Nutrition azotée des associations Pois-Blé d'hiver (Pisum sativum L. – Triticum aestivum L.) : Analyse, modélisation et propositions de stratégies de gestion

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Cette thèse a été réalisée au Laboratoire d'Écophysiologie Végétale et Agroécologie (LEVA) du Groupe ESA. Yves Crozat en a été l'initiateur et l'a encadrée jusqu'à son décès le 20 juin 2007. En conséquence, elle a principalement été dirigée par Marie-Hélène JEUFFROY (UMR 211 Agronomie INRA AgroParisTech) et co-encadrée par Guénaëlle CORRE-HELLOU (LEVA, Groupe ESA Angers).

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« Peut-être les choses que je perçois, les animaux, les plantes, les hommes, les montagnes, les eaux brillantes et courantes,

Les ciels de jour et de nuit, les couleurs, les densités, les formes, peut-être ces choses sont-elles (comme sans doute elles le sont) de simples apparitions, et le vrai quelque chose est encore à connaître »

Calamus, Feuilles d'herbe
WALT WHITMAN
(Traduction de Louis FABULET)

"May-be the things I perceive, the animals, plants, men, hills, shining and flowing waters,

The skies of day and night, colors, densities, forms, may-be these are (as doubtless they are) only apparitions, and the real something has yet to be known"

Calamus, Leaves of grass
WALT WHITMAN

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SOMMAIRE

Introduction générale

De nouveaux défis agricoles

Au sortir de la seconde guerre mondiale, l'enjeu était, pour la France, d'assurer son autonomie alimentaire. La combinaison de moyens tels que la sélection d'innovations variétales, le recours aux intrants chimiques de synthèse et la réorganisation des structures agricoles de production ont rapidement et efficacement permis d'atteindre cet objectif. Dans d'autres régions du monde, cette même démarche, nommée alors « révolution verte », a connu plus ou moins de succès selon le contexte de mise en œuvre (Griffon, 2006). Les systèmes de culture mis au point pour atteindre cet objectif, de plus en plus intensifs et de plus en plus spécialisés, ont, au fil des ans, été à l'origine d'impacts négatifs sur l'environnement : augmentation des émissions de gaz à effet de serre, réchauffement climatique, consommation importante d'énergie fossile, pollutions en nitrate et en pesticides des nappes phréatiques, eutrophisation des côtes et des cours d'eau, *etc.*

Depuis quelques années, une prise de conscience environnementale s'observe dans la société civile, et interpelle le monde agricole et scientifique. Les systèmes de culture et de production sont remis en cause et doivent aujourd'hui répondre à des exigences multicritères combinant niveau élevé de production (dans le but de contribuer à nourrir la population croissante de la planète ; Griffon, 2006 ; Parmentier, 2007), qualité de production, mais aussi rentabilité économique et services écologiques et environnementaux (Griffon, 1999 ; Doré et al., 2006 ; Griffon, 2006 ; Meynard, 2008). Dans ce contexte, il semble nécessaire de substituer en partie le recours aux intrants chimiques de synthèse par l'utilisation de régulations biologiques et écologiques afin d'améliorer le bilan environnemental de la production agricole (Griffon, 1999).

Rôles écologiques des légumineuses à renforcer dans les systèmes de culture face aux enjeux environnementaux actuels

Par leur capacité unique à fixer l'azote de l'air à l'aide de leurs nodosités, les légumineuses constituent une voie privilégiée d'introduction d'azote dans les systèmes de culture. Leur autonomie en azote permet, en réduisant l'utilisation des engrais azotés dans les systèmes de culture, de limiter ainsi les impacts environnementaux directement proportionnels à la quantité d'azote minéral fabriqué, transporté et épandu. L'introduction de légumineuses dans les systèmes de culture offre aussi d'autres atouts environnementaux tels que la réduction des maladies telluriques (Colbach et al., 1996) et des infestations d'adventices (Chauvel et al., 2001) liées à la diversification des successions culturales. Bien que ces cultures puissent contribuer à améliorer sensiblement les impacts environnementaux des systèmes de culture actuels et que la demande en matières riches en protéines pour l'alimentation animale soit très forte, l'agriculture européenne se présente de façon singulière avec moins de 5 % de la surface en grandes cultures allouée aux légumineuses, contre 20 à 30 % en Amérique du Nord et du Sud (Munier-Jolain et al., 2005).

L'amélioration des connaissances sur les impacts environnementaux de l'introduction de légumineuses à graines dans les systèmes de culture et la recherche d'innovations agronomiques permettant de renforcer leurs bénéfices environnementaux sont devenues des nécessités pour les professionnels agricoles et les décideurs politiques demandeurs d'argumentaires en vue de la mise en œuvre de mesures incitatives sur ces cultures.

La valorisation des bénéfices environnementaux des légumineuses est essentiellement aujourd'hui envisagée par leur introduction dans les systèmes de culture en diversifiant les successions de cultures céréalières. L'intérêt notamment du pois comme précédent du blé est ainsi bien connu. Les gains de rendement d'un blé après un précédent pois par rapport à un précédent blé s'élèvent en moyenne à 8 q ha^{-1} (Silsbury, 1990 ; Stevenson et van Kessel, 1996) et les économies d'azote

en moyenne de 20 kg peuvent être réalisées sur la culture de blé. L'absence de fertilisation sur le pois et les économies d'azote sur la culture suivante permettent ainsi de réduire sensiblement la consommation d'énergie fossile et les émissions de gaz à effet de serre. Nemecek et al. (2008) montrent par exemple dans la région du Barrois (France) une réduction de la consommation en énergie fossile de 11 % et une réduction des émissions de gaz à effet de serre de 14 % dans une rotation comprenant du pois par rapport à une rotation sans pois, avec des marges brutes proches entre les deux rotations. Il apparaît néanmoins que les bénéfices environnementaux des protéagineux dans les successions de culture pourraient être encore davantage valorisés par une meilleure adaptation de l'itinéraire technique, notamment de la fertilisation ainsi que du désherbage, en fonction du précédent (Ballot, 2009).

Une autre forme d'introduction des légumineuses dans les systèmes de culture peut être envisagée. Les légumineuses permettent aussi de diversifier les espèces au sein de la parcelle dans le cas d'associations avec d'autres espèces (Altieri, 1999). Ce mode de diversification entraîne une interaction plus forte entre espèces comparé au cas des successions de culture mais reste encore aujourd'hui peu pratiqué en Europe dans le cas d'associations annuelles.

Qu'est-ce qu'une association céréale-légumineuse ?

Une association est définie comme la culture d'au moins deux espèces différentes sur la même surface pendant une période significative de leur développement (Willey, 1979). C'est une pratique plurimillénaire qu'évoquait déjà Pline l'Ancien (23-79 après J.C., dans Schoonhoven et al., 2005) dans son Histoire Naturelle, mais aussi Darwin (1859) dans l'ouvrage qui fonda la théorie de l'évolution. L'association graminée-légumineuse se rencontre aujourd'hui fréquemment dans les régions tropicales et bien sûr dans les prairies pluriannuelles en zone tempérée. La culture d'associations d'espèces annuelles de céréales et de légumineuses a quasiment disparu des systèmes de culture en Europe avec l'intensification de l'agriculture et l'usage massif des intrants chimiques de synthèse. Elle présente un regain d'intérêt, notamment dans les systèmes à faible niveau d'intrants en particulier en agriculture biologique (Hauggaard-Nielsen et al., 2001b; Bellostas et Jensen, 2004).

Des intérêts agronomiques et environnementaux liés à la complémentarité dans l'acquisition de l'azote

Les intérêts d'associations céréale-légumineuse ont largement été démontrés. Des gains de productivité en comparaison des cultures pures ont souvent été observés, ainsi qu'une variabilité interannuelle des rendements inférieure à celle observée en cultures pures (Willey, 1979 ; Jensen, 1996 ; Corre-Hellou et al., 2006). Le taux de protéines des grains de la céréale est généralement plus élevé en association qu'en culture pure (Jensen, 1996 ; Corre-Hellou, 2005 ; Li et al., 2009). Ces avantages en termes de niveau et de qualité de rendement sont attribués principalement à la complémentarité, dans le temps et l'espace, entre céréales et légumineuses, pour l'accès à l'azote, ce qui conduit à une compétition interspécifique inférieure à la compétition intraspécifique (De Wit, 1960 ; Trenbath, 1976). Ainsi, les associations céréales-légumineuses permettent une meilleure efficience d'utilisation des ressources azotées (Ghaley et al., 2005 ; Bedoussac et Justes, 2009). De plus, Jensen (1996) observe, sur plusieurs années, un bilan azoté systématiquement négatif dans des associations pois-orge et intermédiaire entre ceux du pois pur et de l'orge pure. Hauggaard-Nielsen et al. (2003), quant à lui, observent une quantité d'azote lixiviée inférieure ou égale après des cultures associées par rapport à celle observée après des cultures pures.

Face au défi d'une intensification écologique de l'agriculture visant à la fois productivité et réduction des intrants, les associations d'espèces céréales-légumineuses pourraient jouer un rôle intéressant en valorisant des processus écologiques de complémentarité de niche et de facilitation, dans l'objectif d'une meilleure exploitation des ressources naturelles.

Des finalités multiples

Les associations céréale-légumineuse peuvent être cultivées pour de nombreux débouchés différents (UNIP, 2008) : blé meunier cultivé à bas niveau d'intrants, fourrage vert ou en grains, biomasse énergétique. Aujourd'hui surtout valorisées en agriculture biologique, elles peinent pourtant à trouver leur place dans les assolements des exploitations en agriculture conventionnelle. Pourtant elles sont à même de répondre à des finalités multiples :

- dans le cas d'un débouché en grains :
    - produire du blé riche en protéines avec peu d'intrants azotés ;
    - produire du pois sans les problèmes rencontrés en culture pure (verse, adventices,…) ;
    - produire blé et pois en alternative à une culture séparée de deux espèces (niveau de production au moins équivalent et plus stable que la moyenne des cultures pures avec moins d'intrants).
- dans le cas d'un débouché fourrager :
    - produire un fourrage présentant à la fois une forte biomasse et une richesse satisfaisante en MAT (Matières Azotées Totales), tout en n'ayant pas ou peu recours aux intrants (irrigation, fertilisation, protection phytosanitaire). Ces fourrages peuvent contribuer à sécuriser et diversifier les systèmes fourragers

Ces finalités diverses ont en point commun la nécessité de maîtriser la part de chaque espèce associée dans la récolte dans le but d'atteindre les exigences propres à chaque débouché. Or, il y a un manque de connaissances sur les facteurs responsables de la variabilité de la part des espèces cultivées en mélange. C'est aujourd'hui le principal frein à un développement plus large de cette technique dans les agrosystèmes.

Des leviers à identifier pour orienter les associations vers différents objectifs de production

Si les atouts agronomiques et environnementaux des associations annuelles céréale-légumineuse sont aujourd'hui bien connus, peu de références sont disponibles pour optimiser leurs itinéraires techniques (fertilisation azotée, protection phytosanitaire). Il reste difficile d'identifier quels leviers (ou quelles combinaisons de leviers) permettraient d'orienter l'association vers tel ou tel objectif de production. Par ailleurs, l'importance des interactions pour l'acquisition des ressources azotées est bien connue dans les associations, mais on ne dispose pas d'éléments suffisants nécessaires à la construction de règles de décision pour son pilotage.

Enjeux du travail

La compréhension des mécanismes en jeu dans ces systèmes doit permettre de faire émerger des méthodes de pilotage afin d'atteindre des objectifs quantitatifs et qualitatifs de production.
En prenant les associations pois-blé d'hiver comme modèle d'étude, les enjeux de la thèse sont :

1) utiliser les connaissances acquises précédemment sur le fonctionnement dynamique d'une association céréale-légumineuse, ceci dans l'optique d'étudier la pertinence de la fertilisation azotée comme levier pour orienter les performances des associations pois-blé d'hiver pour différents objectifs de production en agriculture conventionnelle;
2) approfondir les connaissances sur le fonctionnement de l'association en réponse à différentes dynamiques de disponibilité en azote minéral (partage des ressources azotées, inhibition et réversibilité de la fixation symbiotique en fonction de la date de fertilisation azotée) ;
3) modéliser le fonctionnement azoté d'une association pois-blé d'hiver, afin de proposer des pistes d'élaboration de règles de décision pour gérer la fertilisation azotée de ces associations pour atteindre différents objectifs de production.

Chapitre 1 :
Problématique et hypothèses

1. Des interactions complexes

Les espèces cultivées en association interagissent indirectement l'une sur l'autre en modifiant les caractéristiques de l'environnement dans lequel elles sont implantées (Figure 1 : Corre-Hellou, 2005 ; d'après Vandermeer, 1989). Cette modification peut concerner la disponibilité en ressources (lumière, eau, azote ; Ofori et Stern, 1987; Jensen, 1996; Tsubo et al., 2001), les caractéristiques biologiques, physiques ou chimiques du sol, la pression en maladies et ravageurs (modification de la propagation des spores ; Kinane et Lyngkjaer, 2002), la perturbation de la traque visuelle ou olfactive d'un ravageur (Altieri, 1993; Finch et Collier, 2000). Cette interaction peut avoir des conséquences négatives sur une des espèces (on parle alors de compétition), ou positives (on parle alors de facilitation).

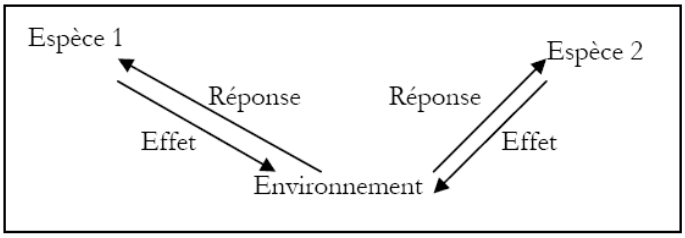

Figure 1 : Principe des interactions entre espèces (Corre-Hellou, 2005 ; d'après Vandermeer, 1989)

2. Des relations de compétition et complémentarité en fonction des caractéristiques de chaque espèce

Les espèces associées peuvent, par leurs caractéristiques biologiques et phénologiques, exploiter des ressources sur des périodes différentes, dans des zones différentes ou encore sous des formes différentes. On parle alors de différentiation de niche ou de complémentarité pour les ressources permettant d'obtenir très souvent une compétition interspécifique inférieure à la compétition intraspécifique.

2.1. Exploitation des ressources sur des périodes différentes : caractéristiques de développement et de croissance du pois et des céréales

Les céréales (l'orge, le blé) et le pois ont des dynamiques de croissance assez proches. On peut noter toutefois une vitesse de croissance de la céréale plus rapide en début de cycle. La céréale prend un avantage dès le démarrage avec une levée plus rapide d'environ 50 dj (degrés-jour) (Corre-Hellou, 2005).

La vitesse de progression du front racinaire des racines de céréale (1.6 mm dj^{-1} ; Paillard, 1991 in Gate, 1995) est aussi plus rapide que celle des racines de pois (0.9 mm dj^{-1} ; Thorup-Kristensen, 1998 ; Corre-Hellou and Crozat, 2005).

L'apparition des feuilles est linéaire en fonction du temps thermique exprimé en degrés-jour (base 0°C pour les deux espèces). Cette vitesse peut varier en fonction des espèces et au sein d'une espèce entre variétés. Dans une étude comparative entre de l'orge et du pois, la vitesse foliaire de l'orge (cv. Scarlett) atteint 0.018 feuilles émises par degré-jour et celle du pois (cv. Baccara) 0.015 feuilles émises par degré-jour (Corre-Hellou, 2005). Le stade épi 1 cm marque chez la céréale l'accélération de la croissance aérienne. La date de réalisation de ce stade est variable pour une variété donnée en fonction de la température et de la photopériode depuis le semis. Chez le pois, la vitesse de croissance s'accélère à partir du stade 6 feuilles (environ 500 degrés-jour après le semis). Les valeurs maximales de LAI (Leaf Area Index) s'élèvent environ à 6 m^2 de feuilles par m^2 de sol pour les deux espèces et sont atteintes au cours de la floraison du pois et au stade « dernière feuille étalée » des céréales (Corre-Hellou, 2005). La période de vitesse maximale de

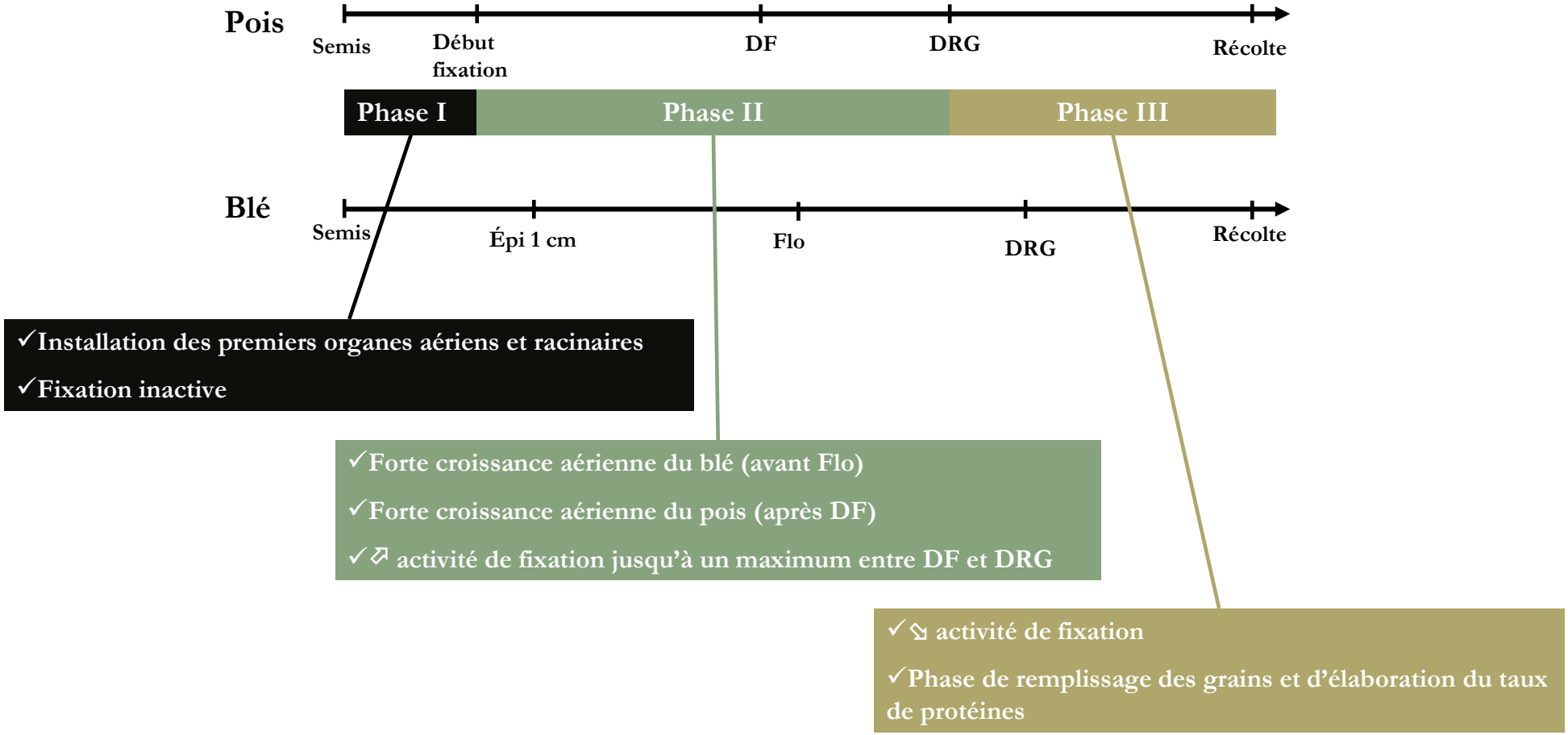

Figure 2 : Juxtaposition des cycles de développement du pois et du blé d'hiver cultivés en association
DF : début floraison du pois ; Flo : floraison du blé ; DRG ; début de remplissage des grains du blé ou du pois

croissance d'une céréale s'observe pendant la montaison, entre les stades 2 nœuds et floraison (Karimi et Siddique, 1991), alors que le pois atteint sa vitesse maximale de croissance entre Début Floraison (DF) et Début de Remplissage des Grains (DRG) (Jensen, 1987).
L'apparition des fleurs le long de la tige de pois est, comme le développement foliaire, linéaire en fonction du temps thermique. La vitesse de progression des fleurs est plus rapide que la vitesse d'émission des feuilles (0.023 fleurs émises par degré-jour, cv. Baccara). Le stade début floraison est atteint environ 800 degrés-jour après le semis, à peu près en même temps que le stade épiaison de la céréale (Figure 2). Le remplissage des grains de pois débute 250 degrés-jour après le début de la floraison. La progression du stade DRG (ou SLA : stade limite d'avortement) le long de la tige est linéaire avec une vitesse légèrement plus rapide (0.024 nœud franchissant SLA par degré-jour, cv. Baccara) que la vitesse de progression de la floraison.

Dans des travaux précédents (Corre-Hellou, 2005), il a été démontré que le développement foliaire et reproducteur de chaque espèce n'était pas modifié en cultures associées par rapport aux cultures pures. Par ailleurs, ces travaux ont aussi montré que la vitesse d'enracinement de chaque espèce était similaire en association et en culture pure.

Les écarts de dynamique de croissance entre céréale et pois purs se retrouvent aussi quand ils sont associés. Dans le cadre d'associations pois-orge, Andersen et al. (2007) ont démontré, en tout début de cycle, que l'orge a une vitesse de croissance supérieure au pois, puis la tendance s'inverse au profit du pois, pour finir en fin de cycle en faveur du blé. Les avantages compétitifs des deux espèces, en termes de rapports de dominance et d'écarts de dynamique de croissance, sont donc variables au cours du temps. L'ampleur des écarts de dynamique de croissance entre les espèces en pur et en association peut varier en fonction des conditions climatiques, des cultivars utilisés, des conditions d'implantation de la culture, plus ou moins favorables à l'une ou l'autre des deux espèces. Les conséquences de ces écarts de dynamiques de croissance entre espèces sur la compétition pour les ressources et les performances finales de l'association n'ont, jusqu'ici, pas été étudiées. Par ailleurs, on manque de connaissances sur les leviers qui permettraient de modifier cette évolution des dominances entre espèces dans l'association.

2.2. Exploitation des ressources dans des zones différentes : caractéristiques d'exploration aérienne et racinaire des céréales et du pois

Dans des associations pois-orge, il a été montré que la croissance en hauteur était proche pour les deux espèces tout au long du cycle ; les écarts étant en moyenne de 5 cm. La croissance en hauteur de chaque espèce était par ailleurs peu modifiée en culture pure et en association (Corre-Hellou, 2005). Sur ces mêmes espèces, il a été aussi montré que la répartition verticale de la surface foliaire dans le couvert différait légèrement entre espèces et évoluait en cours de cycle (Berntsen et al., 2004). Toutefois dans le partage de la lumière dans des couverts plurispécifiques, ce sont les différences de hauteur qui jouent le rôle le plus déterminant. Les différences de répartition verticale affectent beaucoup moins ce partage (Corre-Hellou, 2007).

La vitesse d'enracinement plus rapide de la céréale lui permet d'avoir une exploration racinaire plus profonde que le pois pendant toute la phase végétative. Ces écarts régressent vers le début de la floraison du pois, date à laquelle les deux espèces ont atteint leur profondeur maximale. La répartition verticale des racines dans le profil est similaire pour les céréales et le pois (Greenwood et al., 1982 ; Hamblin et Hamblin, 1985 ; Voisin et al., 2002a ; Corre-Hellou et Crozat, 2005 ; Gregory et al., 1978). Pour le pois comme pour les céréales, plus de 75 % des racines sont situées dans les 40 premiers cm de sol. Corre-Hellou (2005) observe, par contre, que la colonisation latérale diffère entre le pois et l'orge purs. En s'éloignant de la base de la plante, la colonisation racinaire du pois décroît nettement alors que celle de l'orge reste stable.

Les céréales et le pois semblent ne pas se distinguer de façon notoire dans l'exploration de l'espace aérien et racinaire même si une variabilité génotypique existe probablement bien qu'elle ait été rarement caractérisée. Toutefois les écarts importants de vitesse d'enracinement entre la céréale et le pois sont probablement déterminants dans le partage des ressources du sol (cf 3.1).

2.3. Exploitation des ressources par des voies variées

La principale différence biologique entre blé et pois réside dans la capacité du pois à assurer sa nutrition azotée à partir de deux voies : absorption de l'azote minéral du sol et fixation de l'azote atmosphérique. La nutrition azotée du blé ne repose que sur l'absorption d'azote minéral du sol. La fixation de l'azote atmosphérique dont bénéficie le pois est rendue possible grâce à une relation de mutualisme entre le pois et des bactéries du sol (*Rhizobium Leguminasorum* bv. *Vicae*). Ces bactéries sont hébergées sous une forme modifiée (appelées bactéroïdes) dans des nodosités formées sur les racines après infection des poils absorbants du pois par les bactéries.

De la germination à la mise en place des premières nodosités, la nutrition azotée du pois repose sur les ressources de la graine et du sol. Les nodosités se mettent en place progressivement à partir du stade 4 à 6 feuilles du pois jusqu'à floraison (Tricot, 1993). L'activité de fixation augmente progressivement vers un maximum atteint à DF, avant de décroître vers DRG (Voisin et al., 2002b).

Le potentiel inhibiteur des nitrates sur la fixation symbiotique du pois est bien connu (Streeter, 1985a; Streeter, 1985b). Il a été caractérisé sur pois pur en situation de plein champ par l'apport au semis de différentes doses d'azote (Voisin et al., 2002b). La fixation symbiotique décroît linéairement avec une augmentation de la disponibilité en nitrates dans l'horizon labouré et devient nulle dès 48 kg N ha^{-1} pendant les stades végétatifs du pois, et dès 34 kg N ha^{-1} pendant les stades reproducteurs.

Les gains de rendements observés en association par rapport aux cultures pures ont souvent été attribués à la complémentarité entre les deux espèces pour l'azote par l'utilisation de deux sources d'azote (Fujita et al., 1992 ; Hauggaard-Nielsen et al., 2008 ; Hauggaard-Nielsen et al., 2009). Toutefois, les dynamiques différentes d'acquisition des deux voies au cours du cycle sont à bien prendre en compte ainsi que l'impact de la compétition pour la lumière sur la capacité de fixation de la légumineuse dans la compréhension du fonctionnement de l'association.

2.4. Synthèse

La coexistence d'espèces aux phénologies et aux dynamiques de croissance et d'acquisition d'azote différentes permet de distinguer plusieurs phases au cours du cycle concernant la nature des interactions pour les ressources (Figure 2) :

- Phase I (levée - stade 6 feuilles du pois) :
  - l'appareil fixateur du pois n'est pas encore en fonctionnement. Les deux espèces reposent donc sur le même mode d'acquisition de l'azote (absorption de l'azote minéral du sol). C'est aussi la phase de mise en place d'un premier rapport de dominance. La céréale acquiert un avantage compétitif à la fois au niveau aérien par une croissance précoce plus forte et au niveau racinaire par une vitesse plus rapide d'exploration en profondeur.

- Phase II (6 feuilles - début remplissage des grains) :
    - cette phase est bornée par le démarrage de l'activité de fixation symbiotique et le début de remplissage des grains du pois. C'est la phase d'augmentation de la vitesse de fixation jusqu'à un maximum atteint au début de floraison du pois (Voisin et al., 2002b). C'est donc une phase où l'intensité de la compétition pour les ressources azotées du sol diminue du fait de la différenciation de niche permise par la capacité de fixation du pois. Par ailleurs, il s'agit aussi d'une phase de forte compétition pour la lumière (Corre-Hellou et al., 2006) en raison de la période de fermeture du couvert. La croissance et les besoins en azote des deux espèces s'accélèrent.
- Phase III (début de remplissage des grains – maturité) :
    - cette phase correspond au remplissage des grains des deux espèces, qui représentent un puits important d'allocation carbonée et azotée. Chez le pois, l'appareil fixateur, par sénescence ou par diminution d'allocation carbonée, diminue rapidement son activité de fixation jusqu'à l'arrêt (Voisin et al., 2003).

3. Fonctionnement de l'association pois-blé et partage des ressources

3.1. Partage de l'azote du sol

Plusieurs travaux sur les associations céréale-légumineuse ont démontré que la céréale était plus compétitive que le pois pour l'acquisition de l'azote minéral du sol (Fujita et al., 1992 ; Hauggaard-Nielsen et al., 2001a ; Corre-Hellou et Crozat, 2004). La céréale, par son système racinaire plus dense et plus profond a accès à une quantité de ressources en azote minéral plus importante. Toutefois, il a été prouvé que ces écarts d'enracinement n'étaient déterminants que dans le cas d'une faible disponibilité en azote dans le milieu. Dans le cas de disponibilités en azote plus élevées, le partage de l'azote du sol est déterminé essentiellement par les écarts de demande en azote entre espèces (Corre-Hellou et al., 2007).

3.2. Conséquences sur la contribution de la fixation symbiotique à l'accumulation d'azote par le pois

La forte compétitivité de la céréale pour l'azote minéral du sol entraîne une réduction plus rapide et plus forte du pool d'azote minéral en association par rapport au pois pur. Par conséquent, de nombreux travaux mettent en évidence que le pois, en association à une céréale, repose davantage sur la fixation symbiotique pour la satisfaction de ses besoins azotés comparativement au pois pur (Jensen, 1996 ; Hauggaard-Nielsen et al., 2008 ; Hauggaard-Nielsen et al., 2009, Li et al., 2009). Ainsi, la part de l'azote issu de la fixation dans l'azote total accumulé est de 10 à 30 % supérieure chez un pois associé à une céréale que chez un pois cultivé en pur.

3.3. Effet de la disponibilité en azote sur le fonctionnement de l'association

Un apport d'azote est principalement valorisé par la céréale qui voit son statut azoté amélioré et ainsi sa compétitivité aérienne pour la lumière. Le pois voit alors sa photosynthèse réduite et, par conséquent, son potentiel de fixation diminué. Ainsi, la compétition pour la lumière exercée par le blé sur le pois diminue la croissance de ce dernier, ainsi que la quantité d'azote fixé accumulée dans ses tissus (Corre-Hellou et al., 2006 ; Hauggaard-Nielsen et al., 2009). En conséquence, une forte disponibilité en azote minéral favorise le blé au détriment du pois, avec pour effet une diminution de la quantité d'azote fixé par le pois sans forcément diminuer la part de l'azote fixé dans l'azote total accumulé dans les tissus du pois (Ghaley et al., 2005).

Tableau 1 : Liste non exhaustive d'articles abordant l'effet de la fertilisation azotée sur des associations annuelles céréale-légumineuse cultivées en plein champ

Référence	Région d'étude	Espèces	Dose apportée (g N m^{-2})	Date d'apport (JAS, JAL, ou stade)	Gain moyen en comparaison d'une conduite sans fertilisation azotée (%)	
					Rendement ou biomasse totale de l'association	Proportion de céréale dans la récolte
Bedoussac and Justes, 2009	France	*Triticum durum* L. *Pisum sativum* L.	0 - 6 - 8 - 10 - 14 - 18	1 à 3 applications depuis ZGS30 à ZGS37	1%	41%
Corre-Hellou et al., 2006	France	*Hordeum vulgare* L. *Pisum sativum* L.	0 - 3 - 13	semis	3%	45%
Ghaley et al., 2005	Danemark	*Triticum aestivum* L. *Pisum sativum* L.	0 - 4 - 8	semis	-2%	194%
Hauggaard-Nielsen and Jensen, 2001	Danemark	*Hordeum vulgare* L. *Pisum sativum* L.	0 - 4 - 5	semis	13%	34%
Izaurralde et al., 1990	Canada	*Hordeum vulgare* L. *Pisum sativum* L.	0 - 8	durant le printemps avec un semis au 27 mai	1%	13%
Jensen, 1996	Danemark	*Hordeum vulgare* L. *Pisum sativum* L.	0 - 5	1 semaine après JAL	6%	45%
Adu-Gyamfi et al., 1997	Inde	*Sorghum bicolor* L. *Cajanus cajan* L.	0 - 5	0-40 JAS	17%	14%
Baker and Blamey, 1985	Australie	*Sorghum bicolor* L. *Glycine max* L.	0 - 6 - 12	apports fractionnés entre le semis et 37 JAS	109%	15%
Bilalis et al., 2005	Grèce	*Zea mays* L. *Phaseolus vulgare* L.	0 - 6.25 - 20	quelques jours avant semis	12%	-1%
Bilalis et al., 2005	Grèce	*Zea mays* L. *Vigna unguiculata* L.	0 - 6.25 - 20	quelques jours avant semis	15%	1%
Morgado et Willey, 2003	Royaume-Uni	*Zea mays* L. *Phaseolus vulgare* L.	0 - 17.25 - 34.5	apports fractionnés: 26 et 42 JAS	52%	17%
Ofori, 1986	Australie	*Zea mays* L. *Vigna unguiculata* L.	0 - 2.5 - 5 - 10		12%	3%
Rao et al., 1987	Inde	*Sorghum bicolor* L. *Cajanus cajan* L.	0 - 4 - 8 - 12	fertilisation uniquement sur la céréale: apports fractionnés entre 15 JAS et 30JAS	38%	15%
Rao et al., 1987	Inde	*Zea mays* L. *Arachis hypogaea* L.	0 - 5 - 10 - 15	fertilisation uniquement sur la céréale: apports fractionnés entre 15 JAS et 30JAS	127%	67%
Rao et al., 1987	Inde	*Sorghum bicolor* L. *Vigna unguiculata* L.	0 - 4 - 8 - 12	fertilisation uniquement sur la céréale: apports fractionnés entre 15 JAS et 30JAS	32%	20%
Rerkasem et Rerkasem, 1988	Inde	*Zea mays* L. *Vigna umbellata* L.	0 - 5 - 10 - 20	après la levée	26%	5%
Sampaio et al., 2004	Brésil	*Zea mays* L. *Phaseolus vulgare* L.	0 - 1.6	au semis?	14%	-2%
Siame et al., 1998	Zambie	*Zea mays* L. *Phaseolus vulgare* L.	0 - 3 - 6 - 9 - 12	apports fractionnés: au semis et à 42 JAL	128%	-6%
Tobita et al., 1994	Inde	*Sorghum bicolor* L. *Cajanus cajan* L.	0 -2.5 - 5 - 10	avant le semis	86%	169%

JAS : Jours Après Semis ; JAL : Jours Après Levée ; ZGS : « Zadoks Growth Stage »: stade selon l'échelle de Zadoks

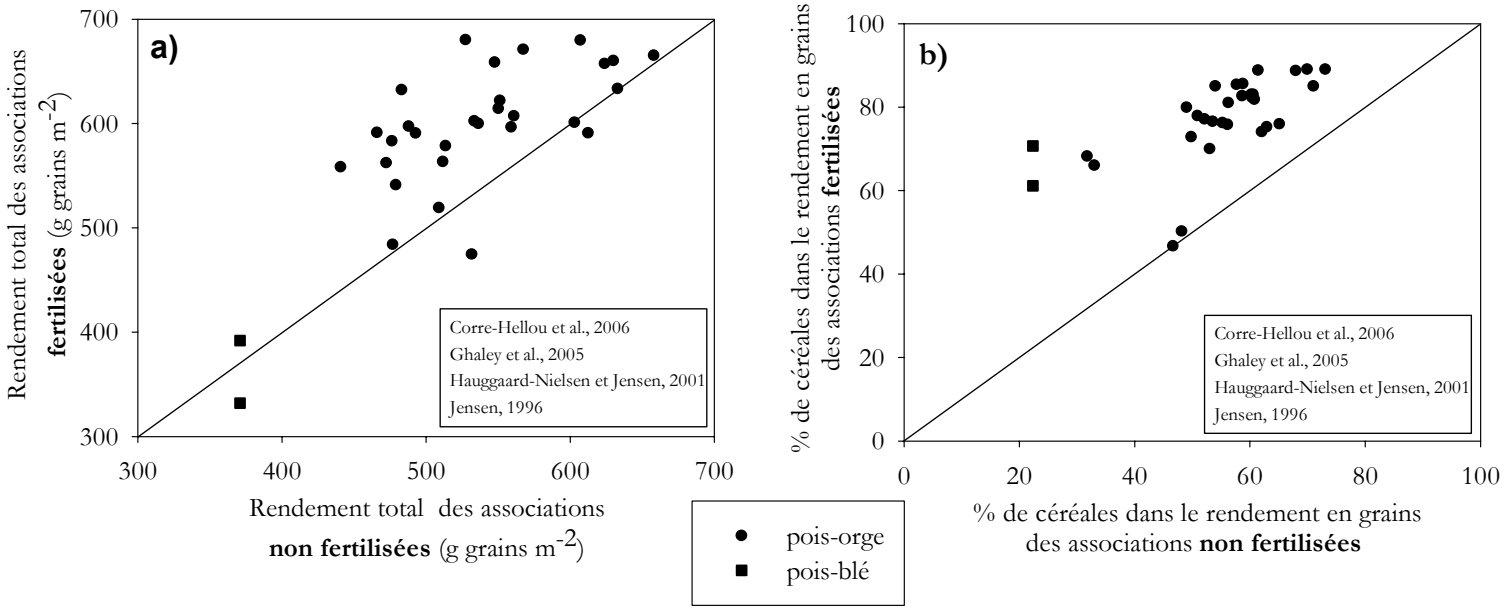

Figure 3 : Effet d'une fertilisation azotée au semis sur les performances d'associations céréale-légumineuse annuelles substitutives*

a) Effet sur le rendement total en grains de l'association

b) Effet sur la proportion de céréales dans le rendement total en grains de l'association

* Les densités de semis des deux espèces distinguent les associations en deux catégories : « substitutives » où la densité de semis de chaque espèce est raisonnée en pourcentage de la densité optimale en culture pure, de sorte que la somme de ces pourcentages soit égale à 100 (De Wit, 1960) ; « additives » où la densité totale dépasse les proportions optimales (Fukai et Trenbath, 1993)

Le fonctionnement dynamique des associations, en réponse à des disponibilités variables en azote minéral du sol, a principalement été étudié à partir d'apports d'azote effectués au semis. L'effet de la date de la fertilisation azotée a été peu abordé. Adu-Gyamfi et al. (1997) ont testé deux dates d'apport sur des associations sorgho-pois cajan. Ils montrent qu'en comparaison d'un apport au semis, un apport azoté réalisé 40 jours après semis affecte moins la croissance de la légumineuse dont la biomasse est proche de celle observée dans l'association non fertilisée.

Des questions demeurent quant à l'impact de différentes dates de fertilisation azotée sur la compétition interspécifique et le partage des ressources dans le cas d'associations pois-blé : quel est l'incidence d'une fertilisation azotée en fonction du stade phénologique des espèces au moment de l'apport et des conditions de croissance de chaque espèce ?

Par ailleurs, si l'effet inhibiteur des nitrates sur la fixation symbiotique du pois est bien connu et a été caractérisé au champ par une gamme d'apports d'azote au semis, il demeure de nombreuses questions sur l'impact d'une augmentation ponctuelle de nitrates en cours de cycle. Fujikake et al. (2002) et Fujikake et al. (2003) ont montré que la fixation symbiotique du soja était réversible après une courte exposition aux nitrates. Jensen (1986) a, quant à lui, étudié l'effet de différentes dates d'apport sur les performances à maturité du pois pur. Il a démontré que l'application d'une faible dose d'azote avant l'apparition des nodules augmentait la biomasse de pois (0.3 g N pot^{-1}), et qu'une forte dose à cette même date (2.4 g N pot^{-1}) diminuait fortement la contribution de l'azote fixé accumulé dans la biomasse de pois. De plus, il a mis en évidence que des apports plus tardifs diminuaient considérablement la contribution de l'azote fixé accumulé dans la biomasse de pois (taux de fixation de 34.2 à 54.4 % en fertilisant entre DF et DRG, contre 82.1 % en situation non fertilisée).
Cependant, il y a un manque de connaissances sur l'analyse dynamique sur pois de l'inhibition des nitrates, à la fois sur la structure et sur l'activité de l'appareil fixateur, et des conditions de réversibilité suite à une courte exposition de l'appareil fixateur aux nitrates à différents stades phénologiques et pour différents niveaux de disponibilité en carbone.

4. Fertilisation azotée et performances à la récolte des associations pois-blé

4.1. Une fertilisation azotée modifie avant tout la proportion d'espèces

De nombreuses études ont analysé l'impact d'une fertilisation azotée sur le rendement des associations céréale-légumineuse et sur la proportion de chaque espèce dans le rendement (Tableau 1). La majorité de ces études démontrent qu'une fertilisation azotée favorise la céréale au détriment de la légumineuse dans le rendement final. De plus, elles mettent en évidence qu'un apport azoté peut fortement augmenter le rendement total d'une association comportant du maïs ou du sorgho, mais qu'il a un plus faible impact sur le rendement d'une association comportant du blé ou de l'orge.

Des expérimentations sur des associations substitutives[†] pois-orge ou pois-blé démontrent que le rendement total de l'association n'est augmenté que de 60 g m^{-2} ± 5 avec un apport azoté (Jensen, 1996 ; Hauggaard-Nielsen et Jensen, 2001 ; Ghaley et al., 2005 ; Corre-Hellou et al., 2006), mais que la proportion de céréales dans le rendement total de l'association, en moyenne de 54% dans une association non fertilisée, augmente en moyenne à 77% ± 10 suite à une fertilisation azotée réalisée au semis (Figure 3). De plus, le taux de protéines des grains de blé

[†] Les densités de semis des deux espèces distinguent les associations en deux catégories : « substitutives » où la densité de semis de chaque espèce est raisonnée en pourcentage de la densité optimale en culture pure, de sorte que la somme de ces pourcentages soit égale à 100 (De Wit, 1960) ; « additives » où la densité totale dépasse les proportions optimales (Fukai et Trenbath, 1993).

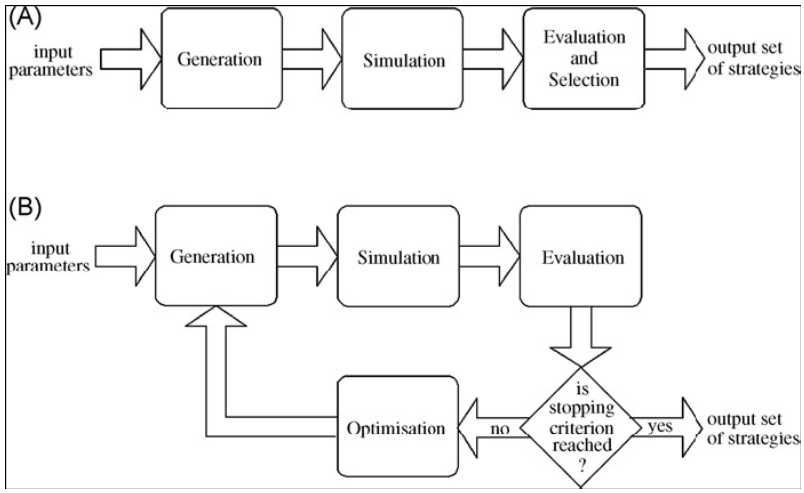

Figure 4 : Deux voies pour générer des stratégies : (A) avant le process de simulation ; (B) pendant le process de simulation en utilisant différentes méthodes d'optimisation pour améliorer la stratégie pas à pas (Bergez et al., 2009)

à la récolte n'est que très faiblement augmenté par des apports azotés au semis en comparaison d'une situation non fertilisée (gain de 0.06 points de teneur en protéines ± 0.39) (Jensen, 1996 ; Ghaley et al., 2005).

4.2. Un manque de références pour proposer des règles de décisions sur la conduite azotée des associations

A l'heure actuelle, des références font défaut pour permettre un réel pilotage du couvert associé. Les études présentées précédemment analysent l'impact d'une fertilisation azotée sur le rendement d'une association céréale-légumineuse dans le cas d'apports principalement réalisés au semis. Cependant, elles ne permettent pas de proposer des règles de décision sur la fertilisation azotée des associations. Par ailleurs, la prédiction et la satisfaction des besoins azotés des deux espèces doivent impérativement s'inscrire dans une approche dynamique. En effet, la proportion des espèces est variable au cours du cycle (Andersen et al., 2007) et dépend de la disponibilité en azote minéral du sol. Un raisonnement de la fertilisation azotée doit donc intégrer qu'un apport d'azote, visant à améliorer le statut azoté des espèces, modifie également la part des espèces, donc les rapports de compétition et, au final, les rapports de demande en azote. Enfin, le fonctionnement autour de deux voies de nutrition azotée à exploiter dans ce type d'association complexifie le raisonnement de la fertilisation d'autant plus que la contribution de la fixation symbiotique dépend de la disponibilité en azote minéral.

4.3. La modélisation : outil de synthèse des connaissances permettant de prolonger la démarche expérimentale vers la proposition de règles de décision

La complexité du fonctionnement dynamique d'un système associé et la diversité de ses débouchés potentiels rendent difficile l'approche de règles de décision sur la conduite azotée par le simple recours aux expérimentations. *A contrario*, l'approche par modélisation permet d'appréhender plus aisément les dynamiques de disponibilité en azote minéral et les dynamiques des besoins de chaque espèce ainsi que la variabilité climatique. Par ailleurs, le raisonnement de la conduite azotée doit être basé sur des combinaisons de facteurs tels que proportion des espèces au semis, date et dose de fertilisation et conditions initiales de la parcelle (reliquat d'azote minéral du sol au semis, potentiel de minéralisation, réserve hydrique affectant la disponibilité de l'azote minéral pour la culture). Il en résulte un très grand nombre de stratégies à tester dans des conditions pédoclimatiques variées, ce qui est difficile à envisager par le biais d'expérimentations de plein champ pour des raisons de coûts et de lourdeur des dispositifs expérimentaux à mettre en œuvre (Jeuffroy et al., 2008).
Enfin, l'approche par modélisation autorise une sélection *ex ante* des stratégies les plus pertinentes à tester expérimentalement et évite de tester, par l'expérimentation, des combinaisons de facteurs présentant *a priori* peu d'intérêt (Bergez et al., 2009) (Figure 4).

5. Proposition d'hypothèses

Compte tenu de l'état des lieux des connaissances évoqué précédemment et des objectifs de thèse, nous proposons de tester les hypothèses suivantes :

<u>Hypothèse 1</u> : Date de fertilisation et dynamiques de croissance

La fertilisation azotée, et notamment la date de l'apport, est un levier efficace pour influencer la dynamique de croissance de chaque espèce dans l'association et modifier ainsi la part des espèces dans le couvert plurispécifique.

Il a été largement démontré que les céréales sont plus compétitives que les légumineuses pour l'acquisition de l'azote minéral du sol au début de la croissance des espèces (Jensen, 1996 ; Andersen et al., 2005 ; Corre-Hellou et al., 2006). Une fertilisation azotée intervenant avant le démarrage de l'accélération de la croissance du blé (*ie* avant ou au moment du stade épi 1 cm) devrait favoriser la croissance de la céréale au détriment de celle de la légumineuse et ainsi modifier la part des espèces au profit de la céréale. Une fertilisation azotée réalisée plus tard dans le cycle (en fin de montaison du blé), devrait moins pénaliser la croissance du pois en lui permettant d'installer ses capteurs racinaires et aériens ainsi que son appareil fixateur sans trop souffrir de la compétition exercée par la céréale.

<u>Hypothèse 2</u> : Partage des ressources

La date de l'apport azoté modifie davantage le statut azoté de la céréale que celui de la légumineuse et donc la compétition pour la lumière entre espèces. De plus, la date de l'apport modifie le partage de l'azote du sol entre les deux espèces en fonction de la demande de chaque espèce.

Tester la date d'apport azoté revient avant tout à tester l'effet des croissances de chaque espèce au moment de l'apport en lien avec leurs phénologies et leurs conditions de croissance respectives qui influencent la réponse à la fertilisation azotée : effet sur le partage de l'azote minéral et sur la réponse de la fixation à la fertilisation.

<u>Hypothèse 3</u> : Inhibition et réversibilité de la fixation symbiotique du pois

L'effet inhibiteur sur la structure et la fonction de l'appareil fixateur du pois est différent selon la date d'exposition aux nitrates. De plus, la fixation symbiotique du pois est réversible après une phase d'inhibition induite par un apport d'azote minéral au cours du cycle. Ce potentiel de réversibilité est fonction :

- du stade du pois au moment de l'exposition ;
- de la disponibilité en photosynthétats.

L'application d'un engrais azoté à différentes dates sur des associations pois-blé induit une perturbation de la fixation symbiotique du pois par un pic ponctuel de nitrates disponibles à différents stades phénologiques du pois. Or, si on connaît bien le caractère inhibiteur des nitrates, il a principalement été étudié dans les premiers stades de développement de la plante. Aussi, il est constaté un manque de connaissance sur l'effet inhibiteur : son impact sur la structure (biomasse et nombre des nodosités) et la fonction (activité de fixation) de l'appareil fixateur des légumineuses, à différents stades d'exposition, est mal connu. De même, peu de résultats disponibles éclairent le potentiel de réversibilité de la fixation symbiotique une fois les nitrates revenus sous le seuil d'inhibition.

<u>Hypothèse 4</u> : Modélisation du fonctionnement dynamique des associations pois-blé d'hiver

Il est possible de modéliser le fonctionnement dynamique d'une association à partir (1) du couplage de modèles de cultures pures, (2) des paramètres des modèles représentant le fonctionnement des espèces pures et (3) de formalismes simples de partage des ressources :

- partage du rayonnement au prorata du kLAI de chaque espèce ;
- partage de l'azote en fonction de la demande de chaque espèce si l'offre est supérieure à la demande et en fonction de l'accès à la ressource (profondeur d'enracinement) si l'offre est limitante ;
- une interaction étroite entre partage de la lumière et des ressources azotées.

<u>Hypothèse 5</u> : Proposition vers des règles de décision de la fertilisation azotée d'associations pois-blé d'hiver adaptées aux différents objectifs de production

Une conduite azotée adaptée (en terme de densité de semis date et dose d'apport) permet d'orienter les caractéristiques de récolte (part d'espèces – qualité protéique) vers les exigences d'une finalité de production donnée.

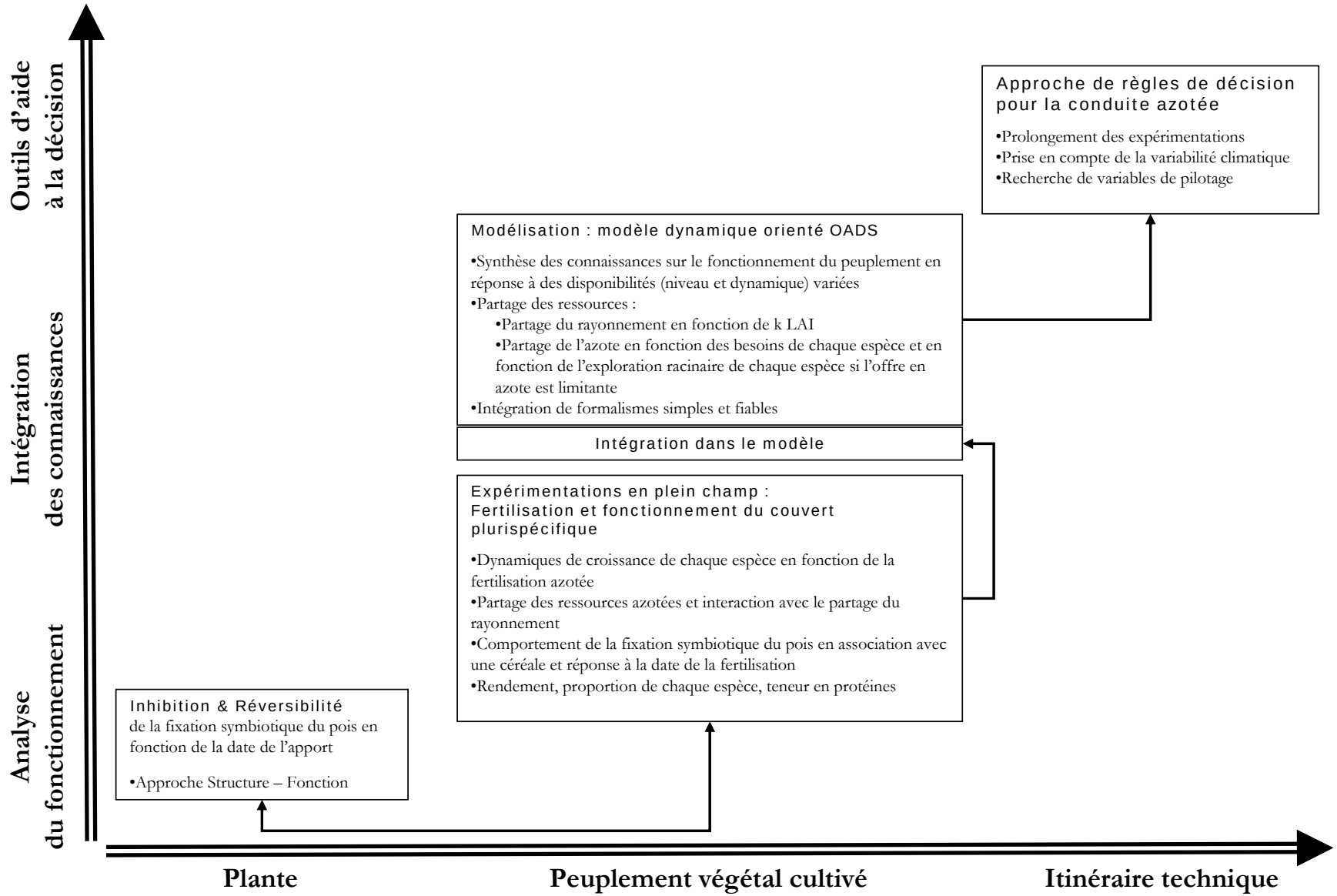

Figure 5 : Schématisation de la démarche générale adoptée
OADS : Outil d'Aide à la Décision Stratégique ; kLAI : coefficient d'extinction * Leaf Area Index

6. Présentation de la démarche

La méthodologie générale adoptée pour réaliser cette thèse s'articule autour de deux approches : une approche par **expérimentations** et une approche par **modélisation**. Elles s'articulent autour d'études à l'échelle du peuplement végétal cultivé, mais aussi à l'échelle de la plante et du système de culture (Figure 5). Les résultats escomptés se situent à l'interface entre questions de recherche (approfondissements de mécanismes) et utilisation des connaissances pour concevoir des systèmes de culture innovants répondant aux problématiques de la profession agricole.
Les **expérimentations** ont eu pour objectifs de tester l'effet de modalités variées de fertilisation azotée sur les performances finales et sur le fonctionnement de l'association.
La **modélisation** a eu pour objectif d'obtenir un outil de synthèse des connaissances sur le fonctionnement de ce type d'association et permettant de quantifier les conséquences de plusieurs stratégies de fertilisation azotée, tout en prenant en considération la variabilité interannuelle du climat.

6.1. Expérimentations

Des expérimentations de plein champ sur deux sites expérimentaux (La Jaillière en Loire-Atlantique et Grignon dans les Yvelines - France) ont eu pour objectif de tester la réponse des associations substitutives pois-blé d'hiver à différentes dates de fertilisation azotée en terme de :

- dynamiques de croissance ;
- partage des ressources ;
- comportement de la fixation symbiotique face à des variations dans le temps de la disponibilité en azote minéral du sol ;
- performances de récoltes telles que niveau de rendement, proportion d'espèces dans le rendement, taux de protéines des grains.

Les détails de ces expérimentations sont exposés dans le Chapitre 2.

Des expérimentations en serre sur pois pur ont été menées afin d'étudier les conditions de réversibilité de la fixation symbiotique après une courte exposition aux nitrates. Les détails de cette expérimentation sont donnés dans le Chapitre 3.

6.2. Modélisation

Des modèles existants mais aux formalismes peu compatibles avec une démarche de développement d'un Outil d'Aide à la Décision Stratégique (OADS)

Les modèles existants adaptés aux associations céréale-légumineuse sont, pour la majorité, construits à partir de modèles initialement développés pour des cultures pures (Corre-Hellou, 2005 ; Caldwell, 1995), et fonctionnant majoritairement avec un pas de temps journalier. Ils ont été adaptés aux associations par ajouts de formalismes pour le partage des ressources.

Ces modèles sont souvent très focalisés sur le partage du rayonnement avec des formalismes parfois très complexes. INTERCOM (Kropff et Laar, 1993) simule la photosynthèse à l'échelle de la feuille trois fois dans la journée. Ecosys (Grant, 1996) calcule des flux de masse et d'énergie à chaque minute. FASSET (Berntsen et al., 2004), NTRM (Ball et Shaffer, 1993), INTERCOM et Ecosys calculent le rayonnement intercepté par le couvert découpé en strates.

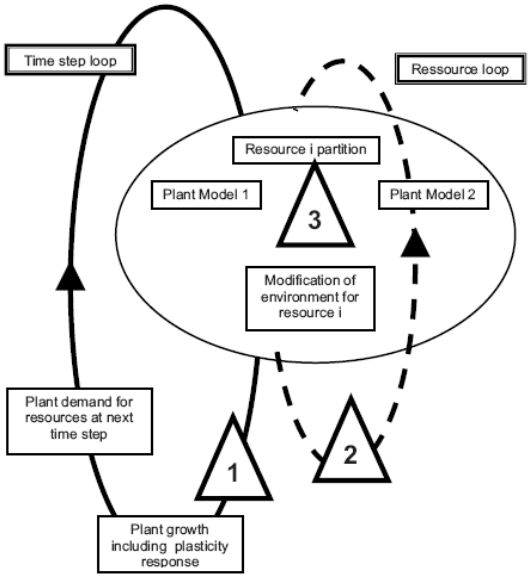

Figure 6 : Modèles dynamiques d'associations de culture : trois points clés pour le partage des ressources : 1) inclure une plasticité adéquate des mécanismes des modèles de fonctionnement des plantes ; 2) résoudre simultanément le partage des différentes ressources ; 3) coupler des modèles de fonctionnement de plantes généralement construits à partir de concepts différents. (Malézieux et al., 2008)

Dans l'adaptation de STICS aux associations pois-orge (Corre-Hellou et al., 2009), des formalismes exigeant une description détaillée de la structure du couvert sont nécessaires (*eg* forme du couvert, zones ombrées et éclairées du couvert, *etc.*). De même, dans ces modèles, le sol est souvent découpé en de très nombreuses couches (Brisson et al., 2004). De tels formalismes nécessitent l'estimation de nombreux paramètres incluant des étapes de calibration inéluctables et parfois complexes.

Plusieurs modèles intègrent aussi le partage de l'azote mais sans lien très étroit entre le partage de la lumière et de l'azote, bien que de nombreuses études démontrent les relations étroites entre les dynamiques de surface foliaire et la satisfaction des besoins azotés (Corre-Hellou et al., 2006 ; Lemaire et al., 2007 ; Lemaire et al., 2008a ; Lemaire et al., 2008b).

Une association étant un système déjà complexe à modéliser, des formalismes trop complexes peuvent au final fortement amputer l'opérationnalité du modèle voulu. Corre-Hellou et al. (2009) conseillent d'ailleurs de recourir à des formalismes simples de partage du rayonnement si l'objectif est le développement d'un modèle orienté vers l'aide à la décision. Par ailleurs, Malézieux et al. (2008) mettent en garde contre les difficultés de couplage de modèles de simulation de cultures pures intégrant des constructions et des concepts différents (Figure 6).

Choix des modèles

Compte tenu des remarques précédentes et des objectifs de notre étude (démarche de modélisation finalisée vers l'aide à la définition de règles de décision), le choix des modèles s'est orienté vers la sélection de deux modèles de cultures pures : Azodyn pour le blé (Jeuffroy et Recous, 1999) et Afisol pour le pois (Vocanson, 2006 ; Biarnès et al., 2009). Ces deux modèles présentent de nombreux avantages par rapport aux objectifs finaux de ce travail :

- ce sont deux modèles de même structure, construits à partir de formalismes et de concepts très proches ;
- ils sont peu exigeants en terme de paramètres d'entrée, ce qui est un avantage pour une orientation OADS ;
- ils ont été développés dans l'objectif de bien simuler la réponse du rendement à des dynamiques de disponibilité en azote minéral, notamment par un lien étroit entre surface foliaire et demande en azote ;
- ils simulent la dynamique du développement racinaire et son fonctionnement de manière simple, nécessitant peu de paramètres.
- Afisol permet la simulation d'un processus clé dans le fonctionnement de l'association céréale-légumineuse : la fixation symbiotique et sa réponse à la disponibilité en nitrates dans le sol

Les connaissances acquises sur le fonctionnement des associations pois-blé ont permis la réalisation d'un module de couplage associant ces deux modèles de cultures pures (Azodyn pour le blé et Afisol pour le pois) afin d'obtenir un nouveau modèle (Azodyn-IC) capable de simuler le fonctionnement d'une association pois-blé et sa réponse à différents régimes de fertilisation azotée. Les paramètres existants pour simuler le fonctionnement des deux espèces en culture pures ont été utilisés. Ce travail de modélisation n'a donc pas nécessité de paramétrage supplémentaire et les données des expérimentations de plein champ ont pu être pleinement exploitées pour l'évaluation du modèle. En dernier lieu, Azodyn-IC a été utilisé sur 26 années climatiques afin d'ébaucher des règles de décision pour la fertilisation azotée combinée au choix de la proportion des espèces au semis dans des associations pois-blé répondant à des objectifs de production contrastés.
Cette étude (développement, validation et utilisation du modèle) est présentée dans le Chapitre 4.

Les résultats s'articulent autour de trois chapitres :

- le Chapitre 2 aborde le fonctionnement du peuplement associé de blé et de pois et de blé sous différentes dates de fertilisation azotées ;
- le Chapitre 3 explore l'inhibition puis la réversibilité de la fixation symbiotique du pois soumis à de courtes expositions aux nitrates, en fonction du stade du pois et de la disponibilité en carbone ;
- le Chapitre 4 présente le modèle Azodyn-IC développé pour la simulation des associations pois-blé, son évaluation et son utilisation en vue d'élaborer des règles de décision sur la conduite azotée des associations pois-blé.

Enfin, une discussion générale est l'occasion d'une synthèse des résultats et d'une présentation des perspectives.

Références bibliographiques

Adu-Gyamfi, J., Ito, O., Yoneyama, T., Devi, G., Katayama, K., 1997. Timing of N fertilization on N2 fixation, N recovery and soil profile nitrate dynamics on sorghum/pigeonpea intercrops on Alfisols on the semi-arid tropics. Nutr. Cycl. Agroecosys. 48, 197-208.

Altieri, M.A., 1993. Ethnoscience and biodiversity: key elements in the design of sustainable pest management systems for small farmers in developing countries. Agr. Ecosyst. Environ. 46, 257-272.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agr. Ecosyst. Environ. 74, 19-31.

Andersen, M.K., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266, 273-287.

Andersen, M.K., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2007. Competition for and utilisation of sulfur in sole and intercrops of pea and barley. Nutr. Cycl. Agroecosys. 77, 143-153.

Ballot, R., 2009. Prise en compte dans les indicateurs de rentabilité de facteurs agronomiques intervenant entre cultures se succédant. Master Thesis, Groupe ESA, Angers France, 166pp.

Ball, D.A., Shaffer, M.J., 1993. Simulating resource competition in multispecies agricultural plant communities. Weed Res. 33, 299-310.

Baker CM, Blamey FPC. 1985. Nitrogen fertilizer effects on yield and nitrogen uptake of sorghum and soybean, grown in sole cropping and intercropping systems. Field Crops Rese.12, 233-240.

Bedoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil, in press, doi: 10.1007/s11104-009-0082-2.

Bellostas, N., Jensen, E.S., 2004. Intercropping of cereals and grain legumes in European organic farming systems - INTERCROP. Grain legumes, pp. 14-15.

Bergez, J.E., Colbach, N., Crespo, O., Garcia, F., Jeuffroy, M.H., Justes, E., Loyce, C., Munier-Jolain, N., Sadok, W., 2009. Designing crop management systems by simulation. European Journal of Agronomy, in press, doi:10.1016/j.eja.2009.06.001.

Berntsen, J., Hauggaard-Nielsen, H., Olesen, J.E., Petersen, B.M., Jensen, E.S., Thomsen, A., 2004. Modelling dry matter production and resource use in intercrops of pea and barley. Field Crop Res. 88, 69-83.

Biarnès, V., Lecoeur, J., Jeuffroy, M.-H., Vocanson, A., Ney, B., 2009. A model which 14 integrates knowledge on pea crop physiology and agronomic diagnosis. In: Physiology of the pea crop. Science Publishers, Enfield, USA, pp 197-208. in press.

Bilalis DJ, Sidiras N, Kakampouki I, Efthimiadou A, Papatheohari Y, Thomopoulos P. 2005. Effects of organic fertilization on maize-legume intercrop in a clay loam soil and Mediterranean climate - Can the Land Equivalent Ratio (LER) index be used for root development? J. Food Agric. Environ. 3, 117-123.

Brisson, N., Bussière, F., Ozier-Lafontaine, H., Tournebize, R., Sinoquet, H., 2004. Adaptation of the crop model STICS to intercropping. Theorical basis and parametrisation. Agronomie 24, 409-421.

Caldwell R M 1995 Simulation models for intercropping systems. In Ecophysiology of tropical intercropping. Eds. H Sinoquet and P Cruz. pp. 353-368. INRA, Paris.

Chauvel, B., Guillemin, J.P., Colbach, N., Gasquez, J., 2001. Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (Alopecurus myosuroides Huds.). Crop Prot. 20, 127-137.

Colbach, N., Maurin, N., Huet, P., 1996. Influence of cropping system on foot rot of winter wheat in France. Crop Prot. 15, 295-305.

Corre-Hellou, G., 2007. Using a modelling approach to study interactions between species in intercropping systems. 6th European Conference on Grain Legumes, Lisbon, Portugal.

Corre-Hellou, G., Faure, M., Launay, M., Brisson, N., Crozat, Y., 2009. Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea-barley intercrops. Field Crop Res., 1-10.

Corre-Hellou, G., 2005. Acquisition de l'azote dans des asociations pois-orge (Pisum sativum L.-Hordeum vulgare L.) en relation avec le fonctionnement du peuplement. Thèse de doctorat, Université d'Angers, Angers, France.

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J.l., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crops Res. 103, 76--85.

Corre-Hellou, G., Crozat, Y., 2004. Interspecific competition for soil N in pea-barley mixtures during the vegetative phase and consequences of N2 fixation. 5th Conference on Grain Legumes, Dijon, France, pp. 65-66.

Corre-Hellou, G., Crozat, Y., 2005. Assessment of Root System Dynamics of Species Grown in Mixtures under Field Conditions using Herbicide Injection and 15N Natural Abundance Methods: A Case Study with Pea, Barley and Mustard. Plant Soil 276, 177-192.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for Soil N and its interaction with N2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208.

Darwin, C., 1859.On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life . Eds John Murray, London, UK.

De Wit, C.T., 1960. On competition. Versl Landbouwk Onderzoek 66, 1-81.

Doré, T., Le Bail, M., Roger-Estrade, J., Martin, P., Jeuffroy, M.H., 2006. L'agronomie aujourd'hui. Quae Editions, Versailles, France

Finch, S., Collier, R.H., 2000. Host-plant selection by insects – a theory based on "appropriate/inappropriate landings" by pest insects of cruciferous plants. Entomol. Exp. Appli. 96, 91-102.

Fujikake, H., Yamazaki, A., Ohtake, N., Sueyoshi, K., Matsuhashi, S., Ito, T., Mizuniwa, C., Kume, T., Hashimoto, S., Ishioka, N.-S., Watanabe, S., Osa, A., Sekine, T., Uchida, H., Tsuji, A., Ohyama, T., 2003. Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. J. Exp. Bot. 54, 1379-1388.

Fujikake, H., Yashima, H., Sato, T., Ohtake, N., Sueyoshi, K., 2002. Rapid and reversible nitrate inhibition of nodule growth and N2 fixation activity in soybean (Glycine max (L.) Merr.). Soil Sci. Plant Nutr. 48, 211-217.

Fujita, K., Ofosu-Budu, K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant Soil 141, 155-175.

Gate, P., 1995. Ecophysiologie du blé: de la plante à la culture. Lavoisier Tec&Doc.

Ghaley, B.B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2005. Intercropping of wheat and pea as influenced by nitrogen fertilization. Nutr. Cycl. Agroecosys. 73, 201-212.

Grant RF, 1992 Simulation of competition among plant populations under different managements and climates. Agron. Abstr., American Society of Agronomy, Madison, Wisconsin, USA.

Greenwood, D., Gerwitz, A., Stone, D., Barnes, A., 1982. Root development of vegetable crops. Plant Soil 68, 75-96.

Gregory P J, McGowan M and Biscoe P V 1978 Water relations of winter wheat. 2. Soil water relations. J. Agr. Sci. 91, 103-116.

Griffon, M., 1999. Développement durable et agriculture: la révolution doublement verte. Cah. Agric. 8, 259-267.

Griffon, M., 2006. Nourrir la planète. (Eds) Odile Jacob, Paris, France.
Hamblin, A.P., Hamblin, J., 1985. Root characteristics of some temperate legume species and varieties on deep, free-draining entisols. Aust. J. Agr. Res. 36, 63-72.
Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001a. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res. 70, 101-109.
Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001b. Reintroducing grain legume-cereal intercropping for increased protein production in European cropping systems. 4th European conference on grain legumes. AEP, Cracow, Poland, pp. 52-53.
Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutr. Cycl. Agroecosys. 65, 289-300.
Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Res., 113, 64-71.
Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.
Hauggaard-Nielsen, H., Jornsgaard, B., Kinane, J., Jensen, E., 2008. Grain-legume-cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agr. Food Syst. 23, 3-12.
Izaurralde RC, Juma NG, McGill WB. 1990. Plant and Nitrogen Yield of Barley-Field Pea Intercrop in Cryoboreal-Subhumid Central Alberta. Agron. J. 82, 295-301.
Jensen, E.S., 1986. The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (Pisum sativum L.). Fert. Res. 10, 193-202.
Jensen, E.S., 1987. Seasonal patterns of growth and nitrogen fixation in field-grown pea. Plant Soil 101, 29-37.
Jensen, E.S., 1996. Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil 182, 25-38.
Jeuffroy, M.-H., Recous, S., 1999. Azodyn: a simple model simulatinng the date of nitrogen deficiency for decision suppoort in wheat fertilization. Eur. J. Agron. 10, 129-144.
Jeuffroy, M.H., Bergez, J.-E., David, C., Flénet, F., Gate, P., Loyce, C., Maupas, F., Meynard, J.-M., Reau, R., Surleau-Chambenoit, C., 2008. Utilisation des modèles pour l'aide à la conception at à l'évaluation d'innovations techniques en production végétales: bilan et perspectives. In: Reau, R., Doré, T. (Eds.), Des systèmes de culture innovants et durables: quelles méthodes pour les mettre au point et les évaluer? Educagri, Dijon, pp. 11-27.
Karimi, M.M., Siddique, K.H.M., 1991. Crop growth and relative growth rates of old and modern wheat cultivars. Aust. J. Agr. Res. 42, 13-20.
Kinane, J., Lyngkjaer, M.F., 2002. Effect of barley-legume intercrop on disease frequency in an organic farming system. In: Efpp, t.C. (Ed.), Plant Prot. Sci. Plant Prot. Sci., Prague, Czech Republic, pp. 227-231.
Kropff, M. J. and H. H. van Laar. 1993. Modelling crop-weed interactions. Wallingford, Great Britain: CAB International and International Rice Research Institute. 274 p
Lemaire, G., Jeuffroy, M.H., Gastal, F., 2008a. Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. Eur. J. Agron., 28, 61-624.
Lemaire, G., Oosterom, E.v., Sheery, J., Jeuffroy, M.-H., Massignam, A., Rossato, L., 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? Field Crop Res. 100, 91-106.
Lemaire, G., van Oosterom, E., Jeuffroy, M.-H., Gastal, F.o., Massignam, A., 2008b. Crop species present different qualitative types of response to N deficiency during their vegetative growth. Field Crop Res., 105, 253-265.

Li, Y.-Y., Yu, C.-B., Cheng, X., Li, C.-J., Sun, J.-H., Zhang, F.-S., Lambers, H., Li, L., 2009. Intercropping alleviates the inhibitory effect of N fertilization on nodulation and symbiotic N_2 fixation of faba bean. Plant and Soil 323, 295-308.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., Tourdonnet, S.d., Valantin-Morison, M., 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agron. Sustain. Dev. 29, 43-62.

Meynard, J.M., 2008. Produire autrement: réinventer les systèmes de culture. In: Reau, R., Doré, T. (Eds.), Des systèmes de culture innovants et durables: quelles méthodes pour les mettre au point et les évaluer? Educagri, Dijon, pp. 11-27.

Morgado LB, Willey RW. 2003. Effects of plant population and nitrogen fertilizer on yield and efficiency of maize-bean intercropping. Pesqui. Agropecu. Bras. 39, 1257-1264.

Munier-Jolain, N., Biarnès, V., Chaillet, I., Lecoeur, J., Jeuffroy, M.-H., Carrouée, B., Crozat, Y., Guilioni, L., Lejeune, I., Tivoli, B., 2005. Agrophysiologie du pois protéagineux. Lavoisier, Paris.

Nemecek, T., von Richthofen, J.-S., Dubois, G., Casta, P., Charles, R., Pahl, H., 2008. Environmental impacts of introducing grain legumes into European crop rotations. Eur. J. Agron. 28, 380-393.

Ofori F, Stern WR. 1986. Maize/cowpea intercrop system: Effect of nitrogen fertilizer on productivity and efficiency. Field Crops Res. 14, 247-261.

Ofori, F., Stern, W.R., 1987. Cereal-legume intercropping systems. Adv. Agron. 41, 41-90.

Paillard, C., 1991. Incidence de l'état structural du sol sur le développement et la croissance racinaire du blé tendre d'hiver. Répercussion sur l'alimentation hydrique et minérale. ENSAIA, Nancy, p. 32.

Parmentier, B., 2007. Nourrir l'humanité, les grands problèmes de l'agriculture mondiale. (Eds) Éditions La Découverte, Paris, France.

Pline l'Ancien (23-79). Historiae Naturalis (Traduction E. Littré). Eds Dubochet et al., Paris, 1850.

Rao MR, Rego TJ, Willey RW. 1987. Response of cereals to nitrogen in sole cropping and intercropping with different legumes. Plant Soil 101, 167-177.

Rerkasem K, Rerkasem B. 1988. Yields and nitrogen nutrition of intercropped maize and ricebean (Vigna umbellata [Thumb.] Ohwi and Ohashi). Plant Soil 108, 151-162.

Sampaio EVSB, Tiessen H, Antonino ACD, Salcedo IH. 2004. Residual N and P fertilizer effect and fertilizer recovery on intercropped and sole-cropped corn and bean in semiarid northeast Brazil. Nutr. Cycl. Agroecosys.70, 1-11.

Schoonhoven, L.M., van Loon, J.J.A., ,Marcel Dicke, M., 2005. Insect-Plant Biology, Eds Oxford Biology, Oxford United kingdom, 421pp.

Siame J, Willey RW, Morse S. 1998. The response of maize/Phaseolus intercropping to applied nitrogen on Oxisols in northern Zambia. Field Crops Res. 55, 73-81.

Silsbury, J.H., 1990. Grain yield of wheat in rotation with pea, vetch or medic grown with three systems of management. Aust. J. Exp. Agr. 30, 645-649.

Stevenson, F.C., van Kessel, C., 1996. A Landscape-Scale Assessment of the Nitrogen and Non-Nitrogen Rotation Benefits of Pea. Soil Sci Soc Am J 60, 1797-1805.

Streeter, J.G., 1985a. Nitrate inhibition of legume nodule growth and activity. I. Long term studies with a continuous supply of nitrate. Plant Physiol. 77, 321-324.

Streeter, J.G., 1985b. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Plant Physiol. 77, 325-328.

Thorup-Kristensen, K., 1998. Root Growth of Green Pea (Pisum sativum L.) Genotypes. Crop Sci 38, 1445-1451.

Tobita S, Ito O, Matsunaga R, Rao TP, Rego TJ, Johansen C, Yoneyama T. 1994. Field evaluation of nitrogen fixation and use of nitrogen fertilizer by sorghum/pigeonpea intercropping on an Alfisol in the Indian semi-arid tropics. Biol. Fert. Soils 17, 241-248.

Trenbath, B.R., 1976. Plant interactions in mixed crop communities. Multiple cropping. Pappendick et al., American Society of Agronomy (ed.), Madison, Wisconsin, pp. 129-169.

Tricot 1993. Mise en place des nodosités du pois protéagineux de printemps (*Pisum sativum* L.). Influence de la nutrition carbonée. Thèse de doctorat, université Paris-Sud, Orsay, 78pp.

Tsubo, M., Walker, S., Mukhala, E., 2001. Comparisons of radiation use efficiency of mono/inter-cropping systems with different row orientations. Field Crop Res. 71, 17-29.

UNIP, 2008. Cultiver des associations céréales-protéagineux : des intérêts agronomiques, économiques et environnementaux à découvrir. Rapport technique final. Appel à Projets Casdar 2005, n°431. 112 pages.

Vandermeer, J., 1989. The ecology of intercropping. Cambridge University Press, Cambridge, UK.

Vocanson A., 2006. Evaluation ex ante d'innovations variétales en pois d'hiver (*Pisum sativum* L.) : approche par modélisation au niveau de la parcelle et de l'exploitation agricole. Thèse de Doctorat, INAPG, Paris, 250p. +annexes.

Voisin, A.-S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002a. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (Pisum sativum L.). Plant and Soil 242, 251-262.

Voisin, A.-S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002b. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (Pisum sativum L.). Plant Soil 243, 31-42.

Voisin, A.S., Salon, C., Jeudy, C., Warembourg, F.R., 2003. Symbiotic N2 fixation activity in relation to C economy of Pisum sativum L. as a function of plant phenology. J. Exp. Bot. 54, 2733-2744.

Willey, R.W., 1979. Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. Field Crops Abstr. 32, 1-10.

Chapitre 2 :

Effet de la date de la fertilisation azotée sur le fonctionnement des associations pois-blé d'hiver

Effet de la date de la fertilisation azotée sur le fonctionnement des associations pois-blé d'hiver.

Ce chapitre vise à étudier l'impact de la date d'une fertilisation azotée sur les dynamiques de croissance des deux espèces et sur leurs performances à la récolte en terme de rendement en grains et de proportion des espèces dans le rendement (article ci-dessous Partie I)

Ce chapitre vise aussi à étudier l'impact de la date d'apport sur les dynamiques d'acquisition de l'azote des deux espèces et sur le partage au cours du cycle de l'azote minéral (somme de l'azote issu du sol et de l'engrais) (article ci-dessous Partie II). Dans cet article, il est également étudié l'inhibition de la fixation symbiotique du pois associé lors d'un apport au cours du cycle ainsi que la réversibilité de la fixation en fonction du stade du pois au moment de l'apport.

Cette étude a été réalisée à partir de deux années d'expérimentations de plein champ en Loire-Atlantique (Station expérimentale ARVALIS de La Jaillière) et d'une année d'expérimentation en région parisienne (INRA de Grignon). Les associations pois-blé étudiées sont des associations substitutives où chaque espèce est semée à 50% de sa densité pratiquée en culture pure. Les espèces sont semées et récoltées à la même date. Elles sont mélangées sur le même rang. Ces associations sont conduites sans fertilisation azotée ou avec un apport d'azote sous forme d'ammonitrate apporté à différentes dates. Par ailleurs, les modalités testées sur chacune des expérimentations ainsi que la variabilité des conditions pédoclimatiques entre sites et années ont créé une variabilité de dynamiques en N minéral ainsi qu'une variabilité du stade phénologique et de la vitesse de croissance de chaque espèce au moment de l'apport.

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TIMING EFFECT OF NITROGEN FERTILIZATION ON WINTER PEA-WHEAT INTERCROPS.

I- GROWTH DYNAMICS AND GRAIN YIELD

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Abstract

Cereal-legume intercrops are a promising way to combine high productivity and several ecological benefits in temperate agrosystems. However the proportion of each species in the mixture at harvest is highly variable, particularly in response to fertilizer application at sowing. The aim of this study was to test whether N fertilization and its timing are an effective way of influencing the dynamic interactions between species during crop growth and how they affect the percentage of each species at harvest. The effects of timing of nitrogen fertilization on crop growth and grain yield were assessed in winter pea-wheat (*Pisum sativum* L.-*Triticum aestivum* L.) intercrops in 2007 and 2008 at two locations in France. Whatever the stage of application, N fertilizer tended to increase wheat growth and decrease pea growth. N fertilization (applied once at different dates from tillering to the end of stem elongation) delayed the decrease in the contribution of wheat to total biomass and maintained the competitive ability of wheat over pea for longer than in unfertilized intercrops. However, the effect of N fertilization was mainly observed just after N application and differences in percentage of wheat in grain yield resulting from N fertilization regimes were lower at harvest than observed on above-ground biomass during crop growth. N fertilization should be combined with other factors such as the proportion of each species at sowing or the choice of cultivar in order to manage cereal-legume intercrops for different specific targets.

Keywords: intercropping; N fertilization; pea; wheat

§ In tribute to Yves Crozat who had taken part in the first planning of this research project before deceased on June 20th, 2007.

1. Introduction

During recent decades it has become obvious that the design of cropping systems has to take into account the environmental impacts of agricultural practices (Altieri, 1989) in order to limit the use of non-renewable natural resources and chemical inputs and to improve their efficiency (Tilman et al. 2002). Concerning nitrogen, cereal-legume intercropping is known to be an interesting way to improve the nitrogen efficiency of agrosystems (Hauggaard-Nielsen, 2001) and limit losses through the environment.

Intercropping is the simultaneous growing of two or more species in the same field, with variations in the species used, the densities of each species and their spatial arrangements (Andrews and Kassams, 1976; Ofori and Stern, 1987; Willey, 1979). Apart from its frequent use in forage pastures, this practice is not very widespread in temperate agrosystems. However cereal-legume intercrops are gaining interest in Europe due to the increasing awareness of environmental damage arising from the intensive use of fertilizers and pesticides and the increasing cost of these inputs (Anil et al., 1998; Bellostas and Jensen, 2004; Hauggaard-Nielsen and Andersen, 2000). Intercrops are mainly widespread in organic farming but may have interesting potential uses in conventional farming systems, namely for the development of multi-use crops grown with low inputs.

This practice is known to increase yields and yield stability and grain N concentration of the cereal, and to decrease weed pressure and N leaching. These advantages are assumed to be mainly linked to the complementary use, in time and space, of N sources by the different components of the intercrop (De Wit, 1960; Jensen, 1996; Trenbath, 1976). Thus intercrops can contribute to the development of cropping systems which combine high productivity and several ecological benefits. Intercrops may be used for silage by producing a high biomass, rich in protein with low inputs (water, nitrogen, pesticides). They may also be harvested at maturity in order to obtain high-protein cereal grain with a low N input, or else grown to produce leguminous grain crops without the problems observed in sole crops: weeds, diseases, lodging and risk of leaching. Therefore farmers may have different targets for the final composition of their mixture. However the percentage of each species in the mixture at harvest is usually highly variable and very difficult to predict, creating problems for exploiting the method. Indeed intercrops are complex dynamic systems involving interspecific interactions throughout crop growth. The species interactions and relative dominance of each species may vary over time and according to environmental conditions (Andersen et al., 2007). Several studies have demonstrated the key role of N acquisition by intercrops in the proportion of species at harvest (Corre-Hellou et al., 2006; Jensen, 1996): few have attempted to define a rule-based management system for N fertilization.

Previous studies have questioned the effect of N applied at sowing on the behaviour of cereal-legume intercrops (Ghaley et al., 2005; Fan et al., 2006). Soil and fertilizer N is mainly used by the cereal to satisfy its N demand, expand its leaf area and thus increase its competitive ability for light. Therefore N increases cereal growth and decreases legume growth (Corre-Hellou et al., 2006). Thus several studies have demonstrated that N fertilization only slightly increases, if at all, the total grain yield of intercrops, but significantly decreases the contribution of the legume in the final yield (Corre-Hellou et al., 2006; Ghaley et al., 2005; Hauggaard-Nielsen and Jensen, 2001; Jensen, 1996; Leitch and Musa, 1998; Waterer et al., 1994). Indeed, experiments on substitutive pea-barley or pea-wheat spring intercrops have shown that the total grain yield of N-fertilized intercrops was only increased by about 60 g m^{-2} ± 5 (means ± standard deviation calculated from means of pooled data previously published) (Corre-Hellou et al., 2006; Ghaley et al., 2005; Hauggaard-Nielsen and Jensen, 2001; Jensen, 1996). In the same experiments, the contribution of cereal grain to the total yield of intercrops was increased from

Table 1: Date of sowing and growth stages of sole crops and intercrops

Reference of experiment	Location	Year of harvest	Crop design	Species	Sowing	Wheat ear inflorescence at 1 cm above tillering node (ZGS 30)	Wheat flowering (ZGS 65) and Beginning of pea flowering	Harvest
A	La Jaillière	2007	SC / IC	Pea Wheat	31-Oct-2006	--- 3-Mar-2007 (1038cdd)	8-Apr-2007 (1364cdd) 11-May-2007 (1889cdd)	All crops (except Pea SC:): 29-Jun-2007 (2724cdd) Pea SC: 13-Jun-2007 (2449cdd)
B	La Jaillière	2008	SC / IC	Pea Wheat	26-Oct-2007	--- 5-Mar-2008 (1001cdd)	30-Apr-2008 (1524cdd) 15-May-2008 (1781cdd)	All crops (except Pea SC:): 04-Jul-2008 (2629cdd) Pea SC: 01-Jul-2008 (2578cdd)
C	Grignon	2008	SC / IC	Pea Wheat	15-Nov-2007	--- 27-Mar-2008 (802cdd)	13-May-2008 (1323cdd) 21-May-2008 (1439cdd)	All crops (except Pea SC:): 15-Jul-2007 (2376cdd) Pea SC: 01-Jul-2008 (2128cdd)

Growth stages are described with Zadoks growth stage scale (ZGS); SC: Sole crops; IC: Intercrops; cdd: cumulated degree-days from sowing.

Table 2: Climatic data on each experiment: mean temperature, cumulated rainfall and cumulated global radiation are calculated for each experiment from sowing to harvest of intercrops

Reference of experiment	Location	Year of harvest	Mean Temperature (°C)	Cumulated Rainfall (mm)	Cumulated Glogal Radiation (MJ.m-²)
A	La Jaillière	2007	11,3	677	2420
B	La Jaillière	2008	10,3	566	2608
C	Grignon	2008	9,7	324	2692

54% ± 12 (means ± standard deviation of 31 results observed on unfertilized intercrops) to 77% ± 10 (means ± standard deviation of 31 results observed on sowing N-fertilized intercrops) due to N fertilization. In all these experiments, unfertilized intercrops were compared with intercrops fertilized at sowing, with no information about the effect of the application date of the fertilizer.

As cereals are known to be more competitive for soil mineral nitrogen than legumes at the beginning of growth (Andersen et al., 2005; Corre-Hellou et al., 2006; Jensen, 1996), it is assumed that delaying N application until after sowing could disturb interspecific relationships by reducing the competitive ability of cereals. As shown by Adu-Gyamfi et al. (1997) on sorghum –pigeon pea intercrops, a late application should have a less deleterious effect on pea growth, allowing the peas to have better shoot and root establishment before the strong competition of the cereal for light.

An analysis of the satisfaction of N demand has rarely been achieved in studies on cereal-legume intercrops whereas the N status of the intercropped cereal may determine its own leaf growth and hence light sharing between species (Corre-Hellou et al., 2006). The development of rule-based management of N fertilization in intercrops needs an understanding of how the N fertilization regime affects the N status of the cereal throughout crop growth and its consequences for light sharing and crop growth between the two species.

The main objective of this study was thus to test whether the timing of N fertilization is an effective way of influencing the dynamic interactions between species during crop growth and affecting the outcome of pea-wheat intercrops. This was achieved through a range of field experiments testing the effect of time of nitrogen fertilization on crop growth, leaf expansion, wheat N status and performance assessed at harvest, namely grain yield and the proportion of each species.

2. Materials and Methods

2.1. Experimental data

General design of experiments

Field experiments were carried out in France in 2006-2007 and 2007-2008 at La Jaillière (Exp A and Exp B respectively) at the experimental station of ARVALIS Institut du Végétal, in Loire-Atlantique, (47°26' N, 0°58' W) and at Thiverval-Grignon (Exp C) at the INRA experimental unit (48.85° N, 1.92° W), in conventional conditions (Table 1).

On Exp A and B, winter field pea (*Pisum sativum* L.) cv. Lucy, and winter wheat (*Triticum aestivum* L.) cv. Cézanne, were sown as sole crops at 80 pl m^{-2} and 260 pl m^{-2} respectively. On Exp C, winter field pea, cv. Cartouche, and winter wheat, cv. Trocadéro, were grown as sole crops (SC) with planned plant densities of 80 pl m^{-2} and 240 pl m^{-2} respectively. In both locations, intercrops (IC) were grown in a substitutive design, each species being sown at half its sole crop optimal density, both species being mixed within the rows. All experiments were arranged in randomised complete block designs with three replicates.

The soil was a sandy clayey loam (16.4 % clay, 57.5 % silt, 26.1 % sand) on Exp A, a clayey sandy loam (27.7 % clay, 42.1 % silt, 27.8 % sand) on Exp B, and a clayey loam (21 % clay, 72.2 % silt, 6.8% sand) on Exp C. Dates of sowing, harvest and main growth stages are given in Table 1.

Crop Management

In all experiments, weeds, pests and diseases were controlled with pesticides when required. No irrigation was provided. Weather data (minimal, maximal and mean temperature, rainfall and global radiation) were recorded daily near experimental sites (Table 2).

Table 3: Treatments, experimental conditions and N-fertilization

Reference of experiment	Location	Year of harvest	Crop design	Treatments	Initial conditions observed in February			Crops conditions at the date of N-fertilization			N-fertilization	
					Soil inorganic N (kg N ha^{-1})	Wheat DW (g m^{-2})	Pea DW (g m^{-2})	Wheat ZGS	Pea GS	Contribution of wheat DW to IC DW (%)	Time	Rate (kg N ha^{-1})
A	La Jaillière	2007	SC	A-Psc	62	—	19	—	—	—	—	0
			SC	A-Wsc N	69	29	—	ZGS30	—	—	14/03; 26/03; 27/04	80; 70; 40
			IC	A-IC N0	64	11	11	—	—	—	—	0
			IC	A-IC1	64	11	11	ZGS30	Veg (11-leaf)	64	14/03	45
			IC	A-IC2	64	11	11	ZGS37	Flowering	61	17/04	45
B	La Jaillère	2008	SC	B-Psc	60	—	12	—	—	—	—	0
			SC	B-Wsc N	55	37	—	ZGS30	—	—	07/03; 20/03; 14/05	80; 70; 40
			IC	B-IC N0	60	21	6	—	—	—	—	0
			IC	B-IC3	60	21	6	ZGS23	Veg (8-leaf)	79	07/02	45
			IC	B-IC4	60	21	6	ZGS30	Veg (10-leaf)	76	07/03	45
			IC	B-IC5	60	21	6	ZGS32	Veg (14-leaf)	72	10/04	45
C	Grignon	2008	SC	C-Psc		—	— —	—	—	—	—	0
			SC	C-Wsc N		—	— —	ZGS30	—	—	19/03; 9/04; 19/05	50; 120; 40
			IC	C-IC N0	56	—	— —	—	—	—	—	0
			IC	C-IC6	55	—	— —	ZGS30	Veg	82	09/04	40
			IC	C-IC7	56	—	— —	ZGS32	Flowering	78	13/05	40

Psc: sole cropped pea; Wsc: sole cropped wheat; N0: no-fertilized crop; N: N-fertilized crop; IC: intercrops; ICx: N-fertilized intercrop; ZGS: Zadoks growth stage (wheat); GS: Growth Stage (pea); DW: dry weight; Veg: Vegetative stages of pea; soil samples were taken from 0–90 cm soil layers in order to determinate the soil inorganic nitrogen at the end of winter

Inorganic soil N, measured in February, varied from 55 to 69 kg N ha^{-1} in the 0-90 cm soil layer (Table 3). The contribution of wheat to intercrop biomass observed on the same date varied from 50 (Exp A) to about 78 % (Exp B).

N was applied as NH_4NO_3 liquid fertilizer on Exp A and B and as solid NH_4NO_3 on Exp C. Several dates of N application were tested, from wheat tillering to the end of wheat stem elongation. Application dates and rates of N fertilizer are shown in Table 3. Pea sole crops were always grown without applied N.

Plant sampling and analytical methods

For all experiments, plants were harvested separately several times during crop growth, from the end of winter to maturity. Above-ground dry weight (DW) was determined after oven drying at 70 °C for 48 h. At harvest, grain and straw were weighed separately. All samples were ground and N content was measured using the Dumas procedure (Hansen, 1989).

On all experiments, soil samples were taken from the 0–90 cm soil layer in order to determinate the soil inorganic nitrogen at the end of winter (Table 3). Nitrate and ammonium were measured after KCl extraction by standard colorimetric methods (Keeney and Wilson, 1989).

Leaf areas of intercropped wheat and pea were determined three times on Exp A and B (from ZGS30 of wheat to wheat flowering), on the same harvested area used for the measurement of above-ground DW. The green leaves were separated from other parts of the plant for each species. The area of a sub-sample of green leaves was determined using a LI3100 area meter (LI-COR Inc., NE, USA). The specific leaf area (the ratio of leaf area to leaf dry weight) was determined on this sub-sample. The Green Leaf Area Index (GLAI, m^2 leaf m^{-2} soil) was calculated from the specific leaf area, the biomass of the sub-sample and the DW observed on the harvested area.

2.2. Calculations and statistics

The nitrogen status of the intercropped wheat was assessed during vegetative stage by calculating a Nitrogen Nutrition Index (NNI) (Lemaire and Meynard, 1997). The NNI is considered as an indicator of the level of satisfaction of crop N demand and a good indicator of the nitrogen nutrition of the crop with regard to leaf growth. The NNI of intercropped wheat was calculated as the ratio between the measured concentration of N in the intercropped wheat above-ground DW and the critical Nc determined from the total dry weight of the intercrop (DWic = intercropped wheat DW + intercropped pea DW; as proposed by Cruz and Soussana (1997) for mixed crops) by the equation proposed by Justes et al. (1997) for wheat:

if DWic < 1.55 t ha^{-1}, Nc = 5.35 %; if DWic > 1.55 t ha^{-1}, Nc = 5.35 x $(DWic)^{-0.442}$; (Eq 1)

and by the equation proposed by by Ney et al. (1997) for pea:

if DWic < 1 t ha^{-1}, Nc = 5.08 %; if DWic > 1 t ha^{-1}, Nc = 5.08 x $(DWMic)^{-0.32}$. (Eq 2)

Normal distribution was tested with the Shapiro–Wilk test. Analysis of variance was performed (type III sum of squares, α=5 %) and means were compared using Tukey's HSD tests (Honest Significant Differences, α=5 %), if a main effect or interaction was significant, using R software (R Development Core Team 2009). NNI values were compared to a reference value (0.9) by using Student t-test (α=5 %). Levels of significance of correlation coefficients calculated from linear regressions were tested using the table proposed by Fisher and Yates (1938).

Table 4: Total grain yield; grain yield of wheat and pea; contribution of wheat in total grain yield according to the fertilization effect and the date of fertilization effect (pooled data from all experiments)

	Total IC grain yield (g m-²)		**Wheat grain yield** (g m-²)		**Pea grain yield** (g m-²)		**Contribution of wheat to total grain yield** (%)	
	mean ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD
SC			**815** ±22,4		**503** ±35,6			
a) Fertilization effect								
IC N0	**594** ±35,1	—	**287** ±16,3	b	**307** ±34,9	a	**49** ±3,3	b
IC N	**637** ±17,8	—	**394** ±16,1	a	**243** ±14,7	b	**62** ±2,0	a
b) Date effect								
IC N (wheat ZGS30)	**632** ±35,5	—	**401** ±22,2	—	**231** ±31,5	—	**64** ±3,7	—
IC N (wheat stem elongation)	**625** ±11,6	—	**365** ±15,8	—	**260** ±12,1	—	**58** ±2,0	—

SC: sole crops of N-fertilized wheat or no-fertilized pea; IC N0: no-fertilized intercrops, IC N: N-fertilized intercrops (whatever the time of N-fertilization); IC N (wheat ZGS30): intercrops N-fertilized when wheat ear inflorescence was at 1 cm above tillering node; IC N (wheat stem elongation): intercrops N-fertilized during wheat stem elongation; Values are means of pooled data of all experiments ± SE. Analysis of variance (type III sum of squares, α=5 %) was carried out to test fertilization and date effects: treatments with the same letter are not significantly different (Tukey's HSD test, α=5 %), "—" signifies that the analysis of variance indicates no significant difference between treatments.

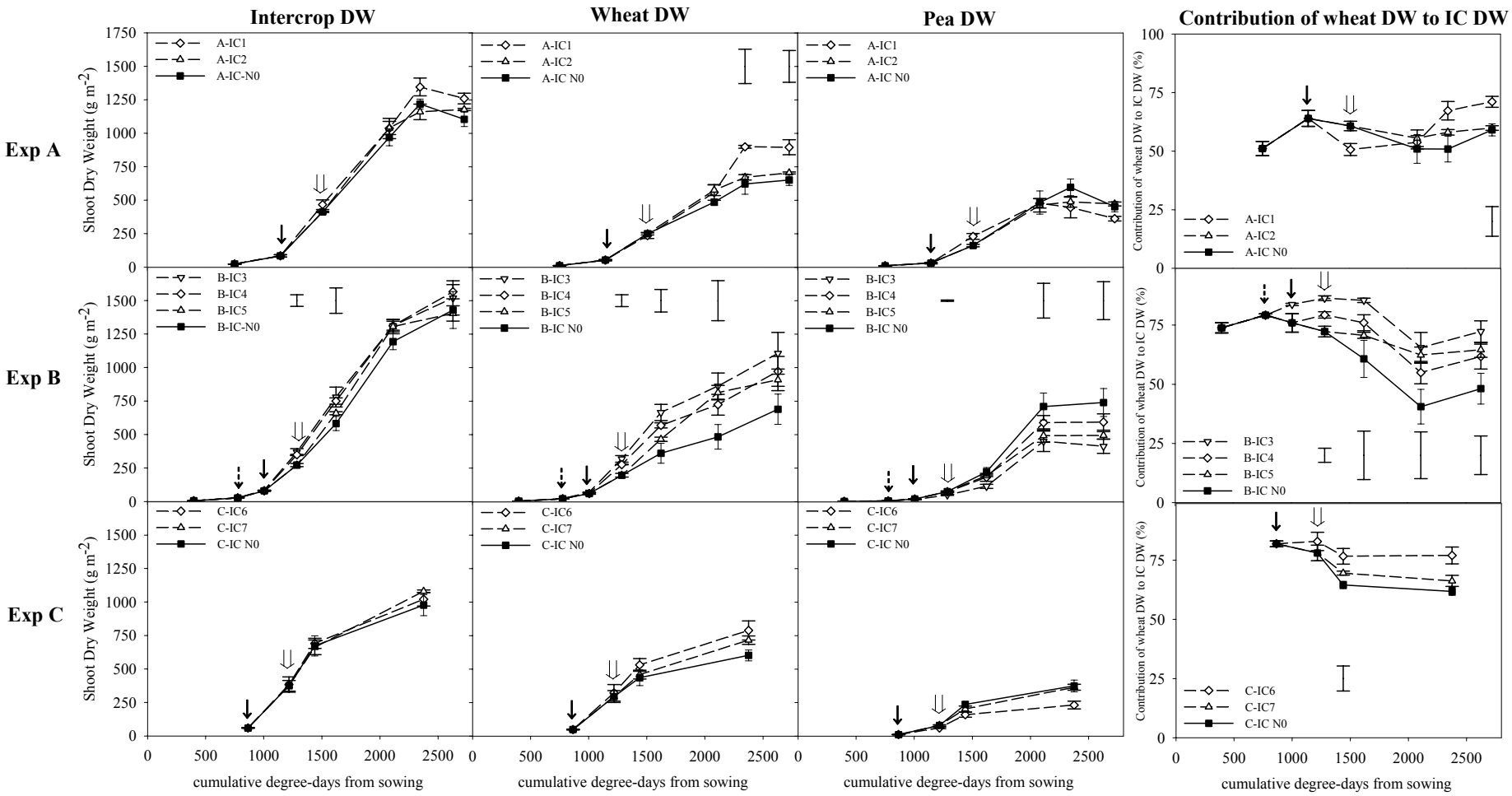

Fig. 1 Growth dynamics of an intercropped canopy, intercropped wheat and peas, and the proportion of wheat DW in the canopy, in unfertilized and N-fertilized conditions
A, B, and C refer to the three experiments. DW: dry weight (g.m^{-2}); IC N0: unfertilized intercrop; ICx: N-fertilized intercrop. Values are means (n=3) ± SE (standard errors bars on plots), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5%) was carried out to compare unfertilized with N-fertilized total intercropped dry weight, intercropped dry weight of wheat, and intercropped dry weight: vertical bars represent Tukey's HSD (α=5%). ⇣: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

3. Results

3.1. Yield and contribution of wheat in the mixed grain

N fertilization did not significantly affect the total grain yield of intercrops (p=0.2368), which in unfertilized and N-fertilized intercrops averaged 594 and 637 g.m^{-2} respectively (Table 4), and was not significantly different from the mean yield of sole crops (p=0.1114). Moreover, N fertilization significantly increased intercropped wheat grain yield (+37 %) and significantly decreased intercropped pea grain yield (-20 %). Thus, N application during vegetative growth increased the contribution of wheat to the total grain yield of intercrops, from 49 % without N to 62 % with applied N.

The date of N application did not significantly affect the grain yield of intercrops (p=0.7115), of intercropped wheat (p=0.2710) or pea (p=0.0926), and thus, the contribution of wheat grain yield to intercrop yield (p=0.0890).

3.2. Effect of N fertilization regimes on crop growth

Growth dynamics of intercropped wheat and pea and the proportions of each species in an intercrop canopy

Mean shoot dry weight (DW) of intercrops gradually increased with thermal time during the vegetative phase from wheat ZGS30 (from 59 to 108 g m^{-2}) to wheat ZGS65 (from 675 to 1015 g m^{-2}) and slowly increased during the reproductive phase (Figure 1). The mean total biomass at harvest varied from 1026 g m^{-2} (Exp C) to 1450 g.m^{-2} (Exp B). N applications never significantly affected total DW of intercrops in Exp A and C. In Exp B, at 1285 and 1623 cumulative degree-days after sowing (cdd), DW of intercrop of B-IC3 (N application during wheat tillering) was higher than that of N-fertilized intercrops at and after wheat ZGS30 (B-IC4 and B-IC5) which were not different from B-IC N0. During reproductive stages, and thus at harvest, DW of intercrops of Exp B did not differ from that of the unfertilized intercrop. In Exp A, intercrop dry weight decreased at the end of growth: this could be explained by losses of pea biomass during final reproductive stages. Indeed, the intercropped peas were mature before intercropped wheat and their grain tended to be shed.

N fertilization generally tended to increase wheat DW and to decrease pea DW. In Exp A, wheat DW of A-IC1 was higher than that of A-IC N0 and A-IC2 during reproductive stages, whereas pea DW was never affected. In Exp B, at 1285, 1623 and 2113 cdd, wheat DW of B-IC3 was higher than that of B-IC N0, but not than that of other N-fertilized treatments (B-IC4 and B-IC5). Pea DW of B-IC3 was higher than that of other treatments (B-IC N0, B-IC4, and B-IC5) at 1005, 1285, 2113 and 2629 cdd. Thus, the earlier the N fertilization, the higher was the wheat biomass and the lower the pea biomass. In Exp C, N application never affected wheat or pea DW.

Before N application, the proportion of intercropped species differed among experiments and among treatments on each experiment (Table 3): at the date of N-application, the proportion of wheat DW in total intercrop DW varied from 61 to 64 % in Exp A, from 72 to 79 % in Exp B, and was about 80 % in Exp C. Without N fertilization the contribution of wheat in intercropped biomass was not constant during crop growth (Figure 1). It varied from about 50% (Exp A) to 80% (Exp B and C) at the end of winter, steadily decreased during spring (from 40 to 65%) and finally increased at the end of growth. N fertilization reduced the size of the observed decrease: the earlier the N fertilization, the smaller the decrease. Indeed, in comparison with unfertilized intercrops, N application significantly increased the contribution of wheat of A-IC1 and B-IC 5 at harvest, of B-IC3 from 1285 cdd to harvest, of B-IC4 at 1285 cdd and of C-IC6 at 1217 cdd.

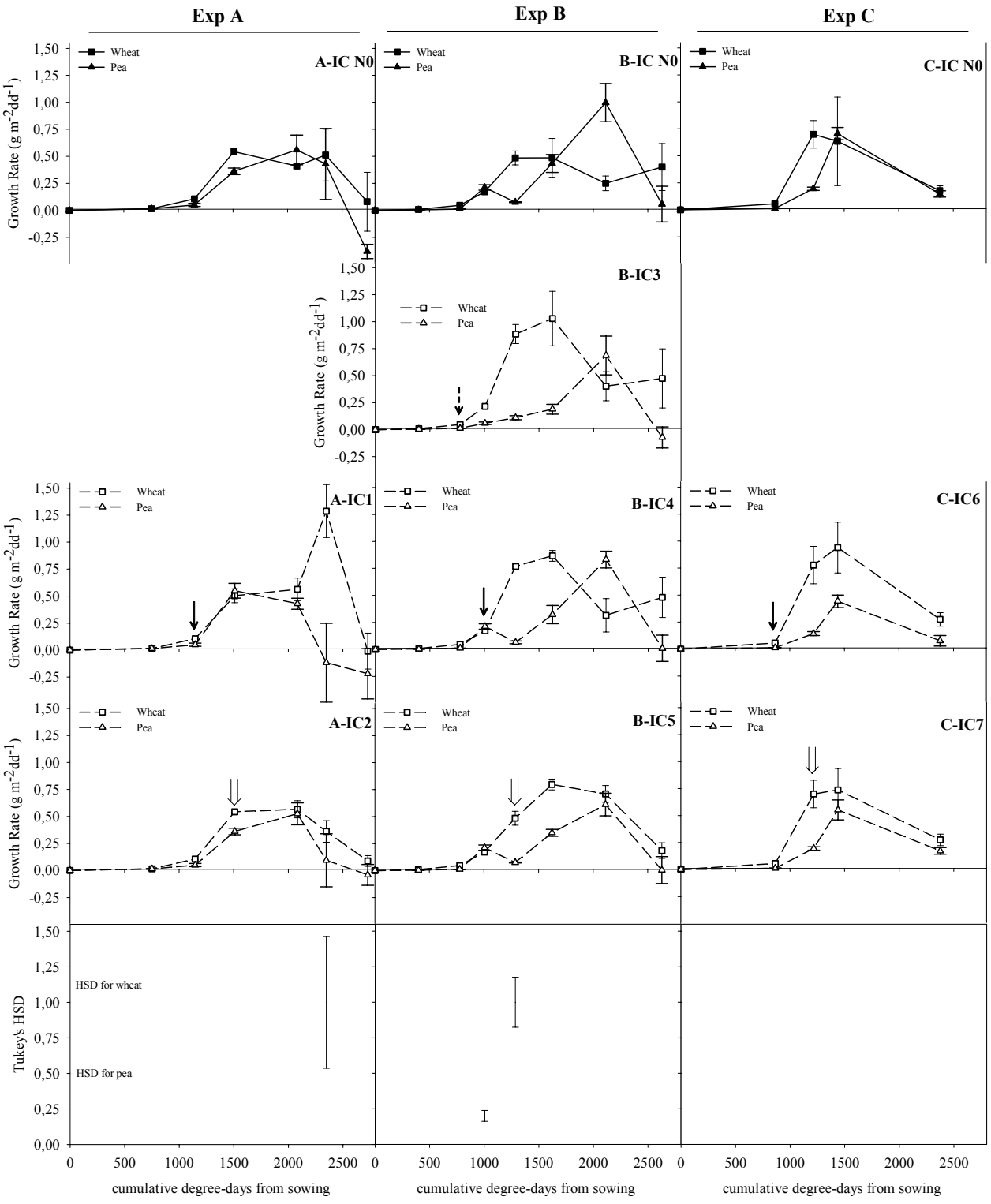

Fig. 2 Growth rate dynamics of intercropped wheat and pea, in unfertilized and N-fertilized conditions
A, B, and C refer to the three experiments. dd: degree-day; IC N0: unfertilized intercrop; ICx: N-fertilized intercrop. Values are means (n=3) ± SE (standard errors bars on plots), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5 %) and Tukey's HSD test were carried out in each experiment to compare growth rate of wheat (HSD for wheat) and of pea (HSD for pea): vertical bars represent Tukey's HSD (α=5 %). ⇣: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

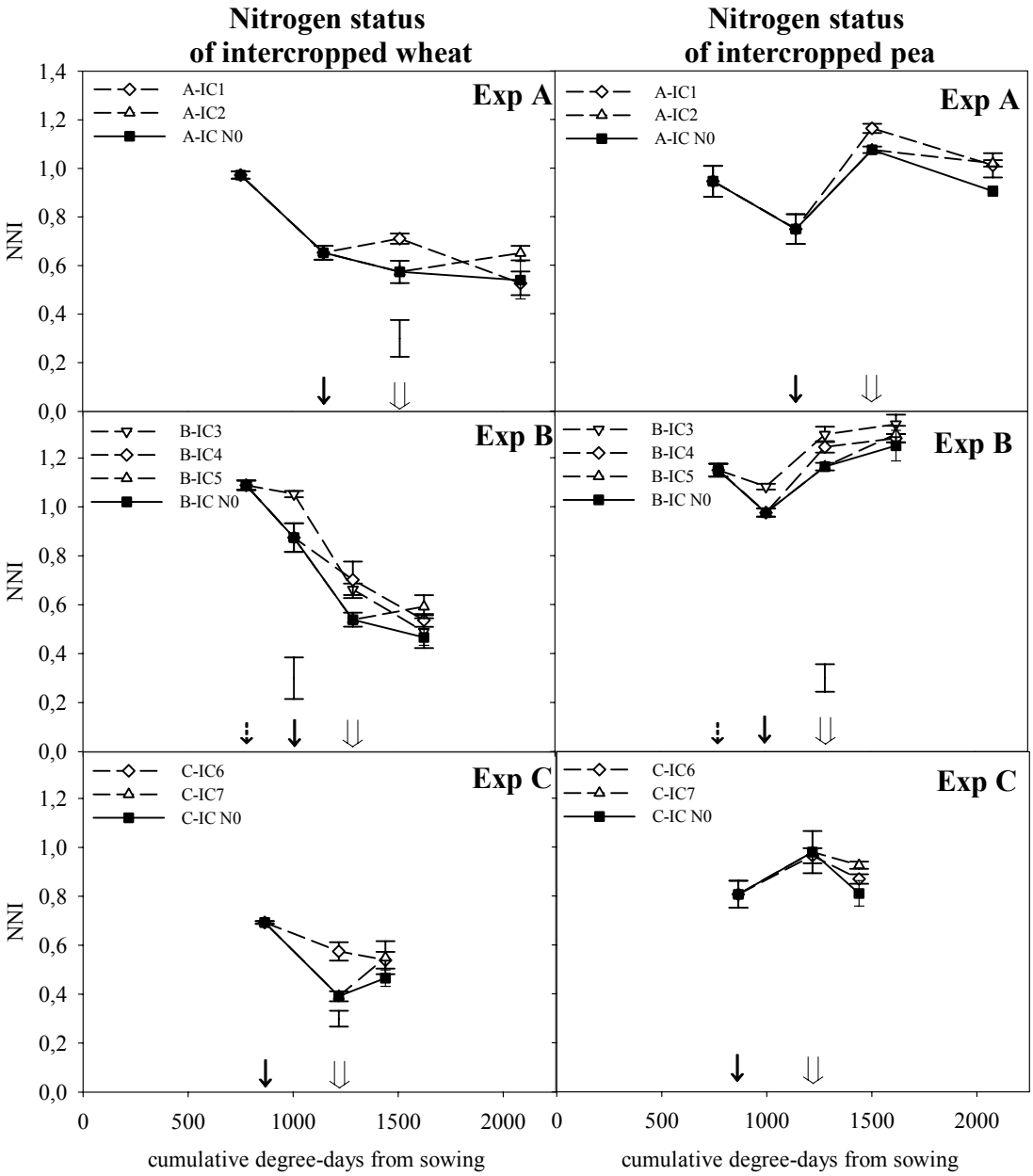

Fig. 3 Nitrogen Nutrition Index (NNI) of intercropped wheat, in unfertilized and N-fertilized conditions
A, B, and C refer to the three experiments. IC N0: unfertilized intercrop; ICx: N-fertilized intercrop. Values are means (n=3) ± SE (standard errors bars on plots), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5%) was carried out to compare unfertilized with N-fertilized NNI of wheat, and of pea: vertical bars represent Tukey's HSD (α=5%). ⇣: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

Growth rates of the intercrop components

In unfertilized conditions, growth rate dynamics of wheat and pea of A-IC N0 had similar patterns: they gradually increased from the end of winter to a plateau (about 0.5 g m^{-2} dd^{-1} which occurred from 1510 to 2348 cdd, and then decreased to zero during reproductive stages (Figure 2). In Exp B, growth rates of wheat and pea of B-IC N0 were similar at the beginning of crop growth. Between 1146 and 1510 cdd, wheat growth rate increased from 0.17 to 0.49 g m^{-2} dd^{-1}, whereas pea growth rate decreased to 0.08. Then, during reproductive stages, the trends were reversed and pea growth rate reached a maximum value of 1 g m^{-2} dd^{-1}, whereas wheat growth rate varied from 0.25 to 0.5 g m^{-2} dd^{-1}. In Exp C, wheat growth rate reached a maximum value (0.7 g m^{-2} dd^{-1}) during stem elongation, whereas that of pea increased a few weeks later to the same value.

During the weeks following N application, N fertilization did not modify patterns of pea growth rate in time but tended to decrease the maximum values in comparison to patterns observed in unfertilized conditions. Hence maximum growth rates of N-fertilized pea decreased by 7 to 23 %, by 17 to 39 %, and by 23 to 38 % in Exp A, Exp B, and Exp C, respectively).

During the weeks following N applications, wheat growth rates were greatly increased by the fertilizer. The earlier the N application, the earlier and higher was the maximum wheat growth rate: these patterns were clear in Exp B (maximum values of 1.03, 0.87 and 0.8 g m^{-2} dd^{-1}, for wheat of B-IC3, B-IC4 and B-IC5, respectively). The same patterns were observed in Exp C (maximum values of 0.94 and 0.74 g m^{-2} dd^{-1}, for wheat of C-IC6 and C-IC7, respectively), but differences were not significant. In Exp A, the situation where the two species had similar growth patterns in no fertilized IC, N fertilization at wheat ZGS30 benefited wheat growth only during reproductive stages, *ie* more than one month after N addition, whereas N addition during wheat stem elongation did not affect the growth patterns of either species.

In all experiments, wheat growth rates observed at ZGS30 (wheat ear 1 cm above tillering node) were higher than those of pea ($p<0.05$). Except in the case of A-IC1 and in Exp C, N fertilization allowed wheat growth rate to remain higher than that of pea until reproductive stages. Moreover, during wheat stem elongation, the earlier N fertilization, the bigger were the differences between the growth rates of the two intercrop components. During the weeks preceding harvest, growth rates of wheat and of pea were never significantly different.

3.3. Effect of N fertilization on wheat N status

The values of the Nitrogen Nutrition Index (NNI) of unfertilized intercropped wheat were highly variable between experiments at the beginning of growth and decreased during vegetative stages (Figure 3): at wheat ZGS30, they varied from 0.65 (A-IC N0 and C-IC N0) to 1.09 (B-IC N0), and decreased to about 0.5 (B-IC N0 and C-IC N0) to 0.57 (A-IC N0) at wheat flowering. N fertilization tended to reduce the intensity of nitrogen deficiency. Indeed, in comparison with the other treatments, and in each experiment before wheat flowering, N application significantly increased wheat NNI of A-IC2 (+24 % at 1510 cdd), of B-IC3 (+20 % at 1005 cdd) and of C-IC6 (+46 % at 1217 cdd). However, in all experiments, NNI values were not significantly different from those of unfertilized wheat at around wheat flowering, and were significantly lower than 0.9 ($p<0.05$) at around wheat flowering and during the month before.

Except once in Exp B (at 1285 ccd), N fertilization never modified NNI of intercropped pea in comparison with that of unfertilized intercropped peas. Moreover, pea NNI was always significantly higher than 0.9.

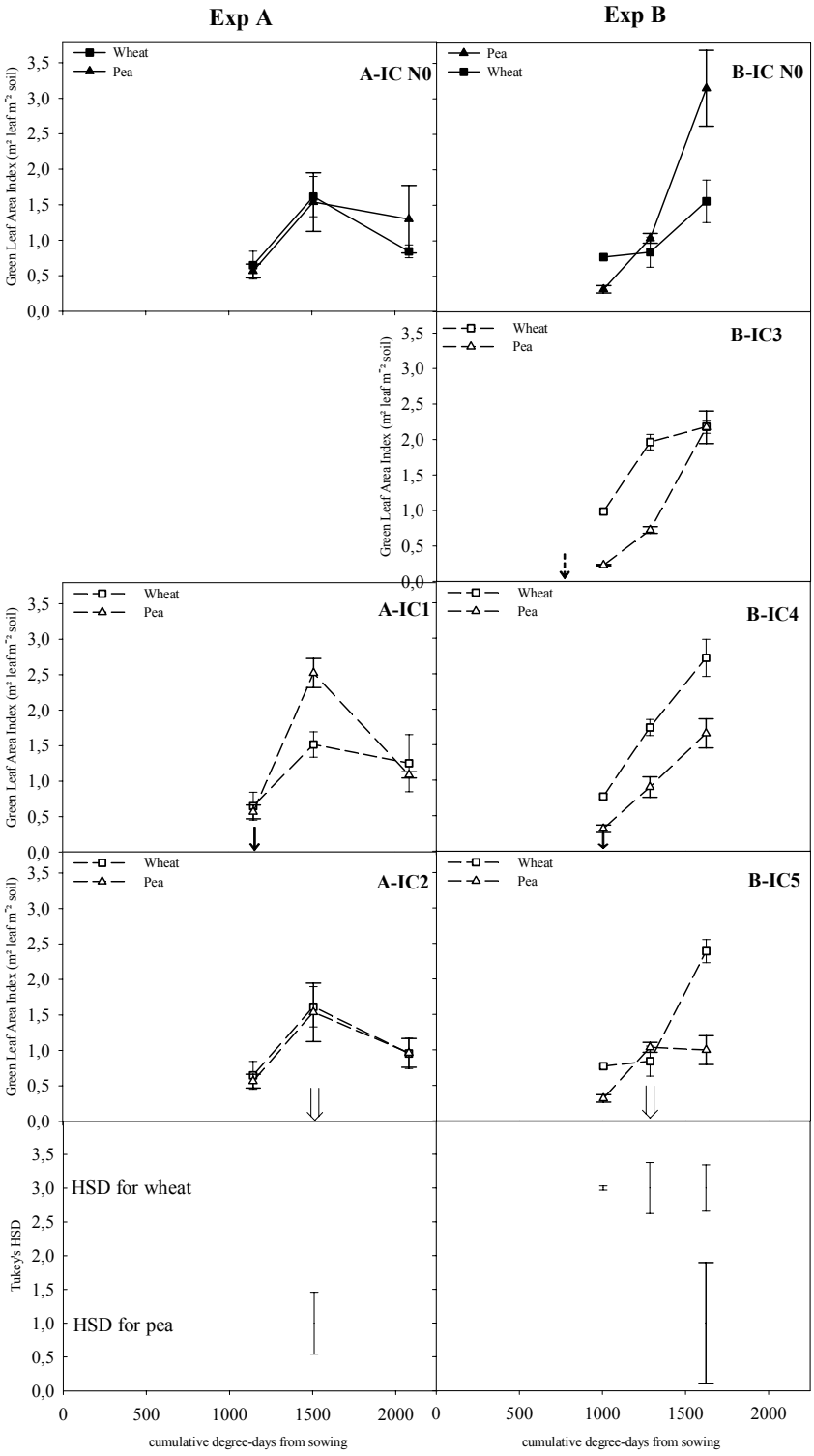

Fig. 4 Green Leaf Area Index (GLAI) of intercropped wheat and pea, in unfertilized and N-fertilized conditions
A, B, and C refer to the three experiments. IC N0: unfertilized intercrop; ICx: N-fertilized intercrop. Values are means (n=3) ± SE (standard errors bars on plots), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5 %) and Tukey's HSD test were carried out in each experiment to compare GLAI of wheat (HSD for wheat), and of pea (HSD for pea): vertical bars represent Tukey's HSD (α=5%). ↓: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

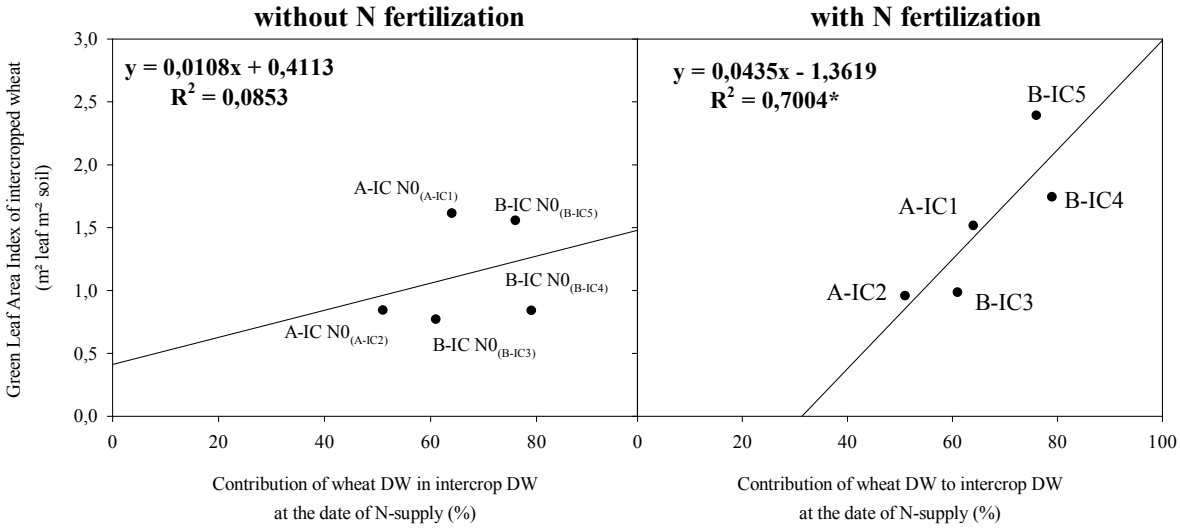

Fig. 5 Correlation Green Leaf Area Index (GLAI) observed one month after N-application and proportion of wheat in total intercropped biomass at the date of N application.
A and B refer to the two experiments. ICx: N-fertilized intercrop observed one month after N application; IC $N0_{(ICx)}$: unfertilized intercrop observed one the month after N application on ICx. DW: dry weight. Values are means (*n*=3) calculated for respective treatments. "*"indicated that linear regression is significant at $p<0.05$.

3.4. Effect of N fertilization on leaf area dynamics

In Exp A, in unfertilized treatments, GLAI of wheat and pea increased from 0.5 (at 1146 cdd) to 1.5 (at 1510 cdd), then decreased to about 1 at 2084 cdd due to the beginning of senescence during reproductive stages of the two species (Figure 4). In Exp B, GLAI of wheat and pea gradually increased from about 0.5 to 1.5 (GLAI of wheat) and to 2.72 (GLAI of pea). Except at 1005 cdd in Exp B, GLAI values of wheat and pea of unfertilized intercrops were never significantly different.

In Exp A, during wheat stem elongation (at 1510 cdd), N fertilization at wheat ZGS30 increased pea GLAI (A-IC1), but late N fertilization never modified wheat or pea GLAI (A-IC2). In Exp B, N fertilization greatly increased wheat GLAI and slightly decreased pea GLAI. Thus, wheat GLAI was significantly higher than that of pea during the weeks following N application ($p<0.05$). Finally, at around the reproductive stage of wheat, GLAI of wheat and pea were not significantly different, except for B-IC4 and B-IC5, where wheat GLAI was significantly higher than pea GLAI ($p=0.03566$ and $p=0.006643$ respectively). Finally, responses of GLAI of wheat to N fertilization were dependent on the proportions of each species at the date of N application. Indeed, GLAI of N-fertilized wheat observed one month after N application were correlated with the contribution of wheat DW to the intercrop DW observed at the date of N application (Figure 5), whereas GLAI of unfertilized wheat observed at the same times did not depend on the contribution of wheat DW to intercrop DW observed at the date of N application. Pea GLAI was not correlated to contribution of species DW to intercrop DW, with or without N fertilization (data not shown).

N addition did not modify total GLAI of the intercrop canopy (data not shown), except for B-IC3 and B-IC4 at 1285 cdd when total GLAI was higher than that of B-IC N0 (2.67 for both B-IC3 and B-IC4 and 1.87 for B-IC N0).

4. Discussion

4.1. Date effects on grain yields

The contribution of each species in intercrops at harvest is variable and is an important consideration for the development of such practices. However, there is a lack of knowledge about factors in the management of intercrops which may influence the relative dominance of each species.

In our experiments, the effect of the different dates of N fertilization confirmed previous results on N-fertilized cereal-legume intercrops. Indeed, compared to unfertilized conditions, N fertilization on intercrops increased wheat grain yield to the detriment of pea grain yield, whatever the date of N application. This is consistent with numerous previous observations on cereal-legume intercrops (Corre-Hellou et al., 2006; Ghaley et al., 2005; Hauggaard-Nielsen and Jensen, 2001; Jensen 1996; Fan et al., 2006; Rao et al., 1987). However, in our experiments, different timing of N fertilization did not lead to big differences in percentage of wheat in grain yield of N-fertilized intercrops: they were not significant at the statistical risk level $\alpha=5$ %, but they were significant at $\alpha=10$ %, indicating a slight effect of the timings we tested. Indeed, at harvest, N-fertilized biomasses were higher than unfertilized but were not different from each other because the effect of N addition on crop growth was mainly observed just after N application. Thus, the present work demonstrates that the analysis of crop growth throughout crop life rather than a single measurement at harvest is necessary to better understand competitive interactions in intercrops in relation to crop management.

4.2. Importance of growth of each species at the date of N-supply

Synchronization of phenological development of intercropped components

The phenological development of each species seems therefore determinant in the response of each of them in the mixture to N fertilization. Differences in weather conditions or cultivars between our experiments may have influenced this phenological development and therefore the relative contribution of each species. Indeed, responses to N fertilization were different according to the experimental conditions and locations. In Exp A, the effects of N fertilizer on wheat of A-IC1 seemed to be delayed. This may be explained by the early start of pea flowering, which occurred one month before wheat flowering, and by the fast pea growth at the beginning of crop growth due to unusually cool temperatures during the autumn of this experiment. Thus pea contributed 40% of the total biomass at the end of the winter in Exp A whereas it contributed only 20-25% in Exp B and C (Table 3). Therefore in Exp A, pea of A-IC1 had a higher N demand at the date of N application compared to the other experiments and N application enhanced its GLAI which became higher than that of intercropped wheat during the month following N application (Figure 4). Thus, competitiveness for light of pea of A-IC1 was improved, leading to a smaller effect of N application on wheat growth.

Peaks of growth rate of wheat and pea did not occur at the same time: in Exp B and C, wheat growth started earlier than that of pea during wheat stem elongation. Then it decreased and pea growth rate increased during pea's reproductive stage while wheat growth rate was falling. Finally, at the end of crop growth, pea growth rate often decreased earlier than that of wheat, indicating that intercropped pea ended its growth cycle before intercropped wheat, as previously observed by Andersen et al. (2007). Indeed, peas were always mature several weeks before wheat.

Contribution of wheat DW to intercrop DW at the date of N application

In pea-wheat intercrops, the contribution of each species to crop growth was not constant over time. At the beginning of crop growth, without N application, wheat contributed 70% (from 63 to 80%) on average of the total biomass in our experiments. Its contribution then fell steadily during the vegetative phase. Hence the contribution of each species to crop growth was different at each N fertilization date: it varied from 51 to 82 % among treatments. This variability in contribution of each species to intercrop biomass before N-application brought about differences in responses of wheat GLAI (Figure 5); but not in pea GLAI. Indeed, the higher the proportion of wheat DW in intercrop DW at the date of N application, the higher was the wheat GLAI one month later. Moreover, as height of wheat and pea were always similar whatever the N fertilization regime (data not shown), GLAI represents competitive ability for light of each species. Therefore, N application always enhanced competitiveness for light of intercropped wheat by increasing its GLAI.

4.3. N fertilization as a way of managing cereal-legume intercrops

N fertilization delayed the decrease in the contribution of wheat to total biomass and maintained high values of wheat growth rate for longer than in unfertilized intercrops. These results are consistent with previous observations on the N fertilization effect at sowing (Ghaley et al., 2005; Corre-Hellou et al., 2006). Adu-Gyamfi et al. (1997) have demonstrated, in sorghum – pigeon pea intercrops, that delaying N fertilization until after sowing increased grain yield of sorghum but not of pigeon pea. However, because of the different species used in their study, probably differing in phenology and interspecific relationships, it is difficult to compare their results with ours. In Exp B, in comparison with earlier date of N-supply, a late date of N-supply bring about a lower biomass of wheat and a lower contribution of wheat to total biomass during the weeks following N application. Late N application also affected pea growth rate. Indeed, timing of our late application occurred during wheat stem elongation and the pea reproductive stage, while N-demand of both intercrop components was increasing. Applying N at this stage, when otherwise

peas would have been able to make rapid growth, meant that the sudden extra growth of wheat restricted the growth of the peas.

We have previously shown that N application increased wheat GLAI and hence its growth generally. The decrease in pea growth can be explained by stronger competition for light from wheat given extra N rather than by an N-deficiency of pea. Indeed, Nitrogen Nutrition Index (NNI) of intercropped peas with and without N fertilizer, were mainly at least at their optimal value of 0.9 (Figure 3). This shows that peas can satisfy their N demand by switching between mineral and atmospheric N resources. However, the response of N_2 fixation to N application at different crop stages and levels of competition for light is not well-known and is worthy of research.

The NNI of wheat given N fertilizer early (during tillering or at ZGS30) was higher than that of unfertilized wheat and allowed an increase in its GLAI. But this small early N application increased the crop's N status and growth rate for only a short time, and led to increased N demand compared with unfertilized conditions. Hence at flowering the NNI of this fertilized wheat was no different from that of the unfertilized crop. Later N application (during stem elongation or around flowering) did not improve significantly the NNI, which did not differ from that of unfertilized wheat.

Further analysis of N dynamics could elucidate the timing effect of N fertilization on N acquisition and partitioning. Moreover, targeting production towards wheat with high grain protein content (e.g. for bread-making) involves raising its NNI near flowering by suitable rates and timing of N fertilization. This could be achieved by using crop models like FASSET (Berntsen et al., 2004), STICS (Brisson et al., 2004) or AZODYN-IC (Malagoli et al., 2009).

5. Conclusions

The total yields of unfertilized or N-fertilized intercrops were not significantly different from those for the means of sole crops. Thus intercrops produced the same yield without fertilizer as the mean of sole crops, which need about 100 kg $N.ha^{-1}$ (185 kg $N.ha^{-1}$ on sole cropped wheat and 0 kg $N.ha^{-1}$ on sole cropped pea) showing the potential value of intercropping to reduce N inputs in cropping systems. The main reason for N fertilization on intercrops is not to increase total yield but to modify the contribution of each species to total biomass or grain yield. Thus N fertilization appears to be an interesting way of managing cereal-legume intercrops for specific production targets in which the proportions of the two components are important. Nevertheless, in our conditions, although the timing of N fertilization significantly modified the crop dynamics of each species in relation to light competition and N status, the effect on final performance was small. It seems to be necessary to test a wider range of rates and timing of fertilization to reveal the full magnitude of responses on final yields. This might be achieved by means of scattered field experiments, but it might more easily be approached by way of modelling. Moreover, N fertilization needs to be combined with other factors such the sowing densities of each species, or cultivars differing in their initial growth rate, in order to influence the outcome of such intercrops for different purposes.

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References

Adu-Gyamfi, J., Ito, O., Yoneyama, T., Devi, G., Katayama, K., 1997. Timing of N fertilization on N2 fixation, N recovery and soil profile nitrate dynamics on sorghum/pigeonpea intercrops on Alfisols on the semi-arid tropics. Nutrient Cycling in Agroecosystems 48, 197-208.

Altieri, M.A., 1989. Agroecology: a new research and development paradigm for world agriculture. Agr. Ecosyst. Environ. 27, 37-46.

Andersen, M.K., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266, 273-287.

Andersen, M.K., Hauggaard-Nielsen, H., Høgh-Jensen, H., Jensen, E.S., 2007. Competition for and utilisation of sulfur in sole and intercrops of pea and barley. Nutr. Cycl. Agroecosys. 77, 143-153.

Andrews, D.J., Kassams, A.H., 1976. The importance of multiple cropping in increasing world food supplies. In: Papendick, R.I. et al. (Eds.), American Society of Agronomy, Madison, Wisconsin, pp. 1-11.

Anil, L., Park, J., Phipps, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53, 301-317.

Bellostas, N., Jensen, E.S., 2004. Intercropping of cereals and grain legumes in European organic farming systems.-INTERCROP. Grain Legumes 39, 14-15.

Berntsen, J., Hauggaard-Nielsen, H., Olesen, J.E., Petersen, B.M., Jensen, E.S., Thomsen A., 2004. Modelling dry matter production and resource use in intercrops of pea and barley. Field Crop Res. 88, 69-83.

Brisson, N., Bussière, F., Ozier-Lafontaine, H., Tournebize, R., Sinoquet, H., 2004. Adaptation of the crop model STICS to intercropping. Theorical basis and parametrisation. Agronomie 24, 409-421.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for Soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208;

Cruz P., Soussana, J.-F. Mixed crops. 1997. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 131–144.

De Wit, C.T., 1960. On competition. Versl Landbouwk Onderzoek 66, 1-81.

Fan, F., Zhang, F., Song, Y., Sun, J., Bao, X., Guo, T., Li, L., 2006. Nitrogen Fixation of Faba Bean (*Vicia faba* L.) Interacting with a Non-legume in Two Contrasting Intercropping Systems. Plant and Soil 283, 275-286.

Fisher, R.A., Yates, F., 1938. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Edinburg.

Ghaley, B.B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2005. Intercropping of wheat and pea as influenced by nitrogen fertilization. Nutr. Cycl. Agroecosys. 73, 201-212.

Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldhal. Acta Agr. Scan. 39, 113-118.

Hauggaard-Nielsen, H., 2001. Competitive interactions, resource use and nitrogen dynamics in annual intercrops in low-input cropping systems. PhD thesis, Risø National Laboratory, Roskilde, pp110.

Hauggaard-Nielsen, H., Andersen, M.K., 2000. Intercropping grain legumes and cereals in organic cropping systems. Grain Legumes 30, 18-19.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.

Jensen, E.S., 1996. Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil 182, 25-38.

Justes, E., Jeuffroy, M.H.,Mary, B., 1997. Wheat, barley, and durum wheat. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 73–91.

Keeney, D.R., Wilson, D.W., 1989. Nitrogen-inorganics forms. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis. Part II: Chemical and Microbiological properties, 2nd edn. American Society of Agronomy, Madison, Wisconsin.

Leitch, M., Musa, M., 1998. Agronomic characteristics and nitrogen balance of mixtures of barley and peas grown in different proportions at two levels of applied nitrogen. In: 3rd European Conference on Grain Legumes, Valladolid, pp 425.

Lemaire, G., Meynard, J.M., 1997. Use of the Nitrogen Nutrition Index for the analysis of agronomical data.In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 45–55.

Malagoli, P., Naudin, C., Goulevant, G., Sester, M., Corre-Hellou, G., Jeuffroy M.H., 2009. Modeling competition for below-ground resources and light within a winter pea (Pisum sativum L.) – wheat (Triticum aestivum L.) intercrop (Azodyn-InterCrop): towards a decision making oriented-tool. 7th European Conference on Precision Agriculture, Joint International Agricultural Conference (JIAC), Wageningen (Pays-Bas). In: van Henten, E.J., Goense, D. Lokhorst C. (Eds), Precision Agriculture,Wageningen Academic publishers. , pp. 659-666.

Ney, B., Doré, T., Sagan, M., 1997. The nitrogen requirement of major agricultural crops: grain legumes. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 107–117.

Ofori, F., Stern, W.R., 1987. Cereal-legume intercropping systems. Adv. Agron. 41, 41-90.

R Development Core Team , 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rao, M.R., Rego, T.J., Willey, R.W., 1987. Response of cereals to nitrogen in sole cropping and intercropping with different legumes. Plant Soil 101, 167-177.

Tilman, D., Cassman, K.G., Matsons, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671-677.

Trenbath, B.R., 1976. Plant interactions in mixed crop communities. In: Pappendick et al. (Eds) Multiple cropping, American Society of Agronomy, Madison, Wisconsin, pp. 129-169.

Waterer, J.G., Vessey, J.K., Stobbe, E.H., Soper, R.J., 1994. Yield and symbiotic nitrogen fixation in a pea-mustard intercrop as influenced by N fertilizer addition. Soil Biol. Biochem. 26, 447-453.

Willey, R.W., 1979. Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. Field Crop Abstr. 32, 1-10.

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TIMING EFFECT OF NITROGEN FERTILIZATION ON WINTER PEA-WHEAT INTERCROPS.

II- N-PARTITIONING AND SYMBIOTIC NITROGEN FIXATION

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Abstract

Cereal-legume intercrops could be a way to combine high productivity and several ecological benefits in temperate farming systems. N fertilization has been shown to be an efficient way to influence the dynamic interactions between species during crop growth and to affect the outcome of cereal-legume intercrops, which is linked with the N nutrition of each species. However setting up a rule-based N management system for such intercrops needs to take account of the influence of N applications on the N-sharing dynamics of the two species. This study aimed at analysing the influence of N fertilization and its timing on pea-wheat intercrops (1) on the dynamics of N acquisition of each species, (2) on the extent of Symbiotic Nitrogen Fixation (SNF) after N application, and (3) on the grain protein content (GPC) of both species. Field experiments were conducted in winter pea-wheat (*Pisum sativum* L.-*Triticum aestivum* L.) intercrops, in 2007 and 2008, at two locations in France. N acquisition dynamics and N sharing between the two species were greatly modified by N fertilization and its timing, suggesting that this technique could be an interesting way of managing cereal-legume intercrops. Crop conditions at the time of N application (growth and phenology of each species, and their proportions in the intercrop biomass) greatly influenced intercrop response to N fertilization. Partitioning between species of soil and fertilizer N were correlated to the proportion of wheat in the total intercrop biomass observed at the date of N application. SNF recovery after N applications was observed only until pea flowering, but was prematurely stopped by N fertilization after this stage.

Keywords: intercropping; N fertilization; pea; wheat; N_2 fixation.

** In tribute to Yves Crozat who had taken part in the first planning of this research project before deceased on June 20th, 2007.

1. Introduction

One way to partly improve sustainability of agrosystems is to limit the use of non-renewable resources and chemical inputs and to improve their efficiency. Intercropping (the growing of two or more crops simultaneously in the same field during a growing season) is known to improve the use of available resources, and to increase yield and its stability compared to sole cropping (Ofori and Stern, 1987; Willey, 1979). Thus, cereal-legume intercrops are gaining interest in Europe in low-input farming systems, especially in organic farming (Anil et al., 1998; Bellostas and Jensen, 2004; Hauggaard-Nielsen et al., 2009; Hauggaard-Nielsen et al., 2008).

This practice could also be useful in conventional agricultural systems to reduce the harm caused by intensive practices by increasing the efficiency of nitrogen fertilizer, and helping to reduce these inputs (Hauggaard-Nielsen et al., 2001), and thus the environmental impacts linked to their use (gaseous emissions particularly). In conventional systems, it has been observed, for example, that pea-wheat intercrops produced as much yield without fertilizer as the mean of sole crops which required about 100 kg N ha^{-1} (185 kg N ha^{-1} on sole cropped wheat and 0 kg N ha^{-1} on sole cropped pea) illustrating their value for combining high productivity with reduced N inputs (Naudin et al., 2009). Nevertheless, N fertilization (even in small amounts) has been shown to be an efficient way to influence the dynamic interactions between species during crop growth and to affect the outcome of cereal-legume intercrops. N application increases cereal growth and decreases legume growth (Corre-Hellou et al., 2006; Ghaley et al., 2005; Jensen, 1996; Rao et al., 1987). Delaying N application may also influence the contribution of each species in the mixture (Naudin et al., 2009, Adu-Gyamfi et al., 1997) and the efficiency of N-fertilized intercrops for grain yield compared to sole crops (Bedoussac and Justes, 2009).

Setting up rule-based management of N fertilization for such intercrops needs a better understanding of the effects of the timing of N applications on inorganic N acquisition, N_2 fixation dynamics and N sharing between the species. Previous studies have demonstrated the key role of N acquisition and the big effect of soil N availability on crop behaviour, mainly in spring pea-barley intercrops and for N applied at sowing. These studies have shown that the advantages of cereal-legume intercrops for yield and grain quality are mainly linked to the complementary use, in time and space, of N by the different components of the intercrop. Faster uptake of soil inorganic nitrogen by the cereal, due to a faster and deeper root elongation and a higher N demand than that of the legume, has been observed, and is assumed to explain the higher N_2 fixation rate usually observed in intercrops compared with sole crops (Corre-Hellou et al., 2007; Fujita et al., 1992; Jensen, 1996). But Symbiotic Nitrogen Fixation (SNF) of pea does not begin at the start of crop growth and hence intercropped peas and wheat are in competition for the same inorganic nitrogen resources during this time. If there is ample soil mineral nitrogen during vegetative growth, the cereal competes strongly with the peas for light. Therefore the growth and the amount of N_2 fixed per plant of intercropped pea could be greatly affected (Corre-Hellou et al., 2006).

The effect of the date of N fertilization on N acquisition has rarely been investigated in cereal-legume intercrops Adu-Gyamfi et al. (1997) have demonstrated that delayed N application increased N fertilizer recovery. Cereals are known to be more competitive for soil N than legumes, especially at the beginning of crop growth, but soil N sharing may vary at later stages according to the changes over time in N demand of each species.

Table 1: Treatments, experimental conditions and N-fertilization

Reference of experiment	Location	Year of harvest	Crop design	Treatments	Initial conditions observed in February			Crops conditions at the date of N-fertilization			N-fertilization	
					Soil inorganic N (kg N ha^{-1})	Wheat DW (g m^{-2})	Pea DW (g m^{-2})	Wheat ZGS	Pea GS	Contribution of wheat DW to IC DW (%)	Time	Rate (kg N ha^{-1})
A	La Jaillière	2007	SC	A-Psc	62	—	19	—	—	—	—	0
			SC	A-Wsc N	69	29	—	ZGS30	—	—	14/03; 26/03; 27/04	80; 70; 40
			IC	A-IC N0	64	11	11	—	—	—	—	0
			IC	A-IC1	64	11	11	ZGS30	Veg (11-leaf)	64	14/03	45
			IC	A-IC8	64	11	11	ZGS32	Beginning of Flowering	51	06/04	30
			IC	A-IC2	64	11	11	ZGS37	Flowering	61	17/04	45
B	La Jaillère	2008	SC	B-Psc	60	—	12	—	—	—	—	0
			SC	B-Wsc N	55	37	—	ZGS30	—	—	07/03; 20/03; 14/05	80; 70; 40
			IC	B-IC N0	60	21	6	—	—	—	—	0
			IC	B-IC3	60	21	6	ZGS23	Veg (8-leaf)	79	07/02	45
			IC	B-IC4	60	21	6	ZGS30	Veg (10-leaf)	76	07/03	45
			IC	B-IC5	60	21	6	ZGS32	Veg (14-leaf)	72	10/04	45
			IC	B-IC9	60	21	6	ZGS65	Beginning of Seed Filling	43	15/05	45
C	Grignon	2008	SC	C-Psc		—	—	—	—	—	—	0
			SC	C-Wsc N		—	—	ZGS30	—	—	19/03; 9/04; 19/05	50; 120; 40
			IC	C-IC N0	56	—	—	—	—	—	—	0
			IC	C-IC6	55	—	—	ZGS30	Veg	82	09/04	40
			IC	C-IC7	56	—	—	ZGS32	Flowering	78	13/05	40

Psc: sole cropped pea; Wsc: sole cropped wheat; N0: no-fertilized crop; N: N-fertilized crop; IC: intercrops; ICx: N-fertilized intercrop; ZGS: Zadoks growth stage (wheat); GS: Growth Stage (pea); DW: dry weight; Veg: Vegