NAR (%)	
N1	NTcc	42.25 ab	59.26 a	66.06 a		55.73 a	69.54 a	89.8 cd	
	CTcc	40.58 abc	57.19 ab	64.37 a		25.78 e	47.49 d	75.66 ef	
	NT	42.27 ab	56.01 ab	62.85 ab		42.41 c	54.37 bc	88.24 de	
	CT	36.25 bc	53.10 b	60.43 abc		31.81 d	48.53 d	80.23 f	
N2	NTcc	50.41 a	44.14 c	61.39 ab		43.37 bc	54.96 bc	70.40 a	
	CTcc	33.40 c	43.50 c	53.75 c		35.19 d	51.45 cd	60.22 bc	
	NT	36.21 bc	44.37 c	56.68 bc		46.16 b	56.06 b	64.55 ac	
	CT	25.62 d	34.65 d	48.64 d		34.23 d	48.16 d	56.28 b	
Analyse of variance		<i>P</i> > <i>F</i>							
Tillage		<0.001 ***	<0.001 ***	<0.001 ***		<0.001 ***	<0.001 ***	<0.001 ***	
N fertilizer		<0.001 ***	<0.001 ***	<0.001 ***		ns	<0.05 *	<0.001 ***	
Tillage×N fertilizer		<0.001 ***	ns	ns		<0.001 ***	<0.001 ***	ns	

Bold: Tillage effect significant over two years. Italics: Fertilizer effect significant over two years (N0, N1 and N2). NTcc: No till with cover crops. NT: No till without cover crops. CTcc: Conventional tillage with cover crops. CT: Conventional tillage without cover crops. N0: no N fertilization. N1: 161 kg N ha⁻¹. N2: 215 kg N ha⁻¹. AEN: N agronomic efficiency. PFPN: N partial factor productivity. NAR: N apparent recovery fraction. Data for each parameter were subjected to variance analysis (Two-way ANOVA). Treatment means were compared using Duncan's new multiple range test at a 95% family-wise confidence level. Means with the same letter are not significantly different. (*, *** = significant at 0.05, 0.001 probability level, respectively). ns = not significant.

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III. RESULTATS ET DISCUSSION

Chapitre 3: No-till improves photosynthesis water use efficiency and photosynthetic nitrogen use efficiency of winter wheat



Chapitre 3. No-till improves photosynthesis water use efficiency and photosynthetic nitrogen use efficiency of winter wheat

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Abstract

In the present study wheat plants were grown under tillage and no-till conditions, with and without cover crops under no or high nitrogen fertilization conditions, to evaluate the combined effect of tilling and N fertilization on photosynthetic nitrogen use efficiency and photosynthetic water use efficiency through their impacts on leaf physiological traits, such as photosynthesis rate, stomatal conductance, transpiration rate, chlorophylls content index, leaf area ratio and specific leaf area. Six years after conversion to no-till, in the presence and in the absence of cover crops, a significant increase in water use efficiency and soil water content was observed both under no and high N fertilization conditions. Moreover, we observed that photosynthetic nitrogen use efficiency, photosynthesis rate and specific leaf area were higher under no-till conditions compared to conventional tillage. Thus, agronomic practices based on continuous no-till appear to be a promising for increasing photosynthetic nitrogen use efficiency and photosynthetic water use efficiency in wheat.

1. Introduction

Crop productivity is mainly determined by the availability and efficiency in the use of limiting resources such as water and nitrogen (N) (Cossani et al., 2012). The efficiency with which these resources are used in the assimilation process is expressed through photosynthetic nitrogen use efficiency (PNUE) and photosynthetic water use efficiency (WUE) (Evans, 1983; Castellanos et al., 2005)

PNUE defined as the ratio of photosynthesis rate to leaf N (Anand et al., 2007), is a key parameter in determining the efficiency with which plants utilise N to achieve growth (Garnier et al., 1995; Poorter and Evans, 1998; Larcher, 2003). High plant growth rate is often associated with a high PNUE (Reich et al. 1995 ; Hikosaka 2004). The meaning of WUE depends on the

unit with which productivity (photosynthesis or biomass accumulation) and water use (transpiration, evapotranspiration or precipitation/irrigation) are expressed, at leaf, plant or canopy level (Salisbury, 1996). High leaf WUE is one of the criteria of water saving strategy in plant growth (Wu and Bao, 2012).

Leaf WUE and PNUE are integration characteristics affected by many leaf physiological traits. Generally, reduction in stomatal conductance (g_s) and transpiration rate (E) result in higher WUE due to the plant adjusting water loss through transpiration and absorption of CO_2 through stomatal regulation (Sharkey et al., 2007; Baker et al., 2007). Regardless of strong correlation between leaves N and photosynthetic rate; the ratio of photosynthesis rate to leaf N (PNUE) is strongly correlated with specific leaf area (SLA) (Poorter & Evans 1998 ; Schulze, 2005), thus high-SLA plants tend to have higher PNUE.

Improving nitrogen and water use efficiency in agriculture appears as an argement imperative for increasing crop productivity (Easterling et al. 2007; Hirel et al. 2011). Conservation agriculture based on no-tillage (NT) becomes a current tendency to enhance crop productivity by improving soil N recovery (Dawson et al., 2008), soil organic matter (SOM) content (Christopher et al., 2009; Lal, 2009; Dalal et al., 2011; Awale et al., 2013; Kahlon et al., 2013; Dimassi et al., 2013b) and soil water contents (Dalal et al., 2013). No-tillage could effectively inhibit the soil evaporation between plants, and increase the rate of evapotranspiration to water consumption (Chen et al., 2013). The absence of tillage conserve soil moisture (Soon et al. 2008; Arshad et al. 1999) due to the fact that the tillage alters both the total pore space and the relationship between macro and micro-pores (Josa March et al., 2010). This soil water conservation under no-till is beneficial for crop after anthesis during grain filling stage (Thomas et al., 2007). It has been shown that water stress during grain filling of wheat reduced nitrogen use efficiency (NUE) by 30% due to reducing nitrogen uptake and N remobilization (Bahrani et al., 2011).

A large number of studies have been focused on improving N fertilizer management practices in order to increase agronomic NUE and WUE in wheat (Shangguan et al., 2000; López-Bellido et al., 2005; Dawson et al., 2008). WUE and NUE have been quantified in a limited number of experiments aimed to compare cropping systems and management practices, particularly in water-limited environments (Delogu et al., 1998; Zhang et al., 1998; Asseng et al., 2001; Wu and Bao, 2012). Among these management practices, no-till effect on PNUE and photosynthetic WUE in wheat through its impact on leaf physiological traits is rarely investigated. In the present study wheat plants were grown under tillage and no- till conditions

with and without cover crops to evaluate the combined effect of tilling, cover crops and N fertilization on PNUE and photosynthetic WUE in wheat through its impact on leaf physiological traits, such as photosynthesis rate (Asat), stomatal conductance (gs), transpiration rate (E), chlorophyll content index (CCI), leaf area ratio (LAR) and specific leaf area (SLA).

2. Materials and methods

2.1. Site description and experimental design

Field experiments were conducted in Woestyne, Northern France (50°44'N, 2°22'E, 40 m above sea level). Physical and chemical soil characteristics are shown in Habbib et al. (2016). Weather-related parameters for this area are as follows: average annual rainfall 675 mm, average annual temperature 10.5°C. The field was managed under a chisel plough and rotary power system until 2010, when the experiment was initiated. The field was managed under a chisel plough and rotary power system until 2010, when the experiment was initiated. In 2010, the experimental field was split into eight treatments with three replicated plots placed randomly: two N fertilization regimes (N0, N1), four tillage/cover crops systems (no-till with (NTcc) or without (NT) cover crops and conventional tillage with (CTcc) or without cover crops (CT)). The conventional tillage in CTcc and CT plots was performed using the moldboard plowing technique followed by the passing of a rotating harrow (Kuhn, France) for shallow tillage.

Wheat (*Triticum aestivum* var BTH intensive EXPERT, Syngenta, Switzerland) was sown in rows 12.5 cm apart using an AS 400 drill (Alpego, Italia). The crop rotation preceding wheat cultivation in 2016 consisted of wheat (*Triticum aestivum* L.) in 2010 followed by pea (*Pisum sativum*) in 2011, maize (*Zea mays*) in 2012, wheat (*Triticum aestivum* L.) in 2013, flax (*Linum usitatissimum* L.) in 2014, beet (*Beta vulgaris* L.) in 2015. This cover crop consisted in a mixture of leguminous and non-leguminous species which were sown as follow: 12 kg ha⁻¹ of Egyptian clover (*Trifolium alexandrinum* L.), 100 kg ha⁻¹ of faba bean (*Vicia faba* L.), 20 kg ha⁻¹ of vetch (*Vicia sativa* L.), 5 kg ha⁻¹ of flax (*Linum usitatissimum* L.), 4 kg ha⁻¹ of phacelia (*Phacelia tanacetifolia* Benth.), 10 kg ha⁻¹ of oats (*Avena sativa* L.).

The amounts of N fertilizer applied under N1 conditions (215 kg N ha⁻¹) were determined according to the N budget method for maize (Machet et al., 1990), based on the predictive balance-sheet method (Software Azobil, INRA, Laon, France). The N fertilizer composed of 50% urea, 25% ammonium, 25% nitrate, applied in a liquid form on the soil

surface through broadcast applications at daybreak or at nightfall. In the applied conditions, we can consider that volatilization is neglected.

2.2. Gas exchange measurements

The flag leaves of four plants during the anthesis period from each treatment were used for leaf gas exchange. Gas exchange parameters were measured on the youngest fully expanded leaf of the main culm, using a LI-6400XT (LI-COR Biosciences, Lincoln, Nebraska 68504, USA) portable photosynthesis system equipped with the 2 cm² LI6400-02B chamber. Photosynthetic activity was measured at a photosynthetic photon flux density (PPFD) of 1100 mmol m⁻² s⁻¹. Leaf temperature was maintained at 21 ± 1.2°C under ambient humidity throughout measurements with sample chamber CO₂ concentration at 400 ppm.

Intrinsic water-use efficiency (WUE_{int}) was calculated as the ratio of light-saturated net CO₂ assimilation (Asat, μmol m⁻² s⁻¹) to stomatal conductance (g_s, μmol m⁻² s⁻¹). Instantaneous water use efficiency (WUE_{ins}, Transpiration efficiency at the leaf level) was calculated as the ratio of CO₂ assimilation (Asat, μmol m⁻² s⁻¹) to transpiration rate (E, mmol m⁻² s⁻¹) (Salisbury, 1996).

2.3. Crop sampling and Plant analysis

After leaf gas exchange measurements, wheat was harvested to assess total leaf area and above ground dry matter. Images of leaves were scanned and images were digitized by the imageJ software in order to determine total leaf area. Then each plant and leaf was dried in an oven for 48 h at 70 °C. Specific leaf area (SLA, cm² g⁻¹) was calculated as the total leaf area divided by the dry mass of leaves. Leaf area ratio (LAR, cm² g⁻¹) was calculated as the total leaf area divided by the dry mass of the whole plant. Leaves were finally ground in a Retsch mill (Retsch zm200, Haan, Germany) to obtain a fine powder (0.75 mm particles). Leaf N contents were quantified using the combustion method of Dumas (Dumas, 1831) using a Flash EA 1112 elemental analyzer, Thermo Electron, Germany. Photosynthetic N-use efficiency (PNUE) was calculated as the ratio of Asat to leaf N content, both expressed per unit leaf area (Lopes and Araus, 2006).

At the same time of gas exchange measurements, chlorophyll content measurements were collected from each flag leaf of each treatment using a portable chlorophyll meter (CCM200, Opti-Science), which calculates a unitless chlorophyll content index (CCI) value from the ratio of optical absorbance at 655 nm to that at 940 nm.

2.4. Soil water content

At the same time of gas exchange measurements, four measures of soil water content (%) at 20 cm depth were determined in each treatment using a soil moisture meter (FieldScout TDR 100, USA).

2.5. Statistical analyses

All statistical analyses were performed in R © version 3.3.0. Data were subjected to non-parametric Kruskal–Wallis one-way analysis of variance (H -value) followed by a Dunn's post hoc test whenever significant (Agricolae package). Correlations between WUE, PNUE, SWC and leaf physiological traits (Asat, g_s , E, CCI, SLA, LAR and Nmass) were computed using a Spearman correlation coefficient at $P < 0.05$ (Hmisc package). Principal component analysis (PCA) (ade4 package) was also carried out to visualize relationships existing among treatments.

3. Results

3.1. Effect of tillage treatment, cover crops and N fertilizer rate on leaf physiological traits

Tillage, cover crops and N fertilizer effect on leaf physiological rate are presented in Table 1. The kruskal-wallis statistical analysis indicated that Asat was significantly different between treatments ($P < 0.001$). Tillage had a significant effect on Asat only under N0 conditions with a higher photosynthetic rate in NTcc than CTcc. Cover crops have a positive impact on Asat with higher photosynthetic rate in NTcc compared to NT in two fertilizer rates and in CTcc compared to CT only under N1 conditions. N application increased significantly Asat in all tillage systems.

g_s was significantly different between treatments ($P < 0.001$). Tillage had a significant effect on g_s under N0 conditions with a higher conductance in NTcc than CTcc. Inversely, g_s was significantly higher in CTcc than NTcc in N1 conditions. Cover crops have a positive impact on g_s only under N0 conditions with higher conductance in NTcc compared to NT. However, cover crops have a negative impact on g_s under N1 conditions with higher conductance in NT compared to NTcc. N application increased significantly g_s in CTcc, NT and CT systems.

Tillage had a significant effect on E under N0 conditions with a higher transpiration in NTcc than CTcc. Inversely, E was significantly higher in CTcc than NTcc in N1 conditions. cover crops have a negative impact on E with higher transpiration in CT than CTcc in N0

conditions and in NT compared to NTcc in N1 conditions. N application increased significantly E in CTcc, NT and CT systems.

Tillage did not modify CCI in both N application rates. Cover crops have a positive impact on CCI only under N0 conditions with higher CCI in NTcc compared to NT. N application increased significantly CCI all tillage treatments.

No-till increased SLA both in N0 and N1 conditions with a higher SLA in NT than CT. Similarly, SLA was higher under NTcc than CTcc in N1 conditions. Cover crops did not modify SLA in both N application rates. N application increased significantly SLA only under NTcc system.

No-till increased LAR in N0 conditions with a higher LAR in NT than CT. No significant effect of tillage system on LAR was observed under N1 conditions. Cover crops increased LAR in N0 conditions with a higher LAR in CTcc than CT. N application increased significantly LAR in NT and CT systems.

Tillage did not modify leaf Nmass in both N application rates. Cover crops increased Nmass in N0 conditions only under no-till systems with a higher Nmass in NTcc than NT. N application increased significantly Nmass in all tillage treatment

Table 1. Impact of tilling, cover crops and nitrogen fertilization on wheat leaf physiological traits.

Source of variance	Leaf physiological traits							
	Tillage	Asat	gs	E	CCI	SLA	LAR	Nmass
N0	NTcc	15.06 ± 0.75 d	0.172 ± 0.005 b	2.03 ± 0.18 bc	11.09 ± 1.08 c	176.22 ± 10.22 bc	3.08 ± 0.31 abc	1.99 ± 0.15 c
	CTcc	10.22 ± 0.47 e	0.113 ± 0.002 a	1.41 ± 0.05 d	9.57 ± 1.18 cd	169.53 ± 4.17 cd	2.88 ± 0.57 bc	1.74 ± 0.09 d
	NT	9.70 ± 1.40 e	0.118 ± 0.027 a	1.62 ± 0.51 bcd	8.86 ± 0.71 d	186.3 ± 5.05 ab	2.87 ± 0.26 c	1.74 ± 0.07 d
	CT	10.87 ± 0.85 e	0.137 ± 0.011 a	2.02 ± 0.17 bc	9.46 ± 1.50 cd	165.45 ± 4.04 d	1.87 ± 0.25 d	1.77 ± 0.03 d
N1	NTcc	21.05 ± 1.23 a	0.171 ± 0.033 b	1.64 ± 0.25 cd	25.45 ± 1.59 b	195.71 ± 16.38 ab	3.80 ± 0.84 ab	2.72 ± 0.05 ab
	CTcc	20.94 ± 2.51 ab	0.259 ± 0.045 c	2.29 ± 0.45 b	32.22 ± 3.03 ab	170.99 ± 14.12 cd	3.77 ± 0.52 ab	2.91 ± 0.12 a
	NT	19.34 ± 0.53 bc	0.246 ± 0.007 c	2.91 ± 0.33 a	34.34 ± 1.86 a	198.21 ± 5.56 a	3.67 ± 0.24 a	2.70 ± 0.14 b
	CT	18.43 ± 0.43 c	0.220 ± 0.008 c	2.66 ± 0.14 a	30.66 ± 2.95 a	170.37 ± 4.16 cd	3.15 ± 0.24 abc	2.85 ± 0.08 ab
<i>H</i>		40.457 ***	36.679 ***	29.590 ***	40.059 ***	24.808 ***	22.583 **	38.658 ***

N0 = no fertilization, N1 = N fertilization, NTcc = No-till with cover crops, CTcc = Conventional tillage with cover crops, NT = No-till without cover crops, CT = Conventional tillage without cover crops. *H*: value of the Kruskal–Wallis test with its significance in brackets (*, **, *** = significant at 0.05, 0.01, 0.001 probability level, respectively). Letters give the result of a Dunn's post hoc test at a 95% family-wise confidence level. Means sharing the same letter are not significantly different.

3.2. Effect of tillage treatment, cover crops and N fertilizer rate on WUE and PNUE

WUE was significantly modified under tillage, cover crops and fertilizer rate ($P < 0.01$) (Fig 1). WUEint was significantly higher under NTcc compared to CTcc in N1 conditions (Fig 1A). In N0 conditions, WUEint was not significantly different between tillage and no-till conditions. Cover crops have a positive impact on WUEint which was higher in CTcc than CT in N0 conditions and in NTcc than Nt in N1 conditions. N application increased significantly WUEint in NTcc system.

WUEins was significantly higher in NT compared to CT in N0 conditions (Fig 1B). Similarly, WUEins was significantly higher in NTcc compared to CTcc in N1 conditions. Cover crops have a positive impact on WUEins. This positive effect was significantly higher under CTcc than CT in N0 and N1 conditions and was higher in NTcc than NT in N1 conditions. N application increased significantly WUEint with higher efficiency in N1 than N0 in NTcc, CTcc, and NT systems.

Tillage had a significant negative impact on PNUE in two fertilizer rates (Fig 2). PNUE was higher under NTcc than CTcc in two fertilizer rates. Similarly, PNUE was higher under NT than CT in N1 conditions. Cover crops have a positive impact on PNUE only in N0 conditions with higher efficiency in NTcc compared to NT. N application increased significantly PNUE with higher efficiency in N1 than N0 in CTcc and NT systems.

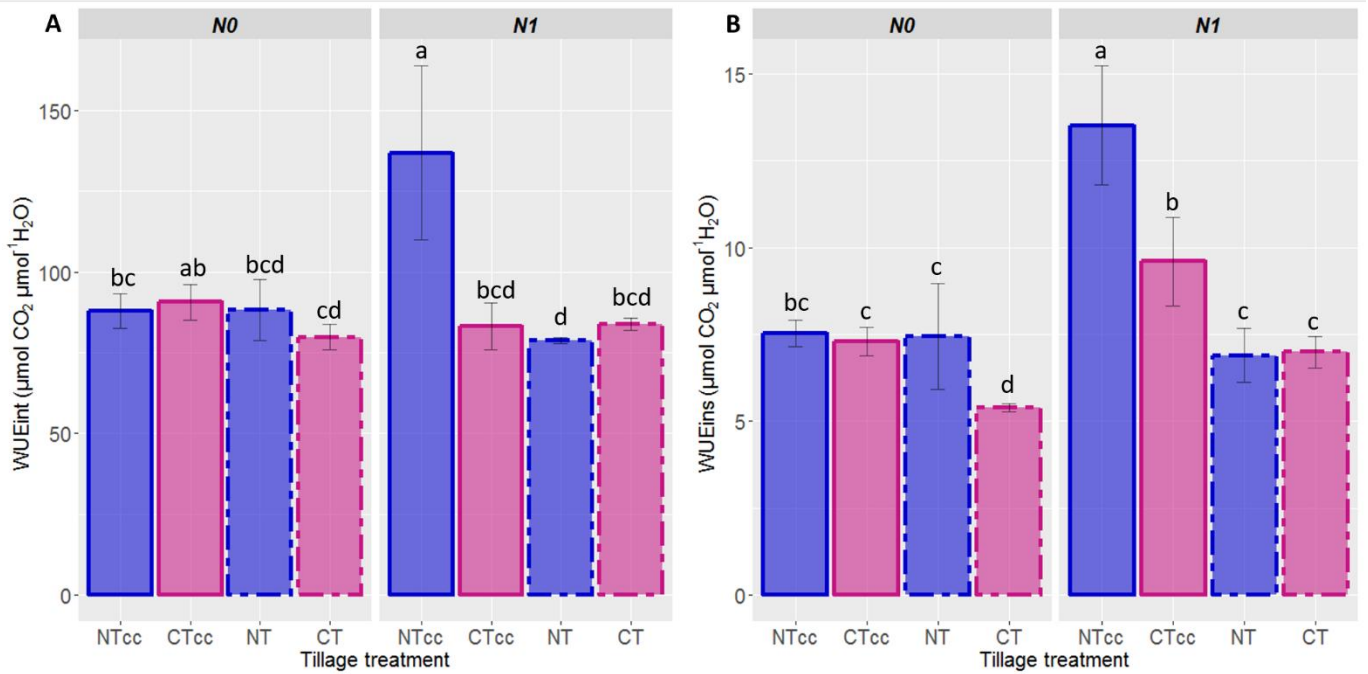


Fig. 1. Impact of tillage and nitrogen fertilization on WUE_{int} (A) and WUE_{ins} (B): (NTcc) No till with cover crops, (NT) No till without cover crops, (CTcc) Conventional tillage with cover crops, (CT) Conventional tillage without cover crops. N0 = no fertilization, N1 = N fertilization. Data for each parameter were subjected to Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

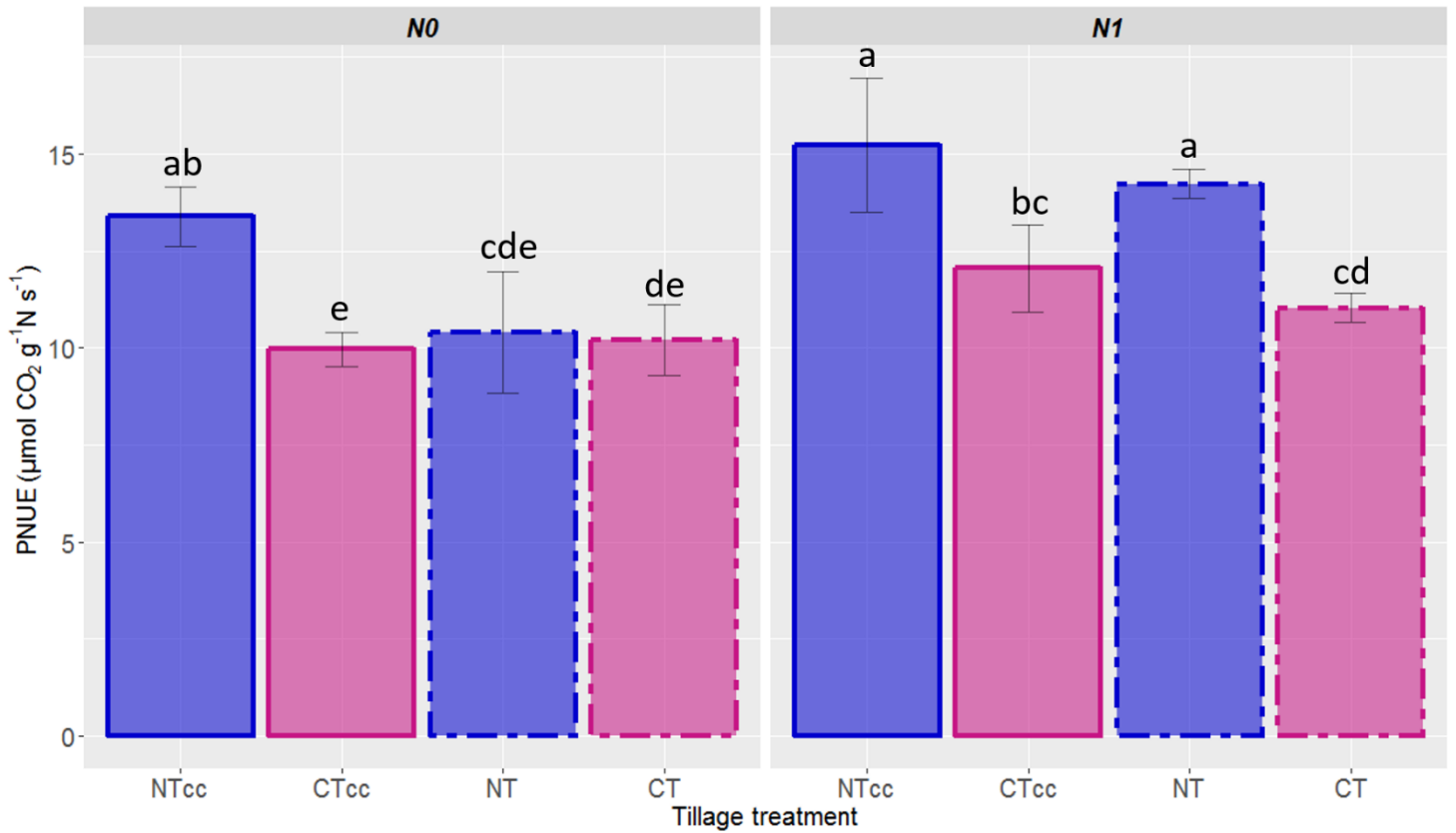


Figure 2. Impact of tillage and nitrogen fertilization on PNUE: (NTcc) No till with cover crops, (NT) No till without cover crops, (CTcc) Conventional tillage with cover crops, (CT) Conventional tillage without cover crops. N0 = no fertilization, N1 = N fertilization. Data for each parameter were subjected to Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

3.3. Effect of tillage treatment, cover crops and N fertilizer rate on soil water content

The kruskal-wallis statistical analysis indicated that SWC was significantly different between treatments ($P < 0.001$) (Fig 3). Tillage had a significant effect on SWC both in N0 and N1 conditions. A significant increase in SWC was observed in NT compared to CT under N0 and N1 conditions and in NTcc compared to CTcc only under N1 conditions. Cover crops have a positive impact on SWC with higher SWC in NTcc compared to NT in two fertilizer rates and in CTcc compared to CT only under N0 conditions. N application decreased significantly SWC with higher content in N0 than N1 in NTcc, CTcc and CT systems.

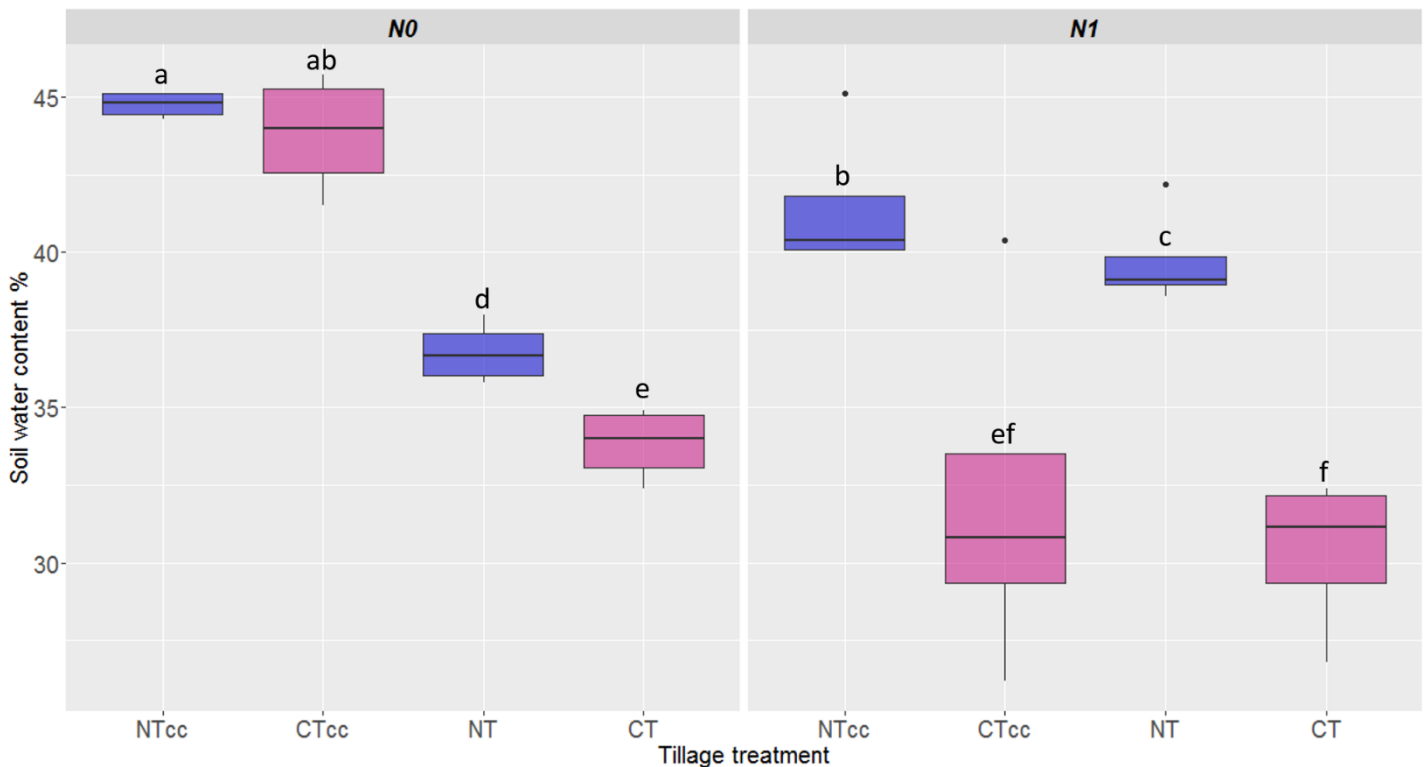


Figure 3. Impact of tillage and nitrogen fertilization on SWC: (NTcc) No till with cover crops, (NT) No till without cover crops, (CTcc) Conventional tillage with cover crops, (CT) Conventional tillage without cover crops. N0 = no fertilization, N1 = N fertilization. Data for each parameter were subjected to Kruskal–Wallis one-way analysis of variance. Treatment means were compared using Dunn's post hoc test at a 95% family-wise confidence level. Means with the same letter are not significantly different.

3.4. Correlation analyses

Spearman correlations between WUE_{int}, WUE_{ins}, PNUE, SWC, Asat, *g_s*, E, CCI, SLA, LAR and N_{mass}, are presented in Fig 4. WUE_{int} was significantly and negatively correlated with *g_s* and E. Conversely, WUE_{int} was significantly and positively correlated with LAR and SWC. WUE_{ins} was significantly and negatively correlated with E and positively correlated with Asat., LAR and CCI. PNUE was significantly and positively correlated with *g_s*, E, LAR, CCI, Asat, SLA, N_{mass} and SWC. A PCA analysis was then performed to obtain a visual representation of the correlations between WUE, PNUE, SWC and leaf physiological traits according to the tillage system and the level of N fertilization (Fig 5). The first two axes of a PCA explained 67.5 % of the variation in the data set. The variables were separated into eight groups corresponding to tillage system, cover crops and fertilizer application rate. Axis.1 (40.6 % of variance explained) was positively correlated with SWC, and negatively correlated with other traits. SWC, WUE_{int}, WUE_{ins}, PNUE, SLA, LAR and Asat were strongly correlated and positively grouped along Axis.2 (26.9 % of variance explained) which matches the Spearman correlation test. Similarly, N_{mass}, CCI, *g_s* and E were strongly correlated and negatively grouped along Axis.2. The N0 and N1 fertilization conditions were clearly separated along the first axis. For N1 conditions, the NT_{cc} and CT_{cc} were clearly separated along the second axis. WUE_{int}, WUE_{ins}, PNUE and SLA were markedly higher in NT_{cc} compared to CT_{cc} under N1 conditions.

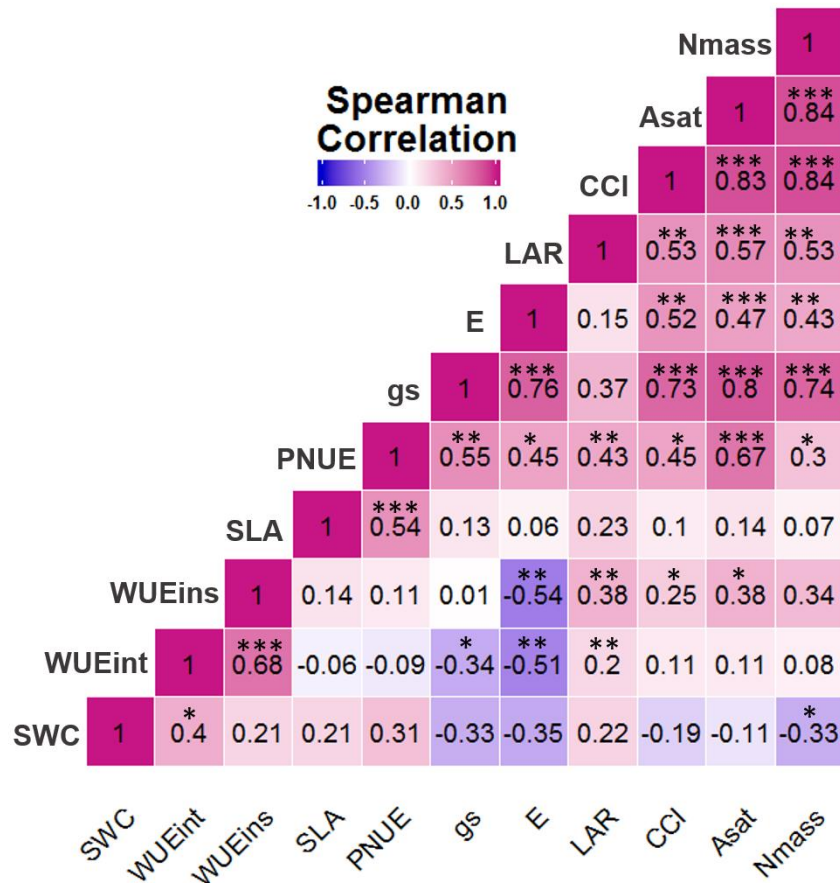


Figure 4. Spearman correlation coefficient r between WUEins, WUEint, PNUE, SWC and leaf physiological traits. (*, **, ***= significant at 0.05, 0.01, 0.001 probability level, respectively.)

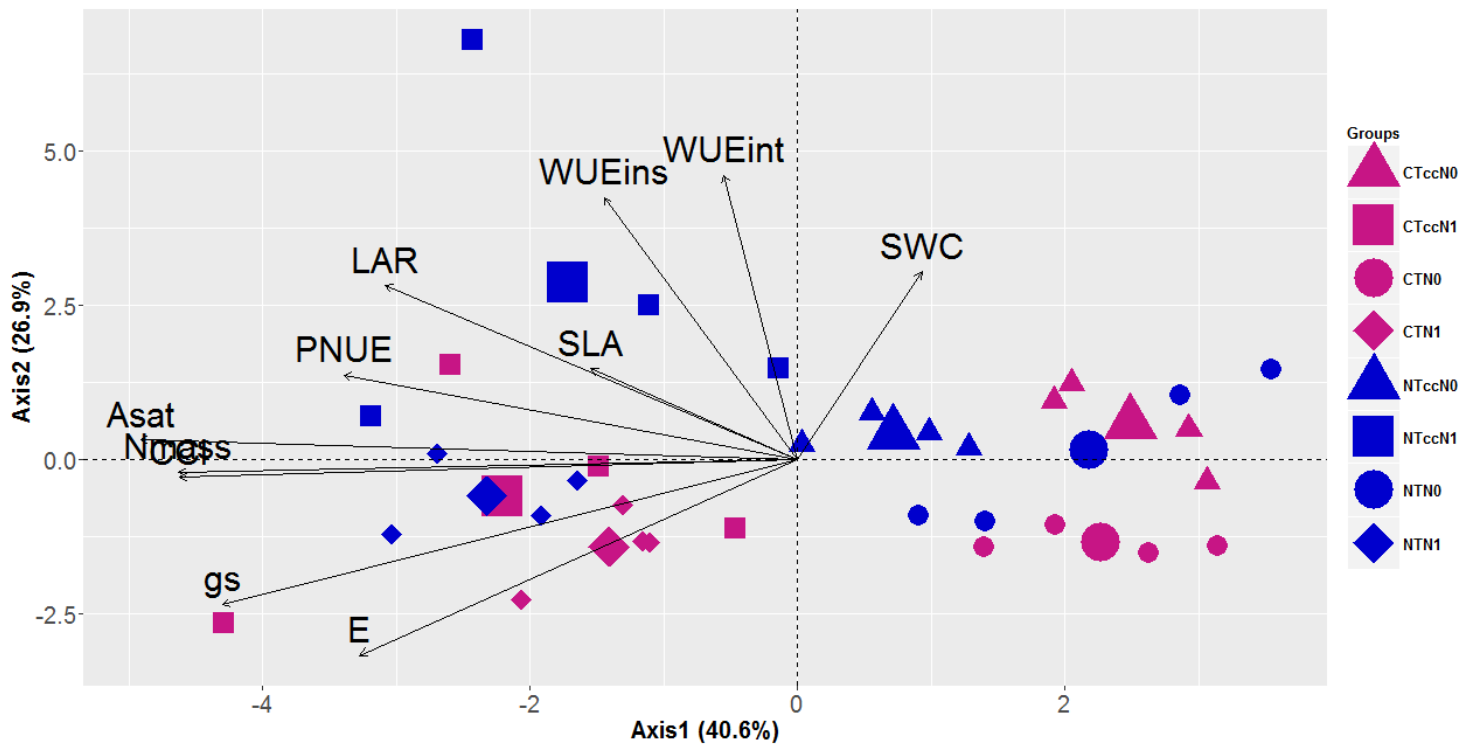


Figure 5. PCA analysis showing the correlations between between WUEins, WUEint, PNUE, SWC and leaf physiological traits according to tilling conditions and N fertilization. Diagrams were defined by the first two axes of the PCA of the variables (n=4). Axis.1 (40.6% of variance explained) and Axis.2 (26.9 % of variance explained). NTccN0 = No-till with cover crops without N fertilization, NTN0 = No-till without cover crops without N fertilization, CTccN0 = Conventional tillage with cover crops without N fertilization, CTN0= Conventional tillage without cover crops without N fertilization, NTccN1= No-till with cover crops and N fertilization, NTN1= No-till without cover crops with N fertilization, CTccN1= Conventional tillage with cover crops with N fertilization and CTN1= Conventional tillage without cover crops with N fertilization.

4. Discussion

In the present study, we found that wheat use the limited resources (N and water) more efficiently under no-till system than conventional tillage system. A higher WUE_{int} was observed in NTcc compared to CTcc. Similarly, WUE_{ins} was higher under NTcc and NT compared to CTcc and CT. This results coincided with a higher photosynthesis rate and lower stomatal conductance and transpiration rate in NTcc than CTcc. These results are in agreement with those obtained with wheat by Hou et al. 2013, who showed that both photosynthesis rate and intrinsic WUE were increased under no-till conditions when compared with CT.

The presence of cover crops improved leaf WUE, due to higher photosynthesis rate and lower transpiration rate in NTcc and CTcc than NT and CT. The higher WUE and Asat in no-till system specially in the presence of cover crops, largely due to the amount of water stored in the soil. In this study, tillage had a negative impact on SWC which was significantly lower following CTcc and CT under N0 and N1 conditions. No-till system increases the infiltration and storage of water in the soil profile (Govaerts et al., 2009), reduces water loss from the soil by evaporation and favoring the development of a deep root system which will produce more efficient use of water (Soon et al., 2008; Ruiz-Colmenero et al., 2013). Several previous studies have shown that water deficit produced negative effects on light-saturated net CO₂ assimilation rate (Asat) (Kumar et al., 2011), due to stomatal closure, which prevents the entry of CO₂ into the leaf (Grassi and Magnani, 2005; Ripley et al., 2007).

PNUE was also higher under no-till system compared to conventional tillage in N0 and N1 conditions, due to higher Asat and higher SLA under no-till. The ratio of photosynthesis rate to leaf N (PNUE) is strongly correlated with specific leaf area (SLA) (Poorter & Evans 1998; Schulze 2005), thus high-SLA plants tend to have higher PNUE. High-SLA plants generally have higher water contents per unit dry mass, lower concentrations of cell wall compounds and C per unit leaf mass, and higher mass based concentrations of N (Poorter et al., 1992). Thus, the variation in PNUE is linked with a suite of traits that determine the growth potential of a crop such as the variation in the proportion of organic N compounds allocated to photosynthetic versus non-photosynthetic functions (Lambers and Poorter, 2004).

N application increased leaf WUE due to a stronger increase in the rate of photosynthesis than in transpiration and stomatal conductance in response to N application. It is likely that N addition accelerate the transport of photosynthetic CO₂ in the leaves, leading to enhanced Asat of the plant. Positive effects of N fertilizer input on leaf WUE have also been shown by Shangguan et al. (2000) and Cabrera-Bosquet et al. (2007).

Similarly, PNUE was higher under N1 conditions than N0 conditions. Under N1 conditions, the rate of photosynthesis increased more than leaf nitrogen concentration, leading to an increase in PNUE. In addition, N application triggered a significant increase in SLA, LAR and CCI which enhance photosynthetic capacity.

Contrary to previous studies reported the negative correlation between WUE and PNUE (Livingston et al., 1999; Maranville and Madhavan, 2002; Cabrera-Bosquet et al., 2007), our results show that a high leaf water use efficiency is not necessarily related with a low PNUE.

PCA analysis allowed a refinement of the correlations observed between WUEint, WUEins, PNUE, SWC, and the various leaf physiological traits such as Asat, gs, E, CCI, SLA, LAR, Nmass and their relationship with the tillage system according to the level of N fertilization (Fig 4). The first axis clearly separated N0 plots from N1 plots. The second axis mainly separated no-till from conventional tillage plots. Remarkably, WUEint, WUEins, PNUE and SLA were markedly higher in NTcc compared to CTcc under N1 conditions. Such an analysis thus confirmed that the no-till system had a positive impact both on leaf WUE and PNUE. Our results indicate that improvement in both physiological WUE and PNUE can be induced by combining agricultural management practices such as no-till and cover cropping.

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IV. DISCUSSION GENERALE ET PERSPECTIVES

Parmi les méthodes agronomiques les plus novatrices, figurent les nouvelles techniques de semis réalisées directement sous couverture végétale permanente permettant la restauration à court terme des niveaux de biodiversité des sols indispensables à une meilleure exploitation de ressources édaphiques naturelles. De plus en plus d'agriculteurs, soucieux de l'environnement, montrent un intérêt particulier pour ces nouvelles techniques de semis direct sous couverture végétale permanente qui constituent des méthodes alternatives de production réputées pour avoir de haute valeur écologique et un plus faible impact environnemental que les systèmes traditionnellement labourés annuellement.

Les céréales ont été les premières cultures à être expérimentées en mode de semis direct du fait de leur facilité d'implantation. A l'heure actuelle, la moitié des céréales produites par trois des plus grands pays agricoles, tels le Brésil, l'Argentine et les États-Unis, sont obtenus au moyen de ces nouvelles techniques de semis direct (Derpsch and Friedrich 2009). Ces pays ont en effet adopté l'agriculture de conservation des sols pour lutter contre l'érosion hydrique et éolienne de leurs sols (Friedrich et al., 2012).

Au plan de l'Etat Français, il s'avère que les systèmes de culture développés et gérés selon les principes de l'agriculture de conservation ou de protection des sols sont encore très peu nombreux. De fait, les études concernant la gestion de l'azote de ces nouveaux systèmes de culture sont quasi inexistantes.

L'objectif principal de la thèse était d'analyser les nouveaux systèmes de cultures économiquement et écologiquement performants basés sur les techniques d'agriculture de conservation de sols au plan de la nutrition azotée. Nous avons choisi d'étudier l'influence des modifications des pratiques culturales (type de travail du sol, couverts végétaux, niveaux d'intensification en azote) sur les divers paramètres affectant la productivité végétale et la nutrition azotée de deux céréales (Blé et Maïs). Dans ce cadre, trois expérimentations ont été mises en place au champ pour mieux comprendre les phénomènes agronomiques et processus écologiques s'opérant lors des changements de pratiques culturales. Les conséquences de la mise en place de couverts végétaux d'interculture et l'arrêt du labour sur la nutrition azotée des cultures ont été comparées aux systèmes de culture traditionnels de type labourés et comportant ou non des couverts végétaux d'interculture. Parallèlement aux deux variables d'entrée des systèmes de culture, « travail du sol x couverture végétale » étudiées, nous avons ajouté une troisième variable d'entrée définissant le niveau d'intensification azotée des systèmes étudiés en prenant comme référence la dose d'engrais azoté X (N₂) telle que définie historiquement par la méthode du bilan prévisionnel de fertilisation azotée pour les systèmes de culture labourés classiques. Une dose N_x-25% (N₁), dite bas-intrants, a également été définie,

permettant de comparer les différentiels de production et de nutrition azotée de deux systèmes de cultures de niveaux d'intensification différents. Puis, en supplément, nous avons défini un troisième niveau d'intensification azoté dit système non intensifié ou système zéro azote (N0). Ce système étant indispensable pour déterminer de nombreux paramètres d'utilisation de l'azote par les cultures. A noter que le système N0 sert également de témoins pour d'autres objectifs non développés dans le cadre de cette thèse, notamment pour comprendre les effets de la fertilisation azotée à long terme sur l'activité biologique des sols et le fonctionnement des cycles biogéochimiques (notamment évolution des teneurs en C et N organique, activités enzymatiques des sols, biomasse microbienne des sols....).

L'objectif finalisé étant de trouver le ou les systèmes de cultures le(s) plus adapté(s) pour obtenir de bons rendements financiers tout en essayant de diminuer, à rendement constant, les impacts environnementaux des apports d'azote minéraux et en maintenant, voire en améliorant simultanément, la fertilité des sols (évolution CN total, biodiversité microbienne, activités biologiques et enzymatiques)

Un essai de deux ans a été effectué sur le terrain pour évaluer l'impact combiné de système du travail de sol avec couverts végétaux et de la fertilisation azotée sur divers caractères agronomiques liés à l'efficacité d'utilisation de l'azote chez le maïs en présence de couverts végétaux permanentes. Les paramètres étudiés dans cet essai sont résumés en (**Figure 1**).

La première expérimentation réalisée sur le maïs a permis de répondre à la question 1. Après 4 années de non travail du sol, on a observé une augmentation significative de l'efficacité d'utilisation de l'azote NUE, de l'indice de récolte d'azote (NHI) ainsi que de l'efficacité de remobilisation de l'azote (NRE), valable pour les systèmes de culture développés avec (N1) et sans fertilisation azoté (N0). Dans nos conditions, au cours des deux années d'expérimentation, le travail du sol a eu un impact négatif sur la quantité d'eau stockée dans le sol. Des résultats comparables montrant que le travail du sol réduisait la quantité d'eau disponible dans le sol ont déjà été publiés (Arshad et al., 1999; Soon et al., 2008). La cause de cette réduction tiendrait au fait que le labour bouleverse la cohésion structurales et altère la porosité et la relation entre micro-macro pores (Josa March et al., 2010). La conservation d'eau dans les modalités de semis direct est bénéfique pour la culture pendant les phases critiques du développement (Thomas et al., 2007). Pendant cette période, la remobilisation de l'azote dépend largement de la disponibilité en eau dans le sol (Sarvestani et al., 2003; Plaut et al., 2004; Ercoli et al., 2008). Cette conservation d'eau dans le système de non-labour améliore la remobilisation de l'azote vers les grains (Thomas et al., 2007) et une meilleur efficacité azotée peut ainsi être observée (Pampana et al., 2007; Bahrani et al., 2011).

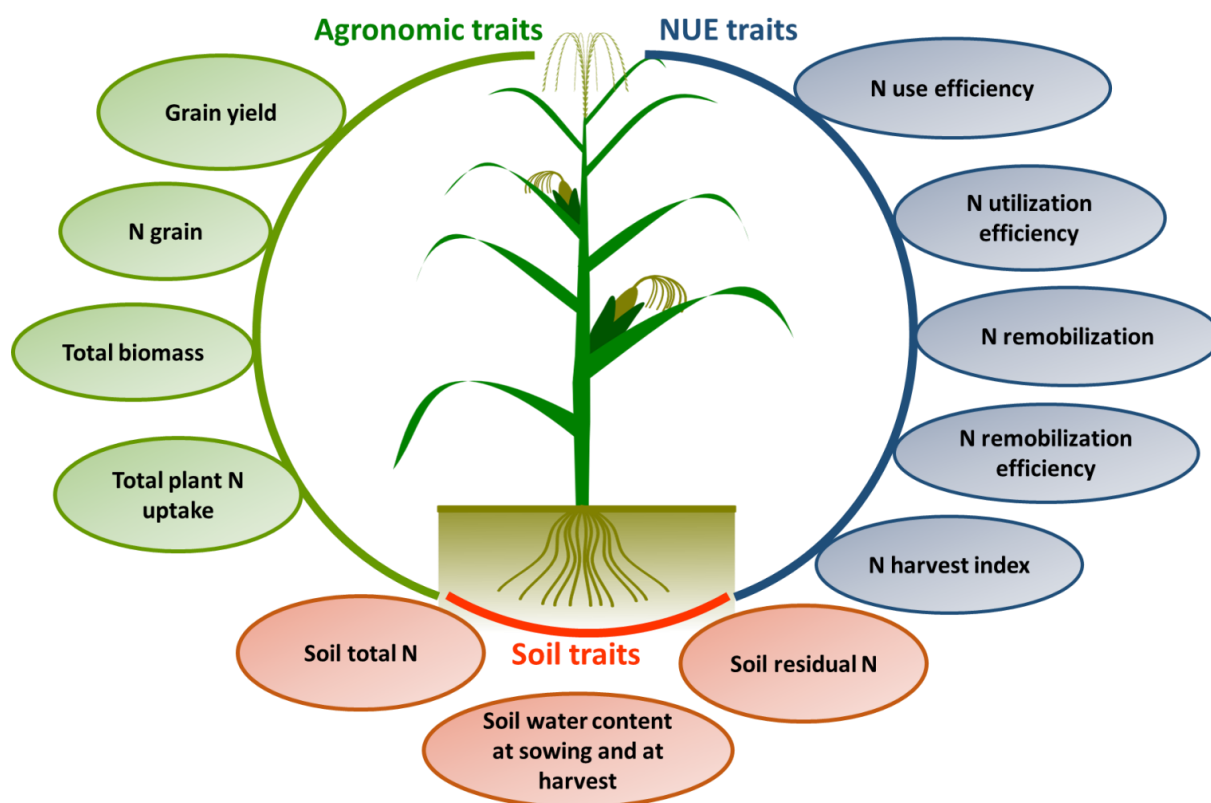


Figure 1. Schéma résumant les paramètres étudiés pour l'efficacité d'utilisation de l'azote chez le maïs au cours des deux années d'expérimentation (2013 et 2014).

Nous avons également progressé dans l'évaluation des nouveaux systèmes de production en montrant dans la deuxième expérimentation, l'importance du système d'agriculture de conservation sur la nutrition azotée du blé et son efficacité d'utilisation de l'azote. Les paramètres étudiés dans ce deuxième chapitre sont résumés en (**Figure 2**). Les résultats obtenus au cours des deux années d'expérimentations ont permis de répondre à la question n°2 et ont également validé l'effet positif de l'agriculture de conservation sur les différents indices d'efficacité d'utilisation de l'azote, telle que, l'efficacité d'utilisation de l'azote (NUE et NUtE), l'efficacité agronomique d'utilisation de l'azote (AEN), le coefficient apparent d'utilisation de l'azote (NAR), le facteur de productivité partielle de l'azote (PFPN) et la remobilisation de l'azote (NRem). A notre connaissance, l'effet du travail du sol vu au travers une approche système de cultures sur l'indice de nutrition azotée n'avait pas encore été évalué chez le blé. Ainsi, ce trait agronomique a été utilisé pour évaluer l'état de nutrition azotée du blé dans les deux traitements de travail du sol, en présence et en absence de couverture végétale et dans les trois systèmes présentant 3 niveaux de fertilisation azotée (N0, N1 et N2). Nous avons montré que l'indice de nutrition azoté (NNI) était plus élevé dans les systèmes de non-labour pour les trois taux d'application d'engrais azoté, par rapport au système du labour

classique. Ces résultats laissent supposer qu'il y a plus de limitation de l'azote au cours de la croissance végétative du blé lorsque le sol est labouré. Cela pourrait être la conséquence de l'augmentation rapide de biomasse végétative dans les modalités du labour conventionnel (Stanislawska-Głubiak and Korzeniowska, 2009). De même secondairement, nous avons pu montrer que les corrélations NUE/NNI étaient différentes en fonction des systèmes « travail du sol » et « couverture du sol ». La NUE est négativement corrélée avec NNI mais à système équivalent en azote, systèmes N2, N1 ou N0, elle reste toujours plus élevée dans les modalités de non-labour par rapport aux modalités du labour conventionnel. Cette efficacité élevée en non-labour peut être attribuée aux meilleures conditions d'absorption de l'azote pendant la croissance végétative du blé comme suggéré par certaines études (Šíp et al., 2009; Maltas et al., 2007)

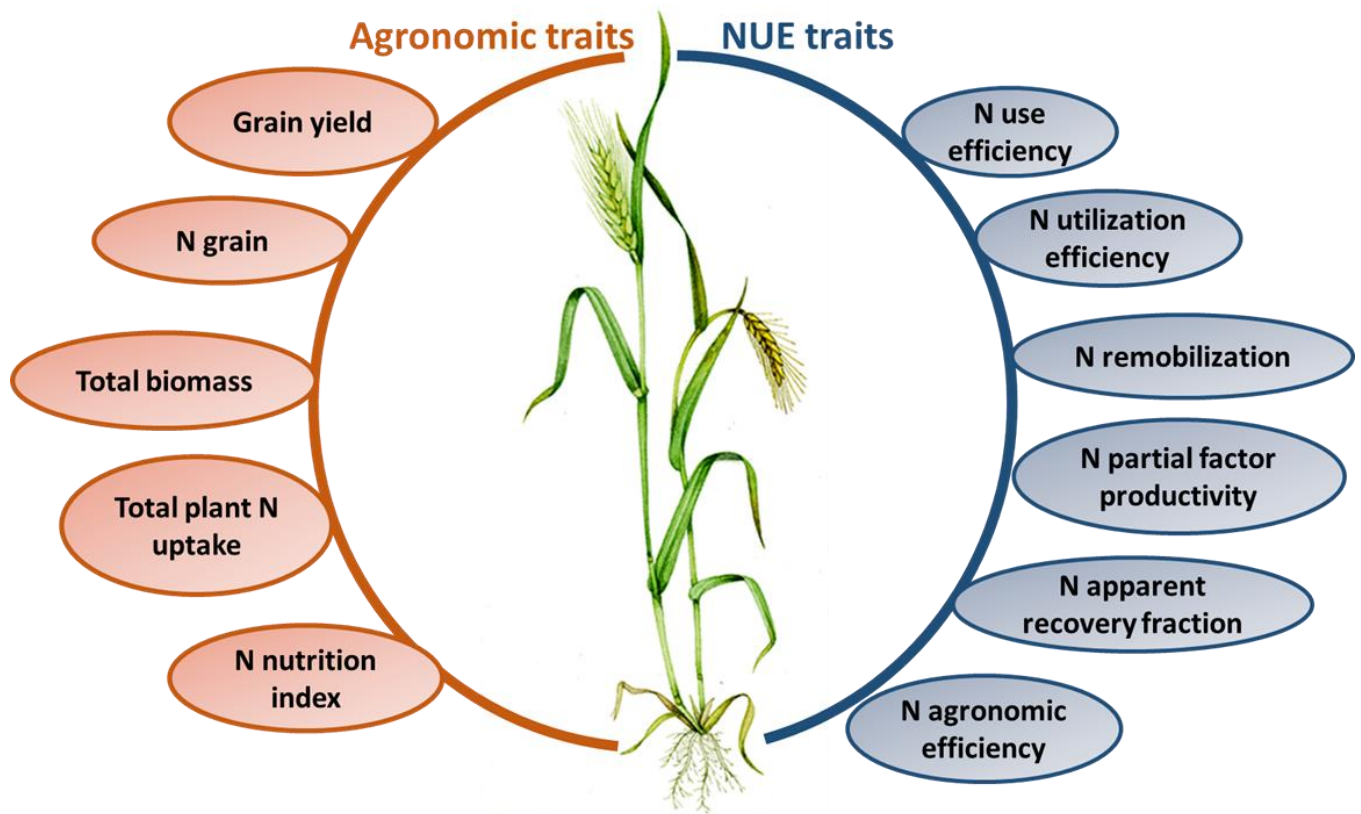


Figure 2. Schéma résumant les paramètres étudiés pour l'efficacité d'utilisation de l'azote chez le blé au cours des deux années d'expérimentation (2014 et 2015).

Nous avons enfin apporté des pistes de recherche pour le développement d'outils d'aide pour évaluer l'efficacité des systèmes de culture différents. Ces outils consistaient en une évaluation des paramètres agrophysiologiques comme l'efficacité photosynthétique d'utilisation de l'azote (PNUE) et l'efficacité d'utilisation de l'eau (WUE). Les paramètres étudiés dans ce troisième chapitre sont résumés en (**Figure 3**). Dans cet essai, le blé a été cultivé selon deux systèmes du travail de sol (Labour et semis direct), en présence et en absence de couverts végétaux, avec et sans fertilisation azotée (systèmes N1 et N0). L'objectif était d'évaluer l'effet combiné de système de culture et de la fertilisation azotée sur la PNUE ainsi que sur la WUE, ou encore la mesure de caractéristiques physiologiques foliaires tels que le taux de photosynthèse (Asat), la conductance stomatique (gs), le taux de transpiration (Tr), l'indice de teneur en chlorophylle (CCI) et la surface spécifique foliaire (SLA).

Nous avons montré que WUE et Asat étaient supérieurs en modalité de non-labour avec couverture végétale par rapport au système du labour conventionnel avec couverture végétale. Cette supériorité apparaît due principalement à la conservation d'eau du sol dans le système de non-labour. Il est connu que le système de non-labour augmente l'infiltration et le stockage de l'eau dans le profil du sol (Govaerts et al., 2009) et réduit la perte d'eau du sol par évaporation et favorise le développement d'un système racinaire profond qui va utiliser l'eau plus efficacement (Bientôt et al, 2008; Ruiz-Colmenero et al, 2013). Plusieurs études ont montré que le déficit de l'eau produit des effets négatifs sur le taux d'assimilation du CO₂ (Asat) (Kumar et al., 2011), en raison de la fermeture des stomates, ce qui empêche l'entrée de CO₂ dans la feuille (Grassi et Magnani, 2005; Ripley et al, 2007). Nous avons montré que la PNUE était également plus élevée dans le système de non-labour par rapport au labour conventionnel dans les deux conditions de fertilisation azotée (N0 et N1), en raison d'une Asat et SLA plus élevée dans ce système. La PNUE est fortement corrélée avec la surface foliaire spécifique (SLA) (Poorter & Evans 1998; Schulze 2005), ainsi les plantes à haute SLA ont tendance à avoir une PNUE plus élevée.

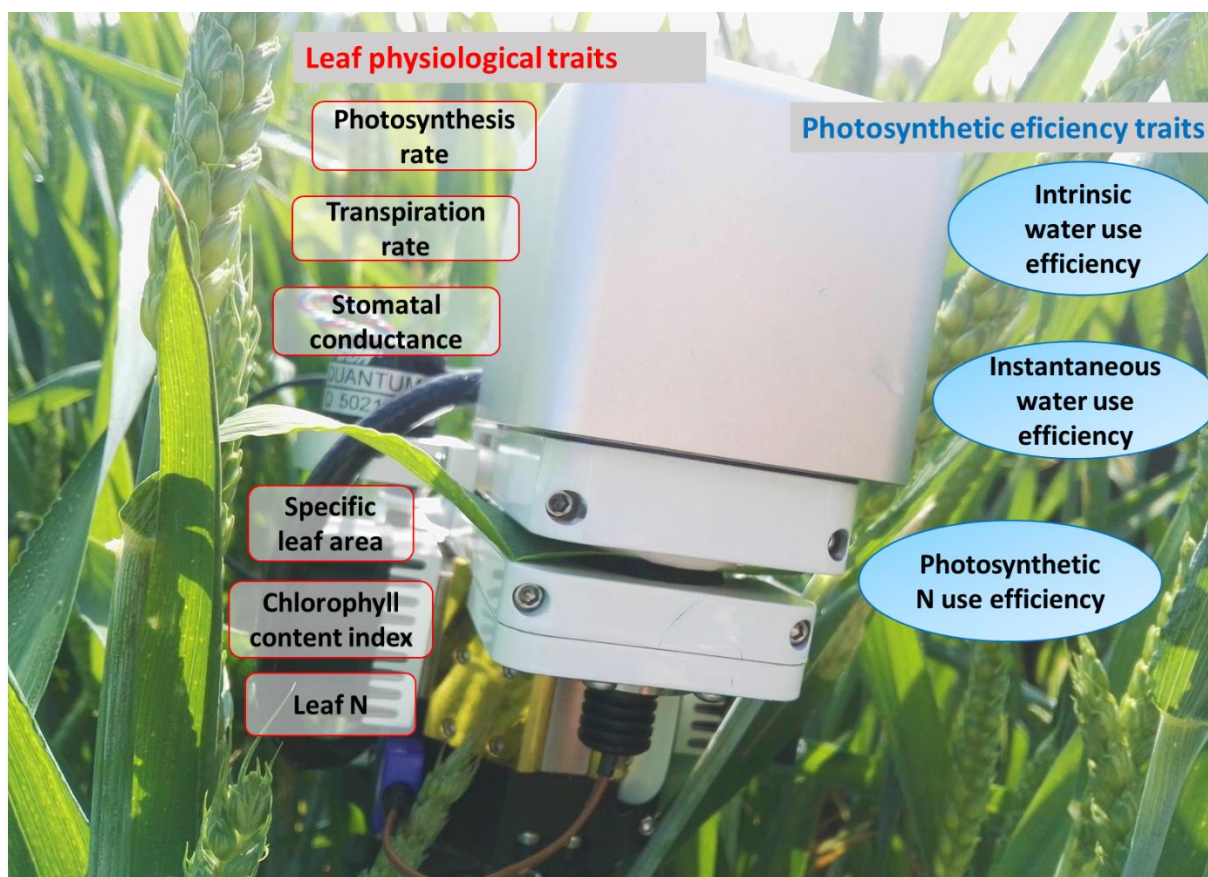


Figure 3. Schéma résumant les paramètres étudiés en 2016 pour l'efficacité photosynthétique d'utilisation de l'eau et de l'azote chez le blé en utilisant un système portable pour la photosynthèse (LI-6400XT).

Plusieurs perspectives peuvent être envisagées sur la base de ces résultats. Tout d'abord, la corrélation entre le rendement blé en 2016 et les résultats issus de l'essai du licor demande à être vérifiée. Normalement, NUE grain est significativement et positivement corrélée avec l'efficacité d'utilisation de l'eau par les cultures (Clay et al., 2001; Ladha et al., 2005), optimisant ainsi l'utilisation de l'eau pour produire plus de rendement grain (Araus et al., 2003), et donc également l'utilisation de l'azote par la culture (Dalal et al., 2013). Néanmoins, si dans nos conditions d'expérimentations réalisées sur limon hydromorphe, nous n'avons pas observé de gain de rendement proportionnel à l'augmentation de la NUE trouvée, il faut signaler que le système racinaire a pu être négativement affecté comme le montre la bibliographie par un niveau de compaction des sols plus important en système de non-labour qu'en système du labour conventionnel.

La durée du programme de recherche (VEGESOL) a été actée, initialement pour 8 années (2010-2018) afin d'intégrer les délais d'inertie de réponses des systèmes biologiques aux changements de pratiques adoptés. Les résultats présentés dans ce travail mettent en évidence une base de données après [3-4 ans (2013 et 2014 chapitre 1), 4-5 ans (2014-2015 chapitre 2) et 6 ans (2016 chapitre 3)] de mise en place des systèmes. Il est évident que les tendances vont évoluer, avec chaque année des modifications apportées au niveau du sol, et notamment au niveau du pôle de ressources organiques facilement dégradables. L'objectif sera donc de mettre en évidence sur le long terme, l'influence des pratiques culturales vues en tant que systèmes de cultures différenciés, sur la nutrition des cultures. De fait, la plupart des études courtes réalisées, concluent généralement par erreur, que les changements de pratique n'ont pas, ou ont eu peu d'effet, alors qu'en réalité les délais d'observation des mécanismes biologiques et écologiques de régulation des réseaux trophiques et des populations vivantes étaient trop courts pour conclure du fait de la résilience des systèmes étudiés.

Dans nos conditions, nous pouvons conclure que le système d'agriculture de conservation est une stratégie efficace pour améliorer l'efficacité azotée tout en préservant le sol et limitant la pollution des ressources naturelles. C'est le principe de l'agriculture raisonnée qui est un pas vers le développement durable. Cette démarche agronomique à la base, est aujourd'hui une option importante pour le changement et l'évolution des méthodes de travail agricoles. Cette évolution implique de nombreuses recherches scientifiques et des adaptations sur le terrain.

Puisque ma thèse fait partie intégrante du programme de recherche VEGESOL, les autres articles publiés dans des revues scientifiques auxquels j'ai contribué comme co-auteur sont présentés dans les annexes 1,2 et 3.

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ANNEXE 1

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Cover crops prevent the deleterious effect of nitrogen fertilisation on bacterial diversity by maintaining the carbon content of ploughed soil



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ABSTRACT

Synthetic nitrogen (N) fertilisers are widely used for enhancing agrosystem productivity and are thus thought to increase organic inputs from crop residues. However, many crop rotations have a low amount of organic residue returned to the soil since the whole aboveground crop biomass is harvested and exported. To compensate for such organic outputs and to improve soil quality, the introduction of winter cover crops in rotations has been suggested. A 4-year controlled field experiment was conducted to quantify the respective and combined effects of chemical N fertilisation and winter cover crops on plant productivity, organic carbon (C) and N inputs from crop residues and cover crops, changes in soil C and N concentrations, C:N ratio, soil mineral N, pH, soil moisture and soil bacterial biodiversity. A ploughing tillage system with low organic input was assessed, for which the main crops were spring wheat, green pea, forage maize, along with cover crops of different legume and non-legume species.

N fertilisation did not have an impact on the aboveground biomass except following forage maize. Cover crops increased the total amount of C and N inputs, irrespective of N fertilisation which had no significant effect. The soil N concentration decreased in all treatments, particularly when N fertilisers were applied under bare fallow conditions. The latter treatment also caused decreased soil C concentrations (slightly increased in the other treatments) and decreased bacterial biodiversity (no change in the other treatments). Bacteria from the Proteobacteria and Bacteroidetes phyla were highly correlated with soil from fertilised bare fallow conditions. While Verrucomicrobia was characteristic of non-fertilised bare fallow soils, Acidobacteria and Cyanobacteria were associated with the high C and N concentrations present in soils following cover crop treatments.

Taken together, these results demonstrate that in ploughing systems, under low organic restitution regimes, intensive N fertilisation decreases the diversity of the bacterial soil community and reduces soil C and N concentrations, but only in bare fallow conditions. There is a protective effect of winter cover crops against the deleterious effect of chemical N fertilisation on soil biodiversity and nutrient cycling, since they can maintain soil C and N concentrations. The use of winter cover crops containing legumes is thus a practice that is able to meet the criteria of a sustainable agriculture.

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1. Introduction

Several studies have reported that there is a decline in soil organic carbon (C) content worldwide and especially in Europe (Capriel, 2013; Heikkinen et al., 2013). Nevertheless, most cultivated soils are still tilled using the moldboard ploughing technique (Higashi et al., 2014; Triplett and Dick, 2008), which is known to provoke deleterious

effects on both soil carbon content and on soil living organisms (Kladivko, 2001; Jacobs et al., 2009; Leite et al., 2009; Nyamadzawo et al., 2009). An increase in the soil organic C pool can be obtained by the use of cover crops, which provide additional C through better energy conversion during crop rotation (Sainju et al., 2007; Smith et al., 2014). In addition, cover crops improve aboveground biodiversity (Balota et al., 2014; Calderon et al., 2016) and provide substantial amounts of N when they are composed of legume species (Kramberger et al., 2014). In conventional crop cultivation systems, the increase in crop productivity by mineral N fertilisers can lead to an increase in N and C inputs into the soil (Sainju et al., 2002, 2006;

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Bakht et al., 2009; Mazzoncini et al., 2011), depending on the type of crop rotation. Intensive N fertilisation can also promote soil organic matter (SOM) mineralisation through modifications of soil microbe communities (Jenkinson et al., 1985; Kuzyakov et al., 2000; Kuzyakov, 2010; Majumder and Kuzyakov, 2010). However, the impact of fertilisers on soil microbial diversity is complex because it involves several factors such as the type and the amount of fertiliser applied, as well as the nature of the soil and the crop rotation (Lupwayi et al., 2012). Generally, N fertilisation induces a loss of microbial diversity and modification of the composition of the bacterial community (Ramirez et al., 2010; Coolon et al., 2013; Cederlund et al., 2014; Willekens et al., 2014; Zhao et al., 2014).

The impact of N fertilisation (Shen et al., 2010; Gomez and Garland, 2012; Zhao et al., 2014; Sun et al., 2015; Zhou et al., 2015; Zeng et al., 2016) and the use of winter cover crops (Carrera et al., 2007; Liu et al., 2007; Nair and Ngouajio, 2012) have not been studied previously in an integrated manner. In particular, soil C sequestration, soil N content and microbial diversity in ploughing-based agricultural systems have received little attention. We thus developed a field experiment to quantify the individual and synergistic effects of cover crops and chemical N fertilisation on the soil microbial community and soil C and N contents under tillage conditions. We found that over a short period of time, intensive N fertilisation reduced both soil C and N contents. We also observed that under high N fertilisation input, the diversity of the soil microbial community decreased. In addition, we found that the detrimental effect of intensive N fertilisation on both soil properties and soil microbial communities can be substantially reduced when using a cover cropping system.

2. Materials and methods

2.1. Site description and experimental design

The field experiment was conducted at the experimental site of La Woestyne, in North France (50°44'N, 2°22'E, 40 m a.s.l.). The average annual air temperature and total rainfall were 10.5 °C and 675 mm, respectively, with amounts of rainfall relatively homogeneous across seasons. Soil particle size composition was characterized by silt 66.8%, clay 21.2% and sand 12%. The concentrations of organic C, total N and SOM before the beginning of the experiment were 13.1 g kg⁻¹, 1.5 g kg⁻¹ and 22.5 g kg⁻¹ respectively (see Table 1 for more characteristics).

Prior to the start of the experiment in 2009, the field was managed using a chisel plough and rotary power system. A crop rotation method for which organic restitution was known for being low and weakly affected by N fertilisers was employed to highlight the effect of N fertiliser on biodiversity and soil C and N concentrations (Fig. 1). It consisted of spring wheat (*Triticum aestivum* L.) in 2010, followed by green peas (*Pisum sativum* L.) in 2011, maize (*Zea mays* L.) in 2012, and spring wheat in 2013. In order to study the effect of fertilisation and cover crops on biodiversity and chemical parameters, the experimental field was split into four treatments with three replicate plots for each: bare

fallow without (BFN0) or with (BFNX) N fertilisation; winter cover crops without (CCN0) or with (CCNX) N fertilisation. BFN0 and CCN0 plots measured 7 × 8 m, while BFNX and CCNX plots measured 14 × 8 m. The cover crops consisted of a mixture of three legumes and three non-legume species, which were sown as follows: 400 seeds m⁻² of Egyptian clover (*Trifolium alexandrinum* L.), 30 seeds m⁻² of faba bean (*Vicia faba* L.), 50 seeds m⁻² of vetch (*Vicia sativa* L.), 80 seeds m⁻² of flax (*Linum usitatissimum* L.), 200 seeds m⁻² of phacelia (*Phacelia tanacetifolia* Benth.), 60 seeds m⁻² of oat (*Avena sativa* L.). Before the main crops were sown, cover crops were buried by a conventional moldboard plough to a depth of 30 cm. Spring wheat was sown at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) combined with a rotating harrow and crop residues were returned to the soil. Green pea was sown at a row spacing of 17 cm using a Turboresem drill (Herriau, France) combined with a rotating harrow. According to European regulations, pea did not receive N fertilisation in NX treatments. Crop residues of green pea were returned to the soil. Maize was sown at a row spacing of 75 cm with a Maxima drill (Kuhn, France) following a rotating harrow. Maize was grown for silage, meaning that all the aboveground residues were removed from the field. The dose of N fertiliser applied in the NX treatments was determined according to the N budget method for maize (108 kg N ha⁻¹) and wheat (160 kg N ha⁻¹). N fertilisation was applied in the form of urea for both maize and wheat and crop protection was ensured conventionally.

2.2. Total biomass and C and N inputs from main crops and cover crop residues

During the experiment, a 3 × 1 linear meter row of the main crop was sampled each year at the time of harvest, in each plot. The part of the plant that is commonly harvested (i.e., grains) was separated from the rest of the aboveground biomass (i.e., crop residues incorporated into the soil). Samples were oven-dried at 65 °C for 3 days and subsequently weighed (± 0.1 g accuracy) to determine total aboveground biomass. Each sample was then ground into a powder prior to C and N analysis using an elemental analyser (Flash EA 1112 series, Thermo Electron, Germany).

For winter cover crops, 3 × 1 m² was sampled each year in each plot, just before ploughing. Samples were oven-dried at 65 °C for three days and subsequently weighed. The total aboveground biomass was determined and then ground into a powder prior to C and N analysis. C and N inputs for each treatment since the beginning of the experiment were measured as the sum of the amounts of C and N in the crop residues plus the C and N in cover crops since 2009.

2.3. Soil sampling and chemical analyses

In late March 2013, 1 month after the last ploughing, 11 months after the last N application and > 1 year after the last cover crop incorporation, six 10 cm deep soil cores were randomly collected using a 2 cm diameter auger in each of the three replicate plots. Samples 10 cm deep were chosen since previous studies indicated that it was an appropriate depth for the assessment of the effects of N additions on microbial communities (Coolon et al., 2013; Zeng et al., 2016), even in ploughed soils (Sun et al., 2015). The six soil cores were composited together into a single sample. Soils were then sieved using a 2 mm mesh and divided into two parts: the first one was stored at 4 °C to await chemical analysis, and the second one was stored at -20 °C until DNA extraction.

The soil moisture concentration was determined by oven-drying at 105 °C. Soil pH was measured using a pH meter FE20-FiveEasy™ (Mettler Toledo, Switzerland) at a ratio of 1:5 (weight/volume) of soil to distilled water, following shaking for 45 min. Nitrate (NO₃⁻-N) and ammonium (NH₄⁺-N) were extracted at a ratio of 20 g fresh soil to 100 mL 1 M KCl. After shaking for 1 h, the extracts were centrifuged for 10 min at 4000 rpm, and the supernatants were analysed by a continuous flow analytical system (San⁺⁺ system, Skalar, Holland). Total

Table 1
Main characteristics of the 0–10 cm soil layer before the beginning of the experiment in 2009.

Parameters (units)	Mean ± SE
pH in H ₂ O	6.28 ± 0.160
CEC ^a (cmol + kg ⁻¹)	11.67 ± 0.272
P ₂ O ₅ ^b (g kg ⁻¹)	0.13 ± 0.008
Organic C (g kg ⁻¹)	13.08 ± 0.042
Total N (g kg ⁻¹)	1.48 ± 0.004
SOM (g kg ⁻¹)	22.50 ± 0.007
Soil C:N ratio	8.85 ± 0.105

SOM: soil organic matter.

SE: standard error of the mean.

^a Cation-exchange capacity (Metson method).

^b Available phosphorus (Olsen method).

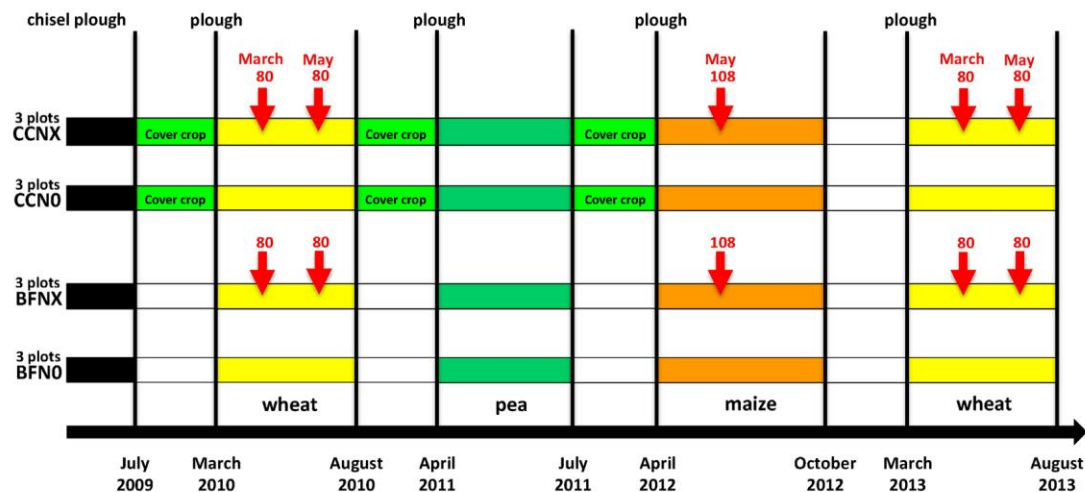


Fig. 1. Chronological representation of crop rotation, ploughing and N fertilisation over the 4-year experiment. CC: winter cover crop treatment with (NX) and without (N0) nitrogen fertilisation. BF: bare fallow treatment with (NX) and without (N0) nitrogen fertilisation; Red arrows indicate nitrogen fertilisation (with month of the year and amount in kg N ha⁻¹). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

soil C and total soil N concentrations were determined using the same elemental analyser as for biomass after drying at 35 °C for 48 h and ball milled using a grinder MM 400 (Retsch, Germany). Since the soil was free of carbonate, the soil organic C was assumed to be equal to the total C. Soil C and N analyses were carried out on samples collected from each of the 12 plots before the beginning of the experiment in 2009 and with the same method than in 2013. Changes in soil C and N concentrations were calculated as the difference between the soil C and N concentrations in March 2013 and the soil C and N concentrations in 2009.

2.4. Soil bacterial community analysis

2.4.1. Soil DNA extraction and PCR amplification

Prior to the pyrosequencing of 16S rRNA gene sequences, DNA was extracted and purified from 0.3 g of defrosted soil using the Fast DNA™ Spin Kit for Soil kit (MP Biomedicals, USA) and the NucleoSpin® gDNA Clean-up XS kit (Macherey-Nagel, Germany), respectively. The total DNA concentration in each sample was fluorometrically quantified with the AccuBlue™ High Sensitivity dsDNA Quantitation Kit (Biotium, USA) using a monochromator based multimode microplate reader (Tecan Infinite® M1000 PRO, Tecan Systems, USA). From the purified DNA, the 16S V3 and V4 region was PCR amplified with the Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3') primers (Herlemann et al., 2011). The forward and reverse primers were designed containing overhang sequences compatible with Illumina Nextera XT index (forward primer overhang: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG, reverse primer overhang: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG). For each sample, 5 ng of DNA was used for a PCR of 25 µL, conducted under the following conditions: 95 °C for 3 min, 25 cycles of 30 s at 95 °C, 55 °C for 30 s and 72 °C for 30 s, followed by 5 min at 72 °C.

2.4.2. Purification and sequencing

Each synthesised amplicon was purified using the AMPure XP beads kit (Beckman Coulter, USA) and subsequently quantified with the AccuBlue™ High Sensitivity dsDNA Quantitation Kit. Illumina Nextera XT Index sequencing adapters were integrated into the amplicons by PCR (95 °C for 3 min; 8 cycles of 30 s at 95 °C, 55 °C for 30 s and 72 °C for 30 s, followed by 5 min at 72 °C for 5 min). The final libraries were purified once again with the AMPure XP beads before quantification with the AccuBlue™ High Sensitivity dsDNA Quantitation Kit. To validate the library, 1 µL of a 1:50 dilution of the final library was used on a Bioanalyzer DNA 1000 chip using a Bioanalyzer 2100 (Agilent

Technologies, USA) to verify the library size and to check for the presence of primer dimer contamination. Purified libraries were pooled at equal molarity, denatured, diluted to 4pM, spiked with a premade PhiX control library at 5% (PhiX control v2, Illumina, USA) and loaded into a MiSeq v2 Reagent Kit (500 Cycles PE, Illumina, USA) to be sequenced in a MiSeq system (Illumina, USA). Illumina sequence reads in the FastQ format were uploaded into the Galaxy instance (v2.3.0) of the Genotoul bioinformatics platform (<http://sigenae-workbench.toulouse.inra.fr>) to be used in the FROGS (Find Rapidly OTU with Galaxy Solution) pipeline (Auer et al., 2015). FLASH (Fast Length Adjustment of SHort reads, Magoc and Salzberg, 2011) was used to merge paired-end reads with a maximum of 10% mismatch in the overlapped region. A filter was set to select the contig sequence with a length between 340 and 450 nucleotides. During the FROGS pre-process, the sequences with ambiguous bases (N) and which did not contain good primers were removed. Cutadapt (Martin, 2011) was used to search and trim the primer sequences with < 10% of differences. Clustering of the sequences was done using the SWARM algorithm (v2.1.5, Mahé et al., 2014). A first denoising step was carried out to build very fine clusters with minimal differences ($d = 1$) and a second one was completed with an aggregation distance of 3. The resulting representative seed for each cluster or OTU (Operational Taxonomic Units) was subjected to chimera detection and removal using the VSEARCH algorithm (v1.9, <https://github.com/torognes/vsearch>). Taxonomic classification of each OTU was performed using RDPClassifier and BLAST tools against the non-redundant small subunit database from SILVA (v123). To identify differences in soil bacterial richness and diversity, Chao 1 (Chao, 1984) and ACE (abundance-based coverage estimators), as well as Shannon–Wiener evenness, Pielou equitability and Simpson diversity indices were computed using the *vegan* package (Oksanen et al., 2015) in R software v. 3.1.2 (R Development Core Team, <http://www.R-project.org>).

2.5. Statistical analysis

All statistical analyses were performed using R v. 3.1.2. Mean values are given with their standard error. Total aboveground biomass, soil C and N concentrations and bacterial diversity indices were compared among treatments using a non-parametric Kruskal–Wallis one-way analysis of variance (H -value) followed by a Dunn's *post hoc* test whenever significant. Correlations between environmental variables (aboveground biomass, C and N inputs, soil C and N) on the one hand and bacterial diversity indices and relative abundances of the most abundant phyla were computed using Pearson's product-moment correlation coefficient (Hmisc package). A non-metric multidimensional

Table 2Total aboveground biomass of main crops (Mg ha^{-1} dry matter; mean $\pm$ standard error of the three plots per treatment).

	H	BFNO	BFNX	CCNO	CCNX
2010 Wheat	2.59 (NS)	9.67 $\pm$ 1.14	11.57 $\pm$ 0.64	10.53 $\pm$ 1.52	12.02 $\pm$ 0.53
2011 Pea	1.34 (NS)	8.35 $\pm$ 0.81	8.22 $\pm$ 0.67	8.40 $\pm$ 0.08	8.33 $\pm$ 0.21
2012 Maize	1.46 (NS)	24.97 $\pm$ 1.09	24.55 $\pm$ 1.58	25.19 $\pm$ 1.90	28.80 $\pm$ 2.90
2013 Wheat	8.44 (*)	6.71 $\pm$ 0.90b	14.45 $\pm$ 1.85a	7.84 $\pm$ 1.28b	15.32 $\pm$ 0.52a

H: value of the Kruskal–Wallis test with its significance in brackets (* $P < 0.05$). Letters give the result of a Dunn's *post hoc* test. BF: bare fallow; CC: winter cover crop; NO: no fertiliser; NX: chemical N fertilisation. NS: non-significant.

scaling (NMDS) of the plot $\times$ phyla (expressed as relative abundances) matrix was employed to visualize the differences in the composition of the bacterial phylum among treatments, in addition to the Bray–Curtis distance and the *vegan* package in R. To see how these compositional differences were related to environmental variables, the significant impacting variables were subsequently fitted onto the NMDS ordination using the 'envfit' function in *vegan*.

3. Results

3.1. Plant and soil parameters

Overall, N fertilisation and winter cover crops had no significant effect on aboveground biomass, except for spring wheat in 2013, when the biomass was doubled following the application of N ($P < 0.05$) and was nearly 1 Mg ha^{-1} greater for CC than for BF treatment, irrespective of N fertilisation (Table 2).

The two treatments including cover crops had significantly higher organic N and C inputs than the two BF treatments (Fig. 2). Overall, the total N input over the 4-year period was approximately 0.55 Mg ha^{-1} from CCNX, 0.50 Mg ha^{-1} from CCNO, 0.25 Mg ha^{-1} from BFNX and 0.20 Mg ha^{-1} from BFNO. C inputs followed the same trend as N inputs, with significantly greater values in CCNO and CCNX than in BFNO and BFNX treatments (8 to 9 Mg ha^{-1} and 5 to 6 Mg ha^{-1} , respectively; $P < 0.05$).

Under bare fallow conditions, (BFNX) N fertilisation decreased soil C and N concentrations, of 0.35 g kg^{-1} (against ca. 0.1 g kg^{-1} for the other treatments) and 1.5 g kg^{-1} (against an increase of 0.2 to 1.8 g kg^{-1} in the other treatments), respectively (Fig. 3). Winter cover crops tended to increase the soil C concentration, an effect that was particularly important in the absence of N fertilisation ($+2 \text{ g kg}^{-1}$ in CCNO). Soil moisture varied from 194 to 207 g kg^{-1} , pH from 6.81 to 6.95 , $\text{NH}_4^+\text{-N}$ from 0.1 to 4.5 mg kg^{-1} and $\text{NO}_3^-\text{-N}$ from 3.54 to 8.45 mg kg^{-1} , but there was

no trend between treatments, nor a statistically significant comparison (data not shown).

3.2. Soil bacterial community structure

The sequencing of the bacterial 16S rRNA gene amplicons resulted in $5,943,622$ sequences with an average length of 394 bp . $>90\%$ of the bacterial sequences were classified into four “major” phyla, namely, Actinobacteria, Chloroflexi, Firmicutes and Proteobacteria. The remaining OTUs were classified within 17 “minor” phyla, of which Acidobacteria, Bacteroidetes, Cyanobacteria, Planctomycetes and Verrucomicrobia represented from 1 to 3% of all bacterial sequences. Although they shared the same phyla, the soil subjected to the four treatments differed by the relative abundances of these phyla, especially in BF treatments (Fig. 4). Compared to BFNO, BFNX showed a clear shift in the relative abundance of Proteobacteria (from 20% to 60%), Actinobacteria (from 40% to 30%), Chloroflexi (from 20% to 5%), Firmicutes (from 8% to 3%), Planctomycetes (from 3% to 1%), Cyanobacteria (from 1.5% to 0.2%) and Verrucomicrobia (from 0.75% to 0.25%). By contrast, soil that had received the two CC treatments exhibited very similar composition.

In addition, N fertilisation had no effect on the bacterial taxonomic richness of the soil but significantly reduced the bacterial diversity, evenness and equitability in BFNX, although no differences were determined between the three other treatments (Table 3).

3.3. Relationships between environmental factors and soil bacterial diversity

The soil C and N concentrations both positively correlated with all the diversity metrics (Fig. 5). Conversely, none of these metrics correlated with either C and N inputs, plant aboveground biomass or other soil parameters. Consistently, soil C and N concentrations correlated

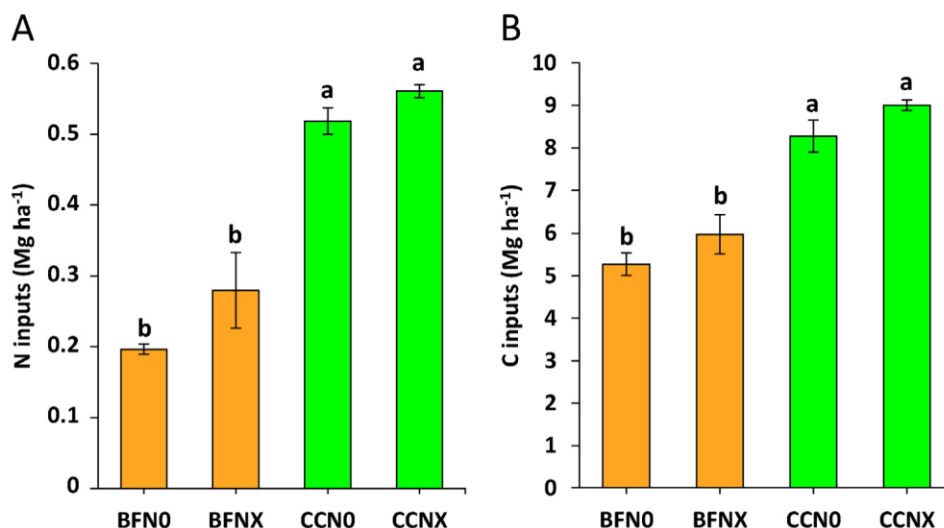


Fig. 2. Bar plots representing the total organic inputs of nitrogen (A) and carbon (B) through main crop and cover crop residues returned to the soil. Letters indicate differences among treatments according to a Dunn's *post hoc* test following a significant Kruskal–Wallis test ($P < 0.05$). BFNO: bare fallow without nitrogen fertilisation. BFNX: bare fallow with nitrogen fertilisation. CCNO: winter cover crop without nitrogen fertilisation. CCNX: winter cover crop with nitrogen fertilisation.

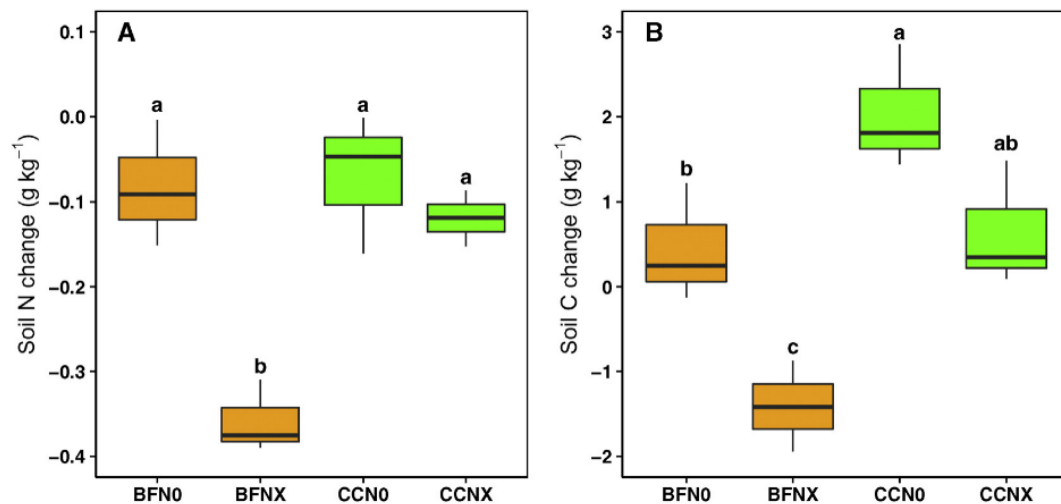


Fig. 3. Box plots representing changes in soil nitrogen (A) and carbon (B) concentrations over the 4-year experiment. Letters indicate differences among treatments according to a Dunn's post hoc test following a significant Kruskal–Wallis test ($P < 0.05$). BFNO: bare fallow without nitrogen fertilisation. BFNX: bare fallow with nitrogen fertilisation. CCN0: winter cover crop without nitrogen fertilisation. CCNX: winter cover crop with nitrogen fertilisation.

positively with the relative abundance of all the phyla with a relative abundance of $>1\%$, except Proteobacteria (negative correlation), Cyanobacteria (no correlation) and Bacteroidetes (non-significant and negative correlation with C and N, respectively). However, there was a positive correlation between organic N input and Acidobacteria, organic C input and Cyanobacteria, NO_3^- -N and Firmicutes/Acidobacteria, NH_4^+ -N and Acidobacteria (Fig. 6). The NMDS revealed that the soils that had received the CCN0 and CCNX treatments contained close bacterial communities. By contrast, BFNO and BFNX soils were strongly separated. The

first axis clearly divided BFNX treated soil plots from the others, due to a greater relative abundance of Proteobacteria and Bacteroidetes phyla. Remarkably, the compositional dissimilarity was much more evident for BFNX soils than for the other treatments, suggesting a greater environmental heterogeneity. The second axis mainly separated CC from BFNO plots and correlated positively with soil C and N concentrations ($P < 0.05$). CC plots were characterized by an increased relative abundance of Cyanobacteria and Acidobacteria, while BFNO plots were characterized by Actinobacteria and Verrucomicrobia. The phylum

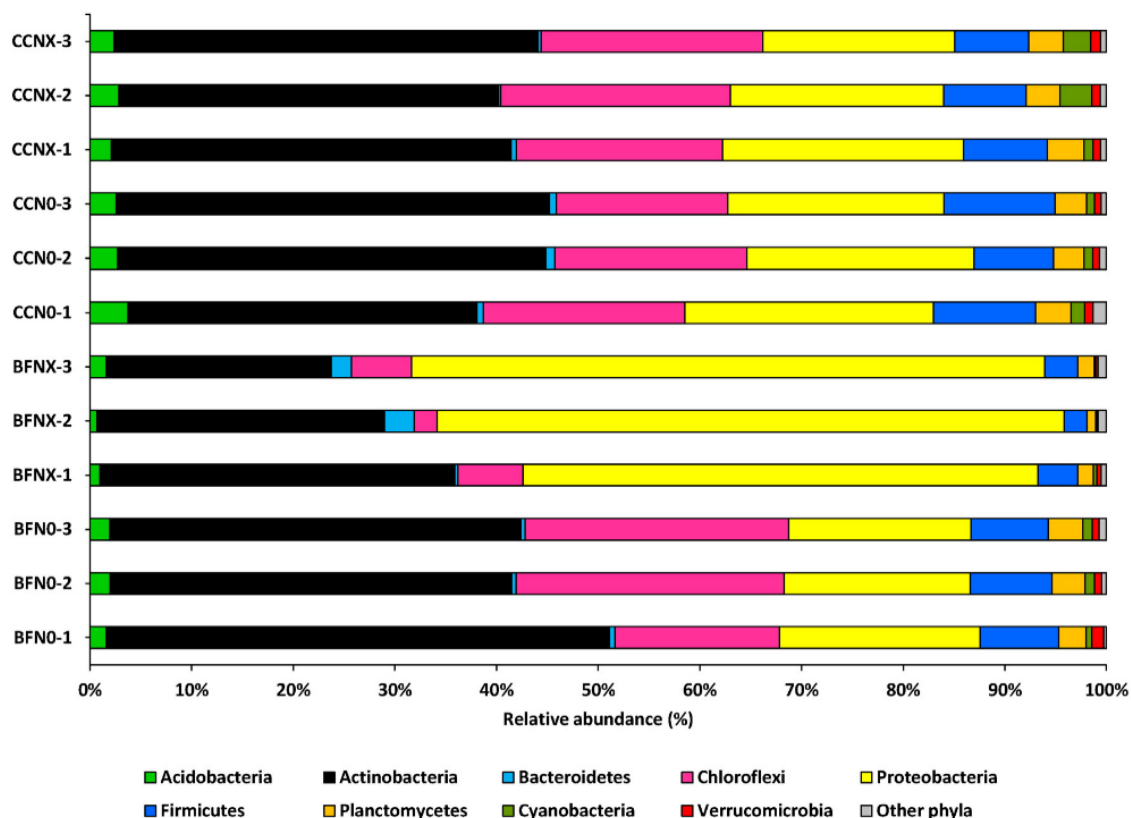


Fig. 4. Relative abundance of the most abundant bacterial phyla in the soil following the different experimental treatments. Sample numbers indicate the different replicates within each treatment. Phylogenetic groups accounting for $<1\%$ of all classified sequences are artificially grouped under "other phyla". BFNO: bare fallow without nitrogen fertilisation; BFNX: bare fallow with nitrogen fertilisation; CCN0: winter cover crop without nitrogen fertilisation; CCNX: winter cover crop with nitrogen fertilisation.

Table 3Indices accounting for soil bacterial diversity (mean $\pm$ standard error).

	H	BFN0	BFNX	CCN0	CCNX
OTUs	6.69 (NS)	808.67 $\pm$ 67.92	601.33 $\pm$ 34.93	822.66 $\pm$ 6.57	831.00 $\pm$ 49.43
Chao 1	6.44 (NS)	966.23 $\pm$ 56.35	732.04 $\pm$ 34.70	988.29 $\pm$ 14.14	968.06 $\pm$ 29.59
ACE	7.81 (NS)	962.92 $\pm$ 49.86	734.23 $\pm$ 22.21	978.27 $\pm$ 13.02	975.70 $\pm$ 24.53
Shannon	9.36 (*)	4.62 $\pm$ 0.002a	3.32 $\pm$ 0.13b	4.81 $\pm$ 0.09a	4.77 $\pm$ 0.03a
Pielou	8.13 (*)	0.69 $\pm$ 0.008a	0.52 $\pm$ 0.02b	0.72 $\pm$ 0.01a	0.71 $\pm$ 0.003a
Simpson	8.74 (*)	0.97 $\pm$ 0.0007a	0.82 $\pm$ 0.02b	0.98 $\pm$ 0.002a	0.98 $\pm$ 0.001a

H: value of the Kruskal–Wallis test with its significance in brackets (* $P < 0.05$). Letters give the result of a Dunn's *post hoc* test. BF: bare fallow; CC: winter cover crop; N0: no fertiliser; NX: chemical N fertilisation. NS: non-significant. OTUs: Operational Taxonomic Units. ACE: abundance-based coverage estimator.

composition was not significantly affected by N fertilisation in the CC treated soils and tended to resemble the BFN0 treatment (Fig. 7).

4. Discussion

In this study, the individual and combined effects of cover crops and chemical N fertilisation on the bacterial community and the C and N content of the soil have been monitored following ploughing. Intensive N fertilisation decreased the diversity of the bacterial community of the soil and reduced the concentration of C and N under bare fallow conditions only. There is thus a protective effect of winter cover crops against

the deleterious effect of chemical N fertilisation on both soil biodiversity and nutrient accumulation.

4.1. Impact of cover crop and N fertilisation on crop productivity

After 4 years of experimentation using a complete crop rotation system, neither winter cover crops nor N fertilisation had any significant effect on the total aboveground biomass production of the main cultivated crop (Table 2). However in 2013, wheat biomass production increased with N fertilisation, when no cover crop was grown, due to the late harvesting of maize. For both maize and wheat, the low impact of N

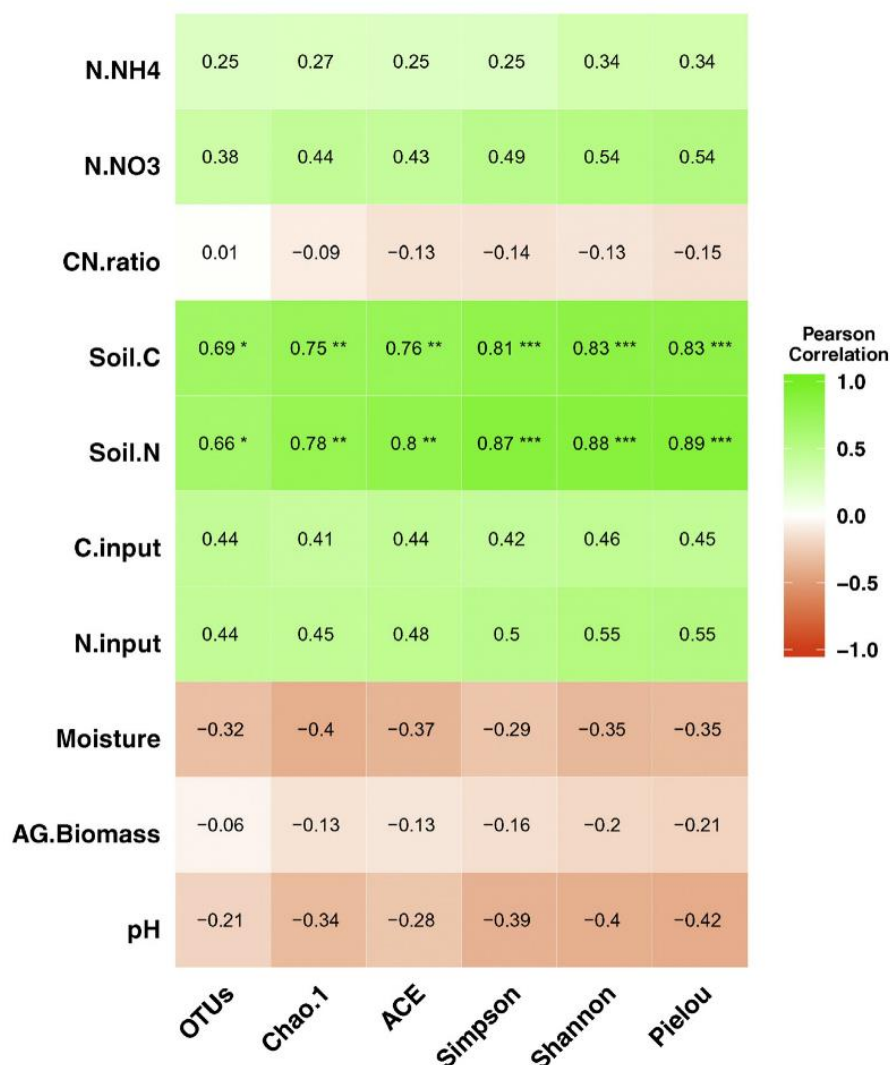


Fig. 5. Pearson correlations between diversity indices and environmental variables. Values of Pearson correlations are coloured by green as positive and orange as negative. Numbers in the squares are correlation coefficients and (*) represent significance level (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). OTUs: operational taxonomic units. ACE: abundance-based coverage estimator. AG Biomass: aboveground biomass. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertilisation on the aboveground biomass can be explained by an increase in N use efficiency, which was approximately two times higher under low N (N0) compared to high N (NX) fertiliser input (Sylvester-Bradley and Kindred, 2009). It is known that the crop cultivated previously (e.g. alfalfa) strongly influences maize yield (Peterson and Varvel, 1989), because the legume provides enough N (Asghari and Hanson, 1984). However for maize, a high level of N fertilisation is required to maintain an acceptable grain yield on the second and third year of cultivation (Stanger and Lauer, 2008). In this study, it is likely that green pea, which provides N to maize for one year, is not able to provide enough N to wheat on the following year.

4.2. Changes in C and N inputs and contents in the soil

In addition, following the CC treatment, the accumulation of C and N in the soil through crop residues and winter cover crops incorporation was higher than after the BF treatment, irrespective of the level of N fertilisation (Fig. 2). Interestingly, the soil C and N content changes were positive in the BFNO treatment, while that of the BFNX treatment were strongly negative (Fig. 3). Since the changes in the soil C and N contents are expressed as a concentration, as has been the case in a number of recent reports (Shen et al., 2010; Geisseler and Scow, 2014; Tian et al., 2015; Zhou et al., 2015), they represent the availability of C and N for microbial processes rather than the actual stocks. The remarkable increase of C concentration observed in CCN0 (Fig. 3) can be explained by root biomass and rhizo-deposition, which were not included in the C and N input measurements shown in Fig. 2. It is likely that an increase of mineralisation under NX conditions was compensated by the organic matter originating from the winter cover crops in the CC treatments, thus stabilising the soil

C and N contents over 4 years. It is known that intensive N fertilisation decreases (Khan et al., 2007; Russell et al., 2009; Zhong et al., 2015), or does not modify (Wilts et al., 2004; Higashi et al., 2014) the soil C content. In certain cases, intensive N fertilisation slows down the rate at which the soil organic C concentration is reduced (Ladha et al., 2011), which could also depend upon the amount of crop residues returned to the soil (Lou et al., 2011). For example, over 15 years in a no-till system, N fertilisation combined with the use of cover crops maintained or even increased soil N and C concentrations (Mazzoncini et al., 2011). Moreover, N fertilisation has been shown to stimulate the decomposition of soil residues (Conde et al., 2005), an effect that is likely to persist over long-term periods (Majumder and Kuzyakov, 2010). Such an effect, called the N-induced priming effect, (Jenkinson et al., 1985; Kuzyakov et al., 2000) accelerates organic matter mineralisation when the soil is enriched with N fertilisers. In the CCNX treatment, it is likely that the N-Induced priming effect was counterbalanced by the cover crop, which was able to provide enough new C for heterotrophic soil microorganisms thus preserving the older SOM from decomposition. In line with this hypothesis, a meta-analysis showed that C originating from the cover crops increased the sequestration capacity of soil C (Poepplau and Don, 2015). Therefore, this study has confirmed that in comparison to bare fallow conditions, winter legume cover crops can play a protective role against C and N losses from the topsoil in annual ploughing systems.

4.3. Soil bacterial community composition and diversity

It has been proposed that maintaining soil C and N stocks is the main factor influencing soil bacterial communities (Sul et al., 2013; Yuan et al., 2013). In this study, the considerable reductions in soil C and N

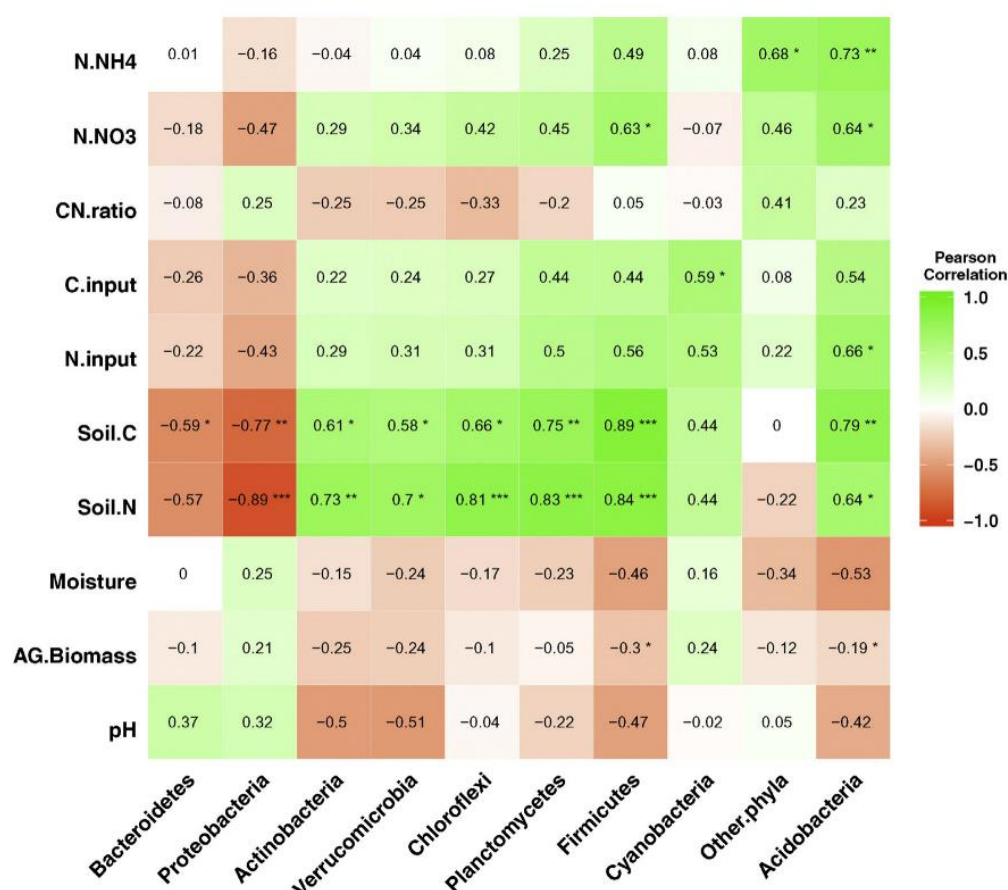


Fig. 6. Pearson correlations between relative abundances of the most abundant phyla and environmental variables. Values of Pearson correlations are coloured by green as positive and orange as negative. Numbers into the squares are correlation coefficients and (*) represent significance level (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). AG Biomass: Aboveground biomass. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations in BFNX were clearly associated with a very low bacterial diversity, in agreement with a number of other studies showing that (1) N enrichment reduces soil bacterial diversity (e.g. Coolon et al., 2013; Geisseler and Scow, 2014; Zhou et al., 2015; Zeng et al., 2016); (2) bacterial diversity is positively correlated with soil N and C concentration (Sul et al., 2013). These results also indicate that the cover crop is able to counteract the negative impact of N fertilisation on soil bacterial diversity. Similarly, Sun et al. (2015) observed a decrease in soil bacterial diversity in N fertilised soils, which did not occur when manure was applied.

In this experimental system, the composition of soil bacteria exhibited important differences between the four different treatments. Moreover, the relative abundances of the dominant phyla were strongly correlated with soil C and N concentrations (except for Bacteroidetes and Cyanobacteria). In agreement with Pascault et al. (2013), fresh organic matter returned to the soil in the CC treatments stimulated oligotrophic phyla (i.e., slow growing bacteria that can use more recalcitrant C sources, such as Acidobacteria and Verrucomicrobia). In addition, some specialised copiotrophic bacteria were present (i.e., fast-growing bacteria such as Actinobacteria and Firmicutes, decomposers of complex C-compounds; Ramirez et al., 2012). The stimulating effect of CCs was maintained even under N fertilisation conditions. By contrast, under bare fallow conditions, there were considerable differences in the bacterial composition of the N0 and NX treated soils, characterised by an enrichment in more copiotrophic phyla (i.e., fast-growing bacteria such as Proteobacteria and Bacteroidetes requiring labile C sources) at the expense of oligotrophic phyla. A notable exception was the copiotrophic Actinobacteria, which were less abundant in BFNX in comparison with the three other treatments. Actinobacteria are important decomposers that play a major role in carbon cycling and nutrient transformation (Jenkins et al., 2009; Fierer et al., 2007; Ventura et al., 2007). It has been shown that their relative abundance is positively correlated with total soil N (Tian et al., 2015; Nacke et al., 2011), and as such we observed that it is lower in the BFNX treatment. This is somehow a paradox that N supply in bare fallow conditions leads to a depletion in the soil C and N contents, thus

promoting a more oligotrophic environment compared to the other treatments.

According to the copiotrophic–oligotrophic hypothesis, N-induced shifts in the microbial community composition should theoretically yield to cognate shifts in the functional and metabolic potentials of the soil microbial community. Such shifts should be able to modify the C decomposition rate (Ramirez et al., 2012) notably for the most recalcitrant compounds (Craine et al., 2007).

5. Conclusion

In this study, a 4-year crop rotation, which included wheat, green pea and maize was conducted in a ploughing system with a relatively low return of organic residues to the soil. Taking together, the results show that under bare fallow conditions, chemical N fertilisers did not increase N and C inputs into crop residues and cover crops. By contrast, chemical N fertilisers strongly depleted the soil C and N concentrations, while reducing bacterial diversity. Importantly, the incorporation of winter cover crops containing legumes between the two main crops, by increasing organic C and N inputs, suppressed the negative impact of N fertilisation, leading to (1) stable soil C and N concentrations and (2) stable bacterial community composition and diversity, while maintaining yields. The use of such practice should be encouraged within the framework of agricultural sustainability.

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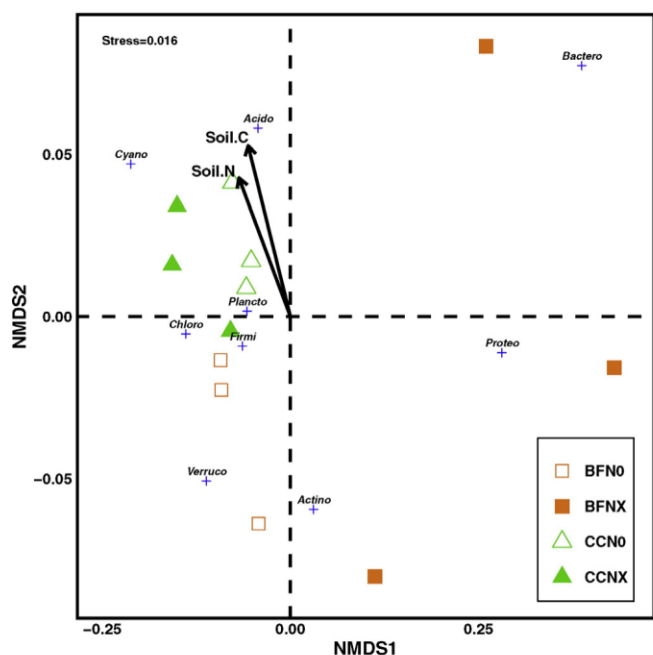


Fig. 7. NMDS diagram defined by the first two axes showing the ordination of the 12 plots (4 treatments × 3 replicates) and 9 bacterial phyla. Only environmental variables significantly fitting onto NMDS axes are shown ($P < 0.05$). Firmi: Firmicutes; Cyano: Cyanobacteria; Actino: Actinobacteria; Verruco: Verrucomicrobia; Bactero: Bacteroidetes; Chloro: Chloroflexi; Proteo: Proteobacteria; Acido: Acidobacteria; Plancto: Planctomycetes. BFN0: bare fallow without nitrogen fertilisation; BFNX: bare fallow with nitrogen fertilisation; CCN0: winter cover crop without nitrogen fertilisation; CCNX: winter cover crop with nitrogen fertilisation.

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ANNEXE 2

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Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization



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ABSTRACT

Agricultural practices such as tillage, cover crops, and nitrogen (N) fertilization affect physico-chemical and biological soil parameters. However, these factors were often studied separately and their combined effects remain unclear, especially with respect to soil microbial functional diversity and carbon (C) and N content. Thereafter, we aim to assess the links between cropping systems and functional response of microbial communities by using a large range of soil chemical and biological measurements. A 5-yr field experiment was conducted in Northern France using a combination of three factors: i) no-till (NT) vs. conventional tillage (CT); ii) with or without winter cover crops (bare fallow; cover crops with a low prevalence of legumes; cover crop with a high prevalence of legumes); and iii) with or without N fertilization.

C and N inputs from cover crops and crop residues, C and N content, enzyme activities, and microbial functional diversity in the topsoil (0–10 cm) were measured over an industrial crop rotation: wheat, pea, corn, wheat, flax. No-till combined with any of the cover crops was characterized by increased total soil organic C and N contents by more than 20% between 2010 and 2015. Dehydrogenase and urease activities were significantly greatest under NT, irrespective of the presence of cover crops. Cover crops without N fertilization under no-till led to higher microbial functional activity (faster carbohydrate and phenolic compound degradation) and diversity. Bare fallow had lower soil microbial functional diversity and C and N contents compared with soil under NT and cover crops. On the other hand, NT associated with cover crops allowed to maintain the soil in both C and N, and to promote microbial activities without N fertilization. In conclusion, winter cover crops and/or NT are sustainable agricultural practices resulting in a greater soil quality index. These results demonstrate that NT and use of standard cover crops or cover crops with legumes for 5 years under a low biomass return in industrial crop production have a positive effect on: i) upper soil C content and microbial enzymes, irrespective of N fertilization regime; ii) soil microbial functional diversity in the absence of N fertilization.

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1. Introduction

Alternative farming practices such as direct seeding mulch-based cropping systems are often employed to reduce the depressive effects of intensive farming on soil fertility. These practices are known to improve ecological relations between

plants, soil, and microorganisms (Chaussod 1996; Alvarez et al., 2002; Marinari et al., 2006), while conventional tillage (CT) depletes physico-chemical properties of soil (Chen et al., 2009; Mangalassery et al., 2015). Nevertheless, the response of soil carbon (C) and nitrogen (N) as well as microorganisms to different farm management patterns are still poorly understood, being dependent on many factors such as pedoclimate and crop rotation. Furthermore, the combined effects of different agricultural practices on the chemical and biological properties of soil received little attention so far (Acosta-Martinez et al., 2011).

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Although no-till (NT) with cover crops has been shown to increase soil enzyme activities (SEA), microbial functional diversity (Gomez et al., 2004; Nannipieri and Eldor, 2009; Lagomarsino et al., 2009; Nautiyal et al., 2010; Mangalassery et al., 2015; Mbuthia et al., 2015), and abundance (Mathew et al., 2012), there is still a lack of information for low organic-input cropping systems (Mbuthia et al., 2015), especially for industrial crop rotations. Indeed, C inputs from cover crops and crop residues shift soil moisture (Jordan, 2004; Bayer et al., 2006), temperature (Kladivko, 2001), and pH (Reganold et al., 1987), inducing short- and medium-term responses from microbial communities. These responses result in an increase of SEA such as dehydrogenase, alkaline phosphatase, and urease, with consistent results across subtropical (Staben et al., 1997), semi-arid (Nautiyal et al., 2010), mediterranean (Marinari et al., 2006; Lagomarsino et al., 2009), and temperate (Salinas-Garcia et al., 1997; Grayston et al., 2001) biomes. In addition, cover crops have been shown to induce priming effects in rhizosphere and detritusphere hotspots, by affecting the turnover of the soil organic matter (SOM) (Kuzyakov, 2010; Kuzyakov and Blagodatskaya, 2015), hence altering soil C and N contents. Increased lignin and cellulose contents, C/N ratio, as well as rhizodeposition (i.e. organic compounds released by roots during plant growth) are also considered as main factors affecting microbial communities (Marinari et al., 2000; Kusliene et al., 2014; Bais et al., 2006; Mangalassery et al., 2015). In low organic restitution cropping systems, one of the main challenges is to compensate the low biomass returned by cover crops originated-C and NT system, thus maintaining C and N contents and improving microbial functions (Mbuthia et al., 2015).

In this way, cover crop species have to be selected according to agronomic objectives, and the potential of different mixtures for improving soil quality needs to be assessed. Indeed the species composition of cover crops is a key factor determining the potential benefits to the soil following their incorporation/mulching. Cover crops enriched in leguminous species were associated with increased soil N content, likely due to N-fixation (Mbuthia et al., 2015). Moreover, the last study reported that vetch cover crops, in comparison with wheat cover crops or bare fallow, improved soil microbial responses such as microbial biomass N or microbial respiration rates. However, according to the level of available N, the inputs of fresh C may negatively correlate with C sequestration through an induced speed-up of C decomposition (Fontaine et al., 2004). Thereby, while the addition of N through fertilizers is commonly described to increase SOM stocks by increasing the rate of residues returned to the soil, long-term application of N fertilization may also cause a SOM depletion by stimulating mineralization.

In the present study, a 5-year experiment is used to investigate, under two N fertilization rates, the combined influence of tillage and cover crops on SEA, soil microbial functional diversity (functional activities and diversity indices), total soil organic carbon (TOC), and nitrogen (TN). We hypothesized that the decrease of soil quality in an industrial crop rotation under conventional soil management could be prevented by no tillage associated with cover crops (with low/high prevalence of legumes). Specifically in these systems, N inputs originated from leguminous cover crops could maintain C and N contents as well as microbial functional activities and diversity despite the absence of N fertilization.

2. Materials and methods

2.1. Site description

The field experiment was conducted at “La Woestyne” experimental site, in Northern France (50°44'N, 2°22'E, 40 m above sea level). The average annual air temperature and total

Table 1

Main characteristics of soil (0–10 cm) before the start of the experiment.

Parameters (units)	
pH in H ₂ O	6.28
CEC ^a (cmol + kg ⁻¹)	11.7
P ₂ O ₅ ^b (g P kg ⁻¹)	0.13
Organic C (g C kg ⁻¹)	13.1
Total N (g N kg ⁻¹)	1.48
Soil C:N ratio	8.85

^a Cation-exchange capacity (Metson method).

^b Available phosphorus (Olsen method).

precipitation between 2010 and 2015 were 11 °C and 620 mm, respectively, with rainfall relatively homogeneous across seasons. Soil particle size composition is characterized by 668 g kg⁻¹ silt, 212 g kg⁻¹ clay and 120 g kg⁻¹ sand (silty clay loam). The main characteristics of the soil before the beginning of the experiment are shown in Table 1.

2.2. Experimental design

Prior to the experiment's establishment, the field was conducted under chisel plough and rotary power system, fertilized conventionally, and cultivated with wheat (*Triticum aestivum*). In 2010, the experimental field was split into twelve treatments with three replicated plots placed randomly: two N fertilization regimes (without (N0) or with (Nx) N fertilization), two tillage systems (no-till (NT) or conventional tillage (CT)), and three cover crops modalities (bare fallow (bf), with cover crop enriched in leguminous species (lcc) or with standard cover crop (cc)). The 18 plots which received the treatment N0 measured 7 × 8 m while the 18 plots which received the treatment Nx measured 14 × 8 m. A 7-m width corridor separated the Nx and N0 plots in order to avoid N contamination.

During the 5-year period of the experiment, the main crop rotation pattern was wheat, pea (*Pisum sativum*), corn (*Zea mays*), wheat and flax (*Linum usitatissimum*). Wheat in 2010 and 2013 was sown in mid October at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) and harvested in late July. Green pea was sown in mid April at a row spacing of 17 cm using a Turbosem drill (Herriau, France) and harvested in early July. Maize was sown in mid May at a row spacing of 75 cm with a Maxima drill (Kuhn, France) and harvested in late September. Flax was sown in early April at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) and harvested in mid July. Only wheat straws were returned as main crop residues since corn and flax were cultivated for forage and fiber respectively. The Nx regime was determined according to the N budget method (Machet et al., 1990) for each rotation crop, and the fertilizer was composed of 50% urea, 25% ammonia and 25% nitrate. Since the beginning of the experiment, 108 kg N ha⁻¹ were added to corn, 160 kg N ha⁻¹ to wheat, and 80 kg N ha⁻¹ to flax. The N0 plots received no N fertilization since 2010. Cover crops “cc” and “lcc” were composed of non-leguminous species: oats (*Avena sativa*), phacelia (*Phacelia tanacetifolia*), flax (*Linum usitatissimum*), as well as leguminous species: vetch (*Vicia sativa*), faba bean (*Vicia faba*), and Egyptian clover (*Trifolium alexandrinum*), but the lcc mixture was particularly characterized by an increase of legume seeding rates. The cc/lcc plots received 160/60 seeds m⁻² of oats, 600/200 seeds m⁻² of phacelia, 80/80 seeds m⁻² of flax, 25/50 seeds m⁻² of vetch, 20/30 seeds m⁻² of faba bean, and 230/400 seeds m⁻² of Egyptian clover. All cover crop seeds were mixed and simultaneously sown in line. Each year, cover crops were sown immediately after the harvest of the previous crop and were terminated by grinding following a frost period. Before the main crops were sown, cover crop residues were

buried by a conventional moldboard plough to a depth of 30 cm in CT plots and left on the ground in NT plots. Crop protection against weed development and pests was ensured conventionally according to recommended local practices, and all the treatments received the same molecules and doses (see Supplementary Table 1). Neither potassium-phosphate nor other elements were applied throughout the experiment.

2.3. Analysis of nitrogen and carbon inputs from cover crop and main crop residues

During the experiment, 3×1 m row of the main crop were yearly sampled at the time of harvest, within each plot. The part of the plant which is commonly returned to the soil through crop residues was separated from the rest of the aerial vegetative part (i.e. exported part). Samples were oven-dried at 65 °C for 3 days, weighed, and then ground into powder for subsequent NC analysis using a Flash Elemental Analyzer 1112 series (Thermo Electron, Germany). Similarly, 3×1 m² of the entire vegetative part of cover crops were yearly sampled within each plot just before sowing the following main crop to measure the total aboveground biomass and NC contents using the same method. The cumulative organic inputs of N and C from both main crop residues and cover crops were calculated as the sum of C and N returned to the soil since 2010.

2.4. Soil sample collection and storage

In March 2015, six 10-cm deep soil cores were randomly collected using a 5-cm diameter auger in each of the three replicate plots by treatment. Soil sampling occurred 1 year after the last plowing in CT and 3 months after the death by frost of cover crop species in cc plots in order to avoid direct effects of soil disturbance and living roots on the measured parameters. We chose to sample to 10 cm since previous studies reported it was an appropriate depth to assess the effects of N additions on microbial communities (Zeng et al., 2016), even in plowed soil (Sun et al., 2015). The six soil cores were composited together as a single sample for each replicate plot. Soil samples were immediately put into polyethylene bags, placed into coolers for transportation to the laboratory, and, after homogenization, they were divided into two subsamples. The first one was stored at –20 °C and returned to 4 °C within 5 days prior to analysis of SEA (Schinner et al., 2012). The second one was stored at 4 °C before the determination of community level physiological profiles (CLPP), total organic carbon (TOC), and total nitrogen (TN).

2.5. Soil pH, total organic carbon (TOC) and total nitrogen (TN)

Soils were 2 mm-sieved and oven-dried at 45 °C for 48 h. Soil pH was measured using a pH meter FE20-FiveEasy™ (Mettler Toledo, Switzerland) and a ratio of 1:5 (mass/volume) of soil to distilled water shaken for 45 min. For TOC and TN, sieved soil was finely ground with a ball-mill (Retsch, MM400) and analyzed using a CN elemental analyzer (Flash EA 1112, Thermo Electron, Germany). Since analysis performed at time of the experiment setup revealed that the soil was free of carbonate, the soil total C was assumed to be equal to the TOC. Soil TOC and TN analyzes from samples taken at the beginning of the experiment with the same sampling method as shown in 2.4 were used to calculate TOC and TN change over the 5-year period.

2.6. Soil dehydrogenase and urease enzyme activities

Before SEA measurements, visible organic residues were removed. Soil dehydrogenase activity was measured as described

by Casida et al. (1964). Soil sub-samples were adjusted with CaCO₃ to a final mass ratio of 100:1 (soil:CaCO₃) using 5.94 g of fresh soil. Then, 1 mL of a solution of 3% 2,3,5-triphenyl tetrazolium chloride and 2.5 mL of ultrapure water was added. After mixing, the tubes were incubated at 37 °C for 24 h. The resulting triphenylformazan (TPF) was then extracted with 30 mL of pure methanol by stirring for 1 min. The solution was filtered in a dark room and the intensity of TPF was measured at 485 nm (Eon spectrophotometer, BioTek Instruments Inc., USA) following Tabatabai (1994). Urease activity was determined using the method of Alef and Nannipieri (1998). A mixture of 5 g of fresh soil with 2.5 mL of 79.9 mM urea was incubated at 37 °C for 2 h before adding 50 mL of 2.0 M KCl. After 30 min of agitation, the suspension was filtered and a solution comprising 1 mL of filtrate, 9 mL of deionized water, 2 mL of 0.1% sodium dichloroisocyanurate and 5 mL of a mixture of 1.06 M sodium salicylate/0.3 M sodium hydroxide/deionized water (1:3/1:3/1:3, vol/vol/vol) was prepared. After decanting for 30 min at room temperature, ammonium was measured at 690 nm using the spectrophotometer.

2.7. Community level physiological profiles (CLPP)

The analysis of CLPP was completed within 5 days after sampling (Schinner et al., 2012) using Biolog EcoPlates™ (Biolog Inc., Hayward, CA) containing 31 different carbon sources plus a control well, in two replications (Huynh, 2009). Five g of fresh, homogenized and organic residue-free soil was incubated with 750 µL of sterile distilled water for 48 h at 26 °C. Then, 50 mL of physiological saline were added and the samples were stirred at 500 rpm for 30 min. The samples were centrifuged at 800 rpm for 10 min and recovered supernatants were centrifuged again at 3000 rpm for 5 min. Then, 150 µL of the supernatant was inoculated into each well of the Biolog EcoPlates and incubated at 25 °C. Data were recorded every 24 h for 196 h at 590 nm and the data recorded at the end of the exponential phase (96 h) were used for further statistical analysis. The optical density (OD) for each well was calculated by subtracting the control well values from each plate to the OD value of the well (Garland and Mills, 1991). Microbial activity in each microplate was expressed using average well color development (AWCD) and calculated following the method of Garland and Mills (1991). The Shannon index representing the soil functional diversity was calculated using an OD of 0.25 as threshold for positive response (Garland, 1997).

2.8. Statistical analysis

All statistical analyzes were performed using R software v. 3.1.2 (R Development Core Team, <http://www.R-project.org>, 2014). Data for each parameter were analyzed using a non-parametric Kruskal-Wallis test. Multiple comparisons among treatments used the Conover post-hoc test ($P < 0.05$) and the PMCMR package (Pohlert, 2016). Values in figures and tables correspond to the average of 3 data ($n = 3$) $\pm$ standard error. For statistical analyzes, the 31 Biolog substrates were grouped into (1) phenolic compounds, (2) amines, (3) amino acids, (4) polymers, (5) carboxylic acids, and (6) carbohydrates. Finally, a redundancy analysis (RDA) was run to visualize the relationships between the 35 functional variables (31 Biolog substrates, 2 enzymatic activities, 2 functional diversity indices), the 7 environmental variables (C, N and C:N ratio inputs from residues, soil TOC, TN, pH and C:N ratio) and the 36 plots, using the *vegan* package (Oksanen et al., 2015). A Monte Carlo permutation test (999 permutations) was used to assess the significance of environmental variables in accounting for the observed variance of the plots. A post-hoc permutation test was further implemented using the *envfit* function in *vegan* (999 permutations) to seek the individual significance of each

environmental and functional variable in accounting for the observed variance of plots.

3. Results

3.1. Carbon and nitrogen inputs from cover crop and main crop residues

In the absence of N fertilization (N0), both C and N inputs from main crops residues were significantly higher in conventional tillage with standard (CTcc) and leguminous cover crop (CTLcc) than in no till with standard cover crop (NTcc) and under bare fallow (NTbf) (Table 2). N input was also higher in conventional tillage with winter bare fallow (CTbf) than in NTbf. These differences disappeared under Nx treatments, into which only the C:N ratio of the main crop residues was significantly higher in NTcc and no till with leguminous cover crops (NTlcc) than in CTcc and CTLcc.

N, C, and C:N inputs from cover crops did not differ among treatments, irrespective of the fertilization level.

3.2. Soil chemical analyzes

Among N0 treatments, soil TOC contents increased between 2010 and 2015 in NTcc and NTlcc, whilst it did not change in NTbf, CTbf, and CTLcc, and it even decreased in CTcc. Soil TN increased in NTcc and NTlcc, showed little change in CTLcc, and decreased in NTbf, CTbf and CTcc (Fig. 1).

Under Nx fertilization, soil TOC increased in NTcc, NTlcc, and, to a lesser degree, in NTbf; it showed little change in CTLcc and CTcc, and decreased in CTbf. Regarding soil TN, the greatest (positive) values were found in NTcc and NTlcc, whilst the lowest (negative) ones were recorded in CTbf and CTcc.

The soil C:N varied from 9.5 to 10.9 and pH varied from 6.7 to 6.9 but they did not differ among treatments (results not shown).

3.3. Soil enzyme activities

A clear trend was found towards greater dehydrogenase and urease values in NT than in CT treatments, irrespective to N fertilization (Fig. 2). All NT treatments showed significantly higher

Table 2

Carbon and nitrogen input from main crop residues and cover crop aboveground biomass over 5 years.

N rate	Crop	Parameter	H (P)	NTbf (Mg ha ⁻¹)	CTbf (Mg ha ⁻¹)	NTcc (Mg ha ⁻¹)	CTcc (Mg ha ⁻¹)	NTlcc (Mg ha ⁻¹)	CTLcc (Mg ha ⁻¹)
N0	Main crop	N input	13.44(0.019)	0.03 ± 0.002 ^a	0.04 ± 0.003 ^{bc}	0.03 ± 0.001 ^{ab}	0.05 ± 0.005 ^c	0.03 ± 0.002 ^{abc}	0.04 ± 0.004 ^c
		C input	12.88(0.024)	2.1 ± 0.12 ^a	2.95 ± 0.29 ^{ab}	2.22 ± 0.14 ^a	3.16 ± 0.31 ^b	2.67 ± 0.09 ^{ab}	3.21 ± 0.13 ^b
		C:N input	NS	79.1 ± 2.82	77.94 ± 2.46	82.98 ± 4.17	68.8 ± 4.41	78.95 ± 1.67	75.66 ± 5.36
	Cover crop	N input	NS			0.22 ± 0.02	0.11 ± 0.02	0.22 ± 0.03	0.2 ± 0.005
		C input	NS			1.95 ± 0.189	1.08 ± 0.17	1.96 ± 0.19	1.79 ± 0.04
		C:N input	NS			8.89 ± 0.271	9.9 ± 0.32	9.03 ± 0.31	8.9 ± 0.08
Nx	Main crop	N input	NS	0.06 ± 0.004	0.06 ± 0.004	0.06 ± 0.004	0.06 ± 0.003	0.06 ± 0.004	0.08 ± 0.004
		C input	NS	4.24 ± 0.27	4.48 ± 0.27	4.82 ± 0.10	4.38 ± 0.23	5.29 ± 0.45	4.74 ± 0.07
		C:N input	11.76(0.038)	73.25 ± 1.51 ^{ab}	69.53 ± 0.69 ^{ab}	82.07 ± 6.24 ^b	71.88 ± 6.31 ^{ab}	82.01 ± 1.55 ^b	60.67 ± 2.96 ^a
	Cover crop	N input	NS			0.23 ± 0.02	0.21 ± 0.02	0.22 ± 0.04	0.21 ± 0.02
		C input	NS			2.03 ± 0.18	1.89 ± 0.14	1.95 ± 0.25	1.89 ± 0.19
		C:N input	NS			8.82 ± 0.10	9.15 ± 0.20	8.87 ± 0.33	9.01 ± 0.13

H: value of the Kruskal-Wallis test with the P value of significance in brackets (NS: non-significant). Letters give the result of the Conover's post-hoc test at $P < 0.05$. CT: conventional tillage, NT: no-till, bf: bare fallow, cc: cover crop, lcc: leguminous cover crop, N0: no nitrogen fertilization, Nx: conventional nitrogen fertilization.

[†] Standard error of the mean.

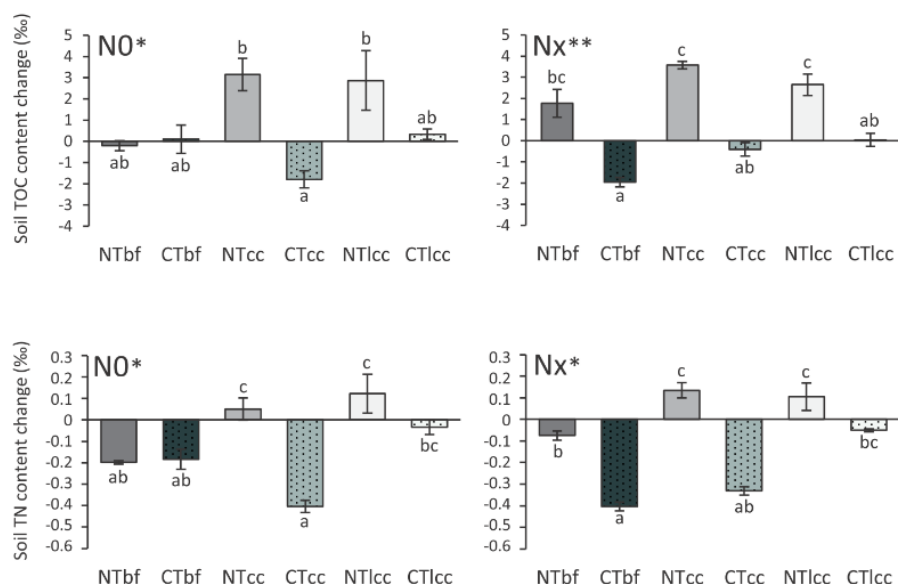


Fig. 1. Changes in soil total organic carbon (top) and total nitrogen (bottom) over the 5-year period. Stars indicate the significance level of the Kruskal-Wallis test (* $P < 0.05$, ** $P < 0.01$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$). TOC: total organic carbon, TN: total nitrogen.

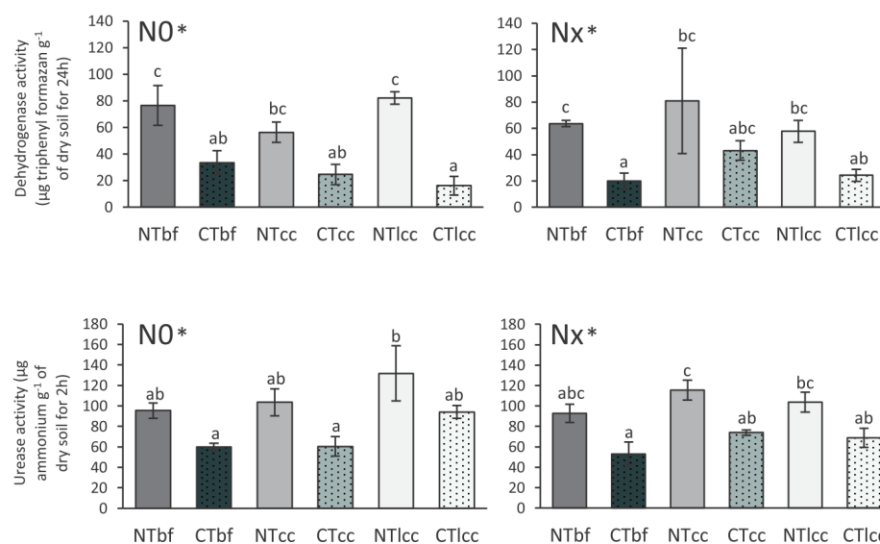


Fig. 2. Enzyme activities of dehydrogenase (top) and urease (bottom). Stars indicate the significance level of the Kruskal-Wallis test (* $P < 0.05$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$).

dehydrogenase activity than CTlcc (under N0) or CTbf (under Nx). Urease activity was greater in NTlcc than in CTbf and CTcc under N0, and greater in NTcc than in CT plots under Nx.

3.4. Community level physiological profiles

3.4.1. AWCD and shannon functional diversity

In the absence of fertilizer (N0), both average well color development (AWCD) and the Shannon index were the lowest in bare fallow treatments (NTbf and CTbf) (Fig. 3). In cover crop treatments, the Shannon index was similar in both CT and NT plots, whilst CTlcc showed lower AWCD values than the three other treatments. No statistically significant differences among treatments were observed under Nx.

3.4.2. Substrate used by the microbial communities

Among N0 treatments, significant differences were found for phenolic compounds, polymers, and carbohydrates, which were

metabolized faster by microbial communities from cover crop treatments. Phenolic compounds were used faster by NTcc than by NTbf communities. Polymers were used faster in CTcc and NTcc than in CTbf and, to a lesser extent, in NTbf communities. Carbohydrates were used faster in NTcc and NTlcc than in bare soil treatments (NTbf and CTbf). In contrast, a lack of difference was found among treatments under Nx (data not shown).

3.5. Relationships between soil chemical and biological parameters

The RDA diagram defined by the first two canonical axes (adjusted $R^2 = 0.25$; cumulated explained variance by 'species data': 40.0%) revealed the relationships between experimental treatments, "functional" and environmental variables (Fig. 4). The variance in the 42 variables (31 Biolog substrates, AWCD, Shannon index, dehydrogenase activity, urease activity, and 7 environmental variables) was significantly related to differences in TOC, TN, N

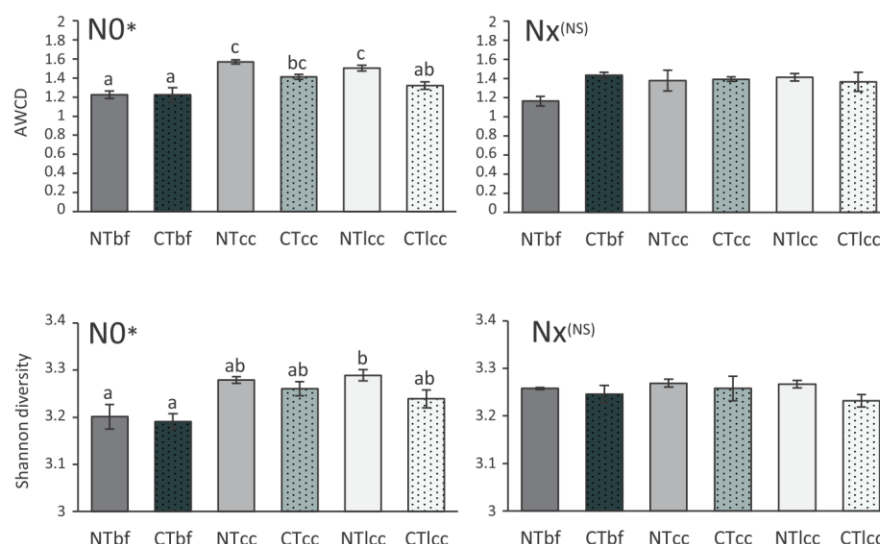


Fig. 3. Microbial functional diversity assessed by total degradation activity (top) and Shannon diversity (bottom) calculated from substrate utilization pattern (31 substrates). Stars indicate the significance level of the Kruskal-Wallis test (NS: non-significant, * $P < 0.05$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$). AWCD: average well color development.

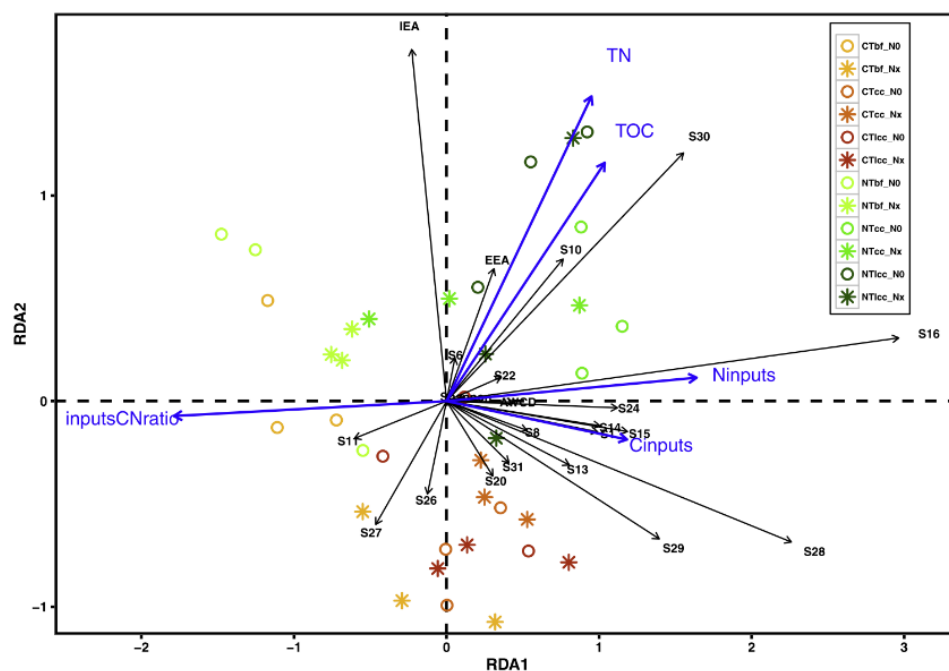


Fig. 4. Redundancy analysis (RDA) of microbial data using soil and input properties as environmental parameters (blue arrows). For the sake of simplicity, only the variables that significantly correlated with canonical axes according to a post-hoc permutation test ($P < 0.05$) are shown. TN: total nitrogen, TOC: total organic carbon, Ninputs: cumulative amount of nitrogen input from crop and cover crop residues, Cinputs: cumulative amount of carbon input from crop and cover crop residues, inputsCNratio: C:N ratio of inputs from crop and cover crops residues. Biolog substrates are **carboxylic acids** (S1: Pyruvic Acid Methyl Ester, S2: D-Galacturonic Acid, S3: γ -Hydroxybutyric Acid, S4: D-Glucosaminic Acid, S5: Itaconic Acid, S6: α -Ketobutyric Acid, S7: D-Malic Acid), **carbohydrates** (S8: β -Methyl-D-Glucoside, S9: D-Galactonic Acid γ -Lactone, S10: D-Xylose, S11: i-Erythritol, S12: D-Mannitol, S13: N-Acetyl-D-Glucosamine, S14: D-Cellobiose, S15: Glucose-1-Phosphate, S16: α -D-Lactose, S17: D,L- α -Glycerol Phosphate), **amino acids** (S18: L-Arginine, S19: L-Asparagine, S20: L-Phenylalanine, S21: L-Serine, S22: L-Threonine, S23: Glycyl-L-Glutamic Acid), **amines** (S24: Phenylethylamine, S25: Putrescine), **polymers** (S26: Tween 40, S27: Tween 80, S28: α -Cyclodextrin, S29: Glycogen), **phenolic compounds** (S30: 2-Hydroxy Benzoic Acid, S31: 4-Hydroxy Benzoic Acid). AWCD: average well color development, IEA: intracellular enzyme activity (dehydrogenase), EEA: extracellular enzyme activity (urease). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inputs, C:N ratio of inputs ($P < 0.001$), and C inputs ($P < 0.01$), as indicated by permutation tests. There was a clear trend towards stronger dispersion among N0 plots compared with Nx plots, suggesting a greater heterogeneity in the values of the response variables.

The first RDA axis (proportion of constrained variance explained: 63.7%) corresponded to a gradient of increasing C and N inputs (but decreasing input C:N ratio) and increasing AWCD/Shannon. It tended to separate bare fallow plots (negative scores) from no tillage-cover crop plots (most positive scores). Regarding substrate utilization preferences, this first axis mostly correlated positively with several carbohydrates (S16: α -D-Lactose, S15: Glucose-1-Phosphate, S14: D-Cellobiose, S13: N-Acetyl-D-Glucosamine, S8: β -Methyl-D-Glucoside) and polymers (S28: α -Cyclodextrin, S29: Glycogen), as well as the phenolic compound 2-Hydroxy Benzoic Acid (S30), the amine Phenylethylamine (S24), and the carboxylic acid Pyruvic Acid Methyl Ester (S1). It negatively correlated with one carbohydrate (S11: i-Erythritol).

RDA axis 2 (18.6%) was associated with a gradient of increasing soil TN, TOC, dehydrogenase activity, and urease activity, and decreasing soil C:N ratio and pH (the last two were non-significant according to permutations test). It clearly separated CT (negative scores) from NT (positive scores) treatments. Among the latter, plots with cover crops receiving no fertilizer exhibited the highest scores. This axis positively correlated with the carboxylic acid α -Ketobutyric Acid (S6), the phenolics 2-Hydroxy Benzoic Acid (S30), and the carbohydrate D-Xylose (S10). It negatively correlated with the polymers Tween 40 (S26) and Tween 80 (S27), the amino-acid L-Phenylalanine (S20), and the phenolic compound 4-Hydroxy Benzoic Acid (S31).

4. Discussion

The present controlled experiment is one of the first to shed light on the combined effects of soil tillage and cover crops, under two N fertilization rates, on chemical and biological properties over an entire industrial crop rotation including cereals (wheat and corn), pea, and flax over a 5 year-period. It reveals clear trends towards (i) increased soil TN and TOC contents, and increased enzymatic activities that are also associated with the use of more diverse substrates when NT with or without N fertilizer is applied compared with CT soils; (ii) increased soil C and N inputs and functional activity/diversity of soil microbes, which are associated with the use of more diverse and complex substrates when a cover crop is associated to NT, compared with winter bare fallow. However, the single time point sampling must be taken into account within the interpretation of the discussion and conclusions related to the results obtained. Hereafter, these findings and their relevance for sustainable agriculture are discussed.

4.1. No tillage coupled with cover crops increases soil C and N contents

Increased soil C and N contents were found in both NTcc and NTlcc plots, independently from the species mixture used in cover crops and irrespective of N fertilization (Fig. 1), a beneficial impact already reported in the literature (Mazzoncini et al., 2011; Mbuthia et al., 2015). Consistently, C and N inputs from cover crops were similar between the two mixtures and among treatments, indicating that a winter cover crop can compensate for the organic C and N losses associated with main crop exportation (Table 2, Verzeaux et al., 2016). NT and cover crops may enhance the soil C

storage by reducing oxidative stress (Mangalassery et al., 2015) and improving aggregate stability (Martens, 2000), thus reducing the aggregate disruption induced by plowing (Six et al., 2000). Among CT plots, only the cover crops enriched in leguminous species (CTlcc) impeded the soil C and N depletion induced by intensive tillage. The use of a mulch enriched with leguminous species has indeed been shown to increase soil N and C contents in soil (Sanginga et al., 1997; Mancinelli et al., 2015), as a plausible consequence of the high N contained in their roots, stems and nodules. Consistently, the positive effect of cover crops disappears under bare fallow, since the soil N content decreased over the rotation's duration, irrespective of fertilization and tillage. The soil C content followed the same trend except in NT plots receiving N fertilizer, where the main crop residues also exhibited the higher C:N input. Interestingly, this was the only case in which fertilization was beneficial.

4.2. No tillage increases soil enzymatic activity

NT plots exhibited greater soil enzymatic activity than CT plots through increased dehydrogenase and urease, irrespective of N fertilization (Fig. 2). This is consistent with Das et al. (2014), who found that the dehydrogenase activity, which reflects microbial activity and oxidation–reduction reactions in soil, was 107% greater under NT than under CT. In association with cover crops, NT improved urease activity as compared with CT without cover crops, a result already reported by Hamido and Kpomblekou (2009). Overall, our results are consistent with Janusauskaitė et al. (2013), since SEA were mainly impacted by soil tillage among other variables. This result is explained by the greater content of organic matter and nutrient availability associated with NT, the lack of disruption of soil layers, and the less oxidizing environment, which can stabilize the pool of extracellular enzymes (e.g. urease) (Melero et al., 2009; Mangalassery et al., 2015). Soil nutrient cycling involves enzymes that are related to certain soil properties such as moisture and organic matter content. Changes in soil enzyme activity may thus affect chemical and biological properties (Bergstrom et al., 1998) and explain the positive correlation between urease activity, TOC, and TN, and to a lesser degree, between dehydrogenase and TN (Costa et al., 2013).

4.3. Cover crops increase microbial substrate use diversity in the absence of N fertilization

Microbial substrate use diversity clearly differed between N0 treatments, being the lowest in bare fallow and the greatest in cover crop–NT plots (Fig. 3). This is explained by the high contents of soil TN and TOC in the latter compared with the other treatments, which may increase the diversity of substrate–richness and thus induce more microbial enzymes (Diosma et al., 2006; Govaerts et al., 2007). This is supported by the greater metabolism of phenolic compounds and carbohydrates (under NT) and polymers (under CT) as carbon sources in plots with a standard cover crop (Fig. 4). This beneficial effect of cover crops disappears in CT treatments, suggesting that microbial functional diversity is impaired by soil tillage-induced disturbance via aggregate disruption, compaction, or buried litter (Mangalassery et al., 2015). Remarkably, neither functional diversity nor substrate preference differed among Nx treatments, indicating a functional convergence of the soil microbial community under N fertilization.

4.4. No tillage together with cover crops ensures sustainable management of agricultural soils

RDA results highlighted the combined effect of N fertilization, plowing, and winter cover crops on soil chemical properties and

biological activity. Overall, treatments including both cover crops and no tillage performed the best with respect to soil C and N contents, enzymatic activity and the diversity of substrates used as C sources, independently from N fertilization (see the top-right part of the RDA diagram on Fig. 4). In these conditions, carbohydrates were heavily used by microorganisms in the topsoil (0–10 cm), which is composed of a rich organic litter and contains sugars released from its decomposition and rhizodeposits, hence conferring greater availability of these compounds for soil microbes. Carbohydrates are also known for maintaining and stimulating soil microbial activities in the rhizosphere and detritosphere (Guinina and Kuzyakov, 2015).

Opposingly, conventionally-tilled bare fallow systems negatively impacted the soil, by reducing C and N contents and enzymatic activity (see the bottom-left part of the RDA diagram in Fig. 4). In bare fallows, the high C:N ratio of inputs (i.e. close to 80) is a consequence of the incorporation of only wheat straw residues, which is not compensated for by the low C:N ratio of cover crops returned to the soil. It has been indeed shown that available N and P released from decomposition of low C:N inputs (i.e. cover crop residues) is used by microorganisms for the decomposition of high C:N inputs (i.e. main crop residues) (Schimel and Haettenschwiler, 2007). It is likely that the nutrient release following cover crop decomposition increases the soil N content and microbial activity, which in turn improves the decomposition of high C:N ratio residues. This hypothesis is supported by the legacy effect of the C:N quality of organic residues on the ability of microbial communities to decompose the subsequent amendments (Marschner et al., 2015). The higher degradation of polymers (e.g. Tween 40, Tween 80, α -Cyclodextrin and Glycogen) observed in CT plots including cover crops and in CTbf with N fertilization is also consistent with this hypothesis, since these substrates represent more stable or recalcitrant C compounds. Especially tween 40 and tween 80 are molecules that do not resemble plant-derived polymers (Nunan et al., 2015), but characterize processed organic matter (Grandy and Neff, 2008). Their greater use may be a consequence of the greater availability of N following N fertilization in CTbf, decomposition of cover crops in CTcc/CTlcc (Marschner et al., 2015), and/or of the aggregate disruption under plowing, which eases the accessibility of old organic matter to microorganisms (Six et al., 2000). For other polymers, such as α -cyclodextrin and glycogen, the observed higher degradation in CT can simply reflect “young” polymers originating from fresh plant inputs (Nunan et al., 2015; Grandy and Neff, 2008).

The first axis of the RDA strongly separated the plots without cover crops (i.e. CTbf, NTbf, see the left part of the RDA diagram in Fig. 4) from others. These plots were characterized by a higher C:N ratio of inputs (only wheat straws were returned to the soil) and by a higher utilization of the carbohydrate i-Erythritol. However, the plots which received cover crops (see the right part of the RDA diagram in Fig. 4) which were characterized by lower C:N ratio of inputs, and overall, by higher C and N inputs, significantly enhanced AWCD and Shannon diversity. In the same plots, the degradation of carboxylic acid Pyruvic Acid Methyl Ester (S1), the carbohydrates D-Cellobiose (S14), Glucose-1-Phosphate (S15), and α -D-Lactose (S16), the amino acid L-Threonine (S22), and the amine Phenylethylamine (S24), was improved. Frac et al. (2012) reported that an increase of soil diversity following organic inputs (i.e. cover crops in our study) resulted from the development of different microbiota. This is consistent with our study since uncovered plots are characterized by the degradation of one sugar alcohol (i-Erythritol) compared with the large panel of substrates listed above which were preferentially degraded in covered plots.

5. Conclusions

In summary, without N fertilization, conventional tillage plus standard cover crops strongly decreased the soil CN content and markedly increased potential soil polymer degradation. Under the same N fertilization, no-tillage with standard and legume-enriched cover crops improved degradation activity of soil microorganisms (AWCD, carbohydrates and phenolic compounds degradation) and their functional diversity. These results indicate that, without N fertilization, cover crops are required to prevent C and N depletion and to maintain microbial functional activities and diversity.

Irrespectively of N fertilization, cover crops enriched with legumes prevented the strong decrease of C and N content caused by plowing. No-till combined with cover crops increases enzyme activities.

Thereby, under a crop rotation characterized by a low organic C input, a gradual reduction of synthetic N fertilizers is compensated with an increase of cover crop N input must be encouraged to reduce the risk of soil fertility depletion associated with conventional farming practices. However, it is known that soil microbial functional responses may vary seasonally, so, studies including sampling collections throughout seasons and years are required to investigate further the links between cropping systems and microbial communities' behavior at long term.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2016.08.004>.

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ANNEXE 3

Verzeaux, J., D. Roger, J. Lacoux, E. Nivelle, C. Adam, **H. Habbib**, B. Hirel, F. Dubois, and T. Tetu. 2016b. In Winter Wheat, No-Till Increases Mycorrhizal Colonization thus Reducing the Need for Nitrogen Fertilization. *Agronomy* 6(2): 38.



Short Note

In Winter Wheat, No-Till Increases Mycorrhizal Colonization thus Reducing the Need for Nitrogen Fertilization

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Abstract: Arbuscular mycorrhizal fungi (AMF) play a major role in the uptake of nutrients by agricultural plants. Nevertheless, some agricultural practices can interrupt fungal-plant signaling and thus impede the establishment of the mycorrhizal symbiosis. A field experiment performed over a 5-year period demonstrated that both the absence of tillage and of nitrogen (N) fertilization improved AMF colonization of wheat roots. Moreover, under no-till conditions, N uptake and aboveground biomass production did not vary significantly between N-fertilized and N-unfertilized plots. In contrast, both N uptake and above ground biomass were much lower when N fertilizer was not added during conventional tillage. This finding strongly suggests that for wheat, no-till farming is a sustainable agricultural system that allows a gradual reduction in N fertilizer use by promoting AMF functionality and at the same time increasing N uptake.

Keywords: winter wheat; tillage; nitrogen fertilization; arbuscular mycorrhizal fungi; nitrogen uptake

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form obligate symbioses (mycorrhizas) with most cultivated plants [1] and provide many benefits to plants throughout their growth [2]. The intraradical colonization of plant roots by AMF results in the formation of some specialized structures including vesicles for nutrient storage and arbuscules for exchanging nutrients with the host plant. Such symbiotic associations significantly enhance the uptake capacity of plants for nutrients [2,3] beyond the depletion zone surrounding a root, especially for the inorganic phosphate ion and the ammonium ion [4]. In current intensive agricultural systems, soil management practices may be detrimental to an efficient fungal-plant symbiotic interaction [5], notably nitrogen (N) fertilization [6], which at the same time may reduce the diversity of fungal communities [7]. Conventional tillage (CT) causes a physical disruption of the hyphal network in the soil, thus reducing the density of propagules in the rooting zone [8]. However, knowledge of the combined effects of N fertilization and tillage on AMF colonization and N uptake in the field remains limited [2], especially for an important crop such as winter wheat. The present study has demonstrated that over a 5-year period, tillage and N

fertilization reduced AMF colonization of wheat roots and that plant N uptake capacity was reduced in the absence of N fertilization under CT conditions.

2. Results and Discussion

In the field experiment, both CT and N fertilization strongly decreased wheat root colonization by AMF ($p < 0.001$). The highest AMF root colonization was obtained with no-till without N fertilization (NTN0, 35% of root length colonized by AMF) and the lowest with conventional tillage with N fertilization (CTNX, 2%) (Figure 1A). It is known that soil disturbance and N enrichment resulting from plowing [8,9] and N fertilization [6] reduce the ability of plants and AMF to form mycorrhizas. Moreover, such a reduction seems to be greater when both plowing and N fertilization are used. In line with this observation, Mbuthia *et al.* [10] reported that no-till (NT) and low N fertilizer application were associated with the presence of more AMF in the soil. This could explain why in the present study there were larger amounts of AMF in the roots following NT without any N fertilization (N0). It is also well known that mycorrhizal functioning is strongly influenced both by plant nutrient status and by soil nutrient availability [6]. When nutrient availability is high, root exudate composition can be qualitatively modified, thus decreasing fungal colonization. Moreover, it has been shown that root exudates can stimulate hyphal growth and branching [11], through an attractive effect [12]. Recently, it has been reported that in sorghum, N fertilization negatively influences the production and exudation of strigolactones that are essential host recognition signaling molecules involved in AMF colonization [13]. It is therefore likely that low N availability induces changes both in the root system and in the composition of the root exudate, thus inducing the different processes controlling AMF colonization.

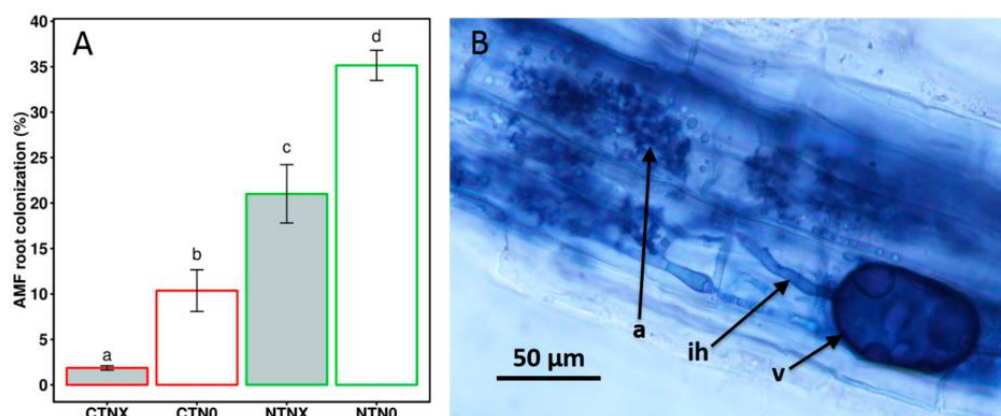


Figure 1. (A) Effect of tillage and nitrogen (N) fertilization on the percentage of wheat root length colonized by arbuscular mycorrhizal fungi (AMF). Values are means $\pm$ standard error. Letters, a, b, c and d indicate differences among treatments according to the Conover post-hoc test ($p < 0.05$), following a significant Kruskal-Wallis test ($p < 0.001$). CT: conventional tillage; NT: no-till; NX: chemical N fertilization; N0: without N fertilization; (B) Wheat root colonized by arbuscular mycorrhizal fungi. a: arbuscule, v: vesicle, ih: intraradical hypha.

The wheat aboveground (AG) biomass at anthesis was the highest under CTNX and NTN0, and was the lowest under conventional tillage without N fertilization (CTN0, $p < 0.05$; Table 1). In addition, there was a much lower AG biomass production in the absence of N fertilizer under CTN0, compared to CTNX. In contrast, total AG plant biomass remained similar between NX and N0 under NT conditions. It was therefore not surprising to observe the same differences for plant N uptake between the different soil treatments and N fertilization conditions ($p < 0.05$; Table 1). When the intraradical colonization of plant roots by AMF is established, the formation of specialized structures for the exchange of nutrients such as arbuscules, enhances the absorbing capacity of the root for water and nutrients [14] and,

as such, speeds up plant growth [15,16]. Moreover, the mycorrhizosphere, as a spatial extension of the rhizosphere associated with the hyphosphere [17], is known to increase the soil volume in which the N-cycling processes can occur. This could partly explain why N uptake decreased only when there was no N fertilization and when root colonization by AMF was reduced under CT condition (CTN0). In contrast, under NT conditions, in the absence of N fertilization, N uptake was maintained by the plant-fungus symbiosis. The extraradical hyphae of AMF are able to take up and assimilate inorganic N [18,19], originating from N fertilizer or released following the decomposition of patches of organic matter. Furthermore, Hodge *et al.* [4] provided evidence that AMF were also able to acquire N directly from the soil organic material, which is able to stimulate hyphae growth. In this study, the higher root colonization of wheat by AMF under NT could be explained by the fact that these soils are characterized by higher contents of fresh organic matter in the upper layer because there is no soil inversion. In the absence of N fertilization, the hyphosphere may prospect for organic nutrients, thus maintaining whole plant N uptake capacity. However, we observed that soil N and C contents were not higher in NT compared to CT (Table 1). Such a finding can be explained by the fact that fresh organic matter was eliminated by sieving before our analysis and by the absence of cover crops during winter periods from the beginning of the experiment. Nevertheless, the soil C:N ratio was significantly higher in NT plots ($p < 0.001$), suggesting a higher availability of C originating from the crop residues present in the upper soil layer. Taken together, our results suggest that the ecological impact of N transfer from AMF to wheat might be higher when the physical environment enhances spore germination and hyphae growth (*i.e.*, lack of physical disturbance in NT) and when mineral N availability is low (*i.e.*, lack of intensive N fertilization in N0), thus maintaining the emission of chemical signals by roots. It can be concluded that direct-seeding mulch-based cropping systems, by stabilizing soil structure and by promoting organic nutrient utilization instead of using inorganic fertilizers, appears to be suitable for sustainable wheat production. In the future, long-term studies will be required to fully assess the impact of cropping systems and the mode of N fertilization on plant-AMF interactions, including the recognition of signaling molecules between the fungi and its host.

Table 1. Impact of nitrogen fertilization and tillage on agronomic traits and soil parameters of wheat plants.

	H (p)	CTNX	CTN0	NTNX	NTN0
AG biomass (g·plant ⁻¹)	10.61 (0.014)	7.02 ± 0.43 ^b	4.88 ± 0.27 ^a	6.07 ± 0.30 ^{ab}	7.13 ± 0.65 ^b
Plant N concentration (mg·g ⁻¹)	NS	10.35 ± 0.45	9.56 ± 0.40	10.89 ± 0.66	9.68 ± 0.55
Plant N uptake (mg·plant ⁻¹)	9.25 (0.03)	72.37 ± 4.95 ^b	46.98 ± 4.06 ^a	66.83 ± 7.03 ^{ab}	68.02 ± 5.37 ^{ab}
Soil N (g·kg ⁻¹)	12.49 (0.006)	1.46 ± 0.01 ^b	1.45 ± 0.01 ^b	1.40 ± 0.03 ^b	1.31 ± 0.02 ^a
Soil C (g·kg ⁻¹)	NS	13.15 ± 0.17	12.93 ± 0.10	14.10 ± 0.33	13.17 ± 0.35
Soil C:N ratio	17.64 (0.0005)	9.01 ± 0.08 ^a	8.93 ± 0.10 ^a	10.08 ± 0.14 ^b	10.04 ± 0.21 ^b
Soil compaction (MPa)	NS	0.13 ± 0.01	0.11 ± 0.03	0.23 ± 0.05	0.20 ± 0.03

In H: Values of the Kruskal-Wallis test with its probability in brackets. Letters, a, ab and b indicate differences among treatments according to the Conover post-hoc test ($p < 0.05$), following a significant Kruskal-Wallis test ($p < 0.001$). CT: conventional tillage; NT: no-till; NX: with chemical N fertilization; N0: without N fertilization. NS: not significant. AG biomass: aboveground biomass. Values correspond to the mean ± standard error of plant and soil parameters among the four treatments.

3. Materials and Methods

3.1. Site Description and Experimental Design

The field experiment was conducted at the experimental site of *La Woestyne*, in Northern France (50°44' N, 2°22' E, 40 m above sea level). The average annual air temperature and total rainfall were 10.5 °C and 675 mm respectively, with amounts of rainfall relatively homogeneous across seasons. The soil particle size composition was as follows: silt 66.8%, clay 21.2%, and sand 12%.

Prior to the establishment of the field experiment in 2009, the field was managed with chisel plowing and a rotary power system. In order to study the effect of tillage and N fertilization on wheat colonization by AMF, the experimental field was split into four replicate plots for each of the four treatments: conventional tillage with (CTNX) or without (CTN0) N fertilization; no-till with (NTNX) or without (NTN0) N fertilization. CTN0 and NTN0 plots measured 7 m × 8 m while CTNX and NTNX

plots measured 14 m × 8 m. The crop rotation before the sampling date consisted of green peas (*Pisum sativum* L.) in 2010, maize (*Zea mays* L.) in 2011, winter wheat (*Triticum aestivum* L.) in 2011–2012, flax (*Linum usitatissimum* L.) in 2013, sugar beet (*Beta vulgaris* L.) in 2014 and winter wheat in 2014–2015. As maize was grown for silage and flax for fiber, all the aboveground structures were removed from the field. Pea haulms, wheat straw and beet leaves were returned to the soil. In all the NX plots, maize received 108 kg·N·ha⁻¹, wheat 160 kg·N·ha⁻¹, flax 80 kg·N·ha⁻¹ and sugar beet 160 kg·N·ha⁻¹ (50% urea, 25% ammonium, 25% nitrate). Green peas did not receive any N fertilization in NX plots according to European policies. The N0 plots have not been fertilized for the 5 years of experiment. In October 2014, the winter wheat used for crop sampling was sown at 12.5 cm of row spacing and 250 seeds m⁻² using an AS 400 drill (Alpego, Italia) and was fertilized in two times with 80 kg·N·ha⁻¹ in March and May 2015.

3.2. Sample Collection and Analyzes

In June 2015, at the anthesis stage of winter wheat, six plants were randomly sampled in each of the four replicate plots for each treatment (CTNX, CTN0, NTN0 and NTN0). The root system was collected by extracting 15 cm depth and 5 cm diameter soil cores directly over the cut stems of the six selected plants. Six 15 cm deep soil cores were also collected for soil N and C analysis using a 2 cm diameter auger.

The aboveground structures of plant samples were dried at 65 °C for 3 days and subsequently weighed (± 0.1 g accuracy) to determine total aboveground biomass. Each sample was then ground into a fine powder for plant total N and C analysis. Soil samples were sieved using a 2 mm mesh, dried at 35 °C for 48 h and ball-milled using a grinder MM 400 (Retsch, Haan, Germany). Soil total plant and soil N and C contents were determined using an elemental analyzer (Flash EA 1112 series, Thermo Fisher Scientific, Waltham, USA). Since the soil was free of carbonate, the soil organic C was assumed to be equal to the total soil C content.

AMF colonization of wheat was monitored in 30 root subsamples of 1 cm length by plant. Subsamples were stained with trypan blue according to Koske and Gemma [20]. Mycorrhizal infection was quantified using the method of McGonigle *et al.* [21], with 150 intersections counted for each sample.

Near the plant and soil sampling areas, penetration resistance was measured by using a penetrometer (Eijkelkamp, Giesbeek, The Netherlands) fitted with a 60 deg and 1 cm² base area cone.

3.3. Statistical Analysis

All statistical analyzes were performed using the R software (v. 3.1.2, R Development Core Team). Mean values are given with their standard error. Plant and soil parameters, as well as AMF colonization, were compared among treatments by using a non-parametric Kruskal-Wallis one-way analysis of variance followed by a Conover post-hoc test whenever significant (PMCMR package, [22]).

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Conflicts of Interest: The authors declare no conflict of interest.

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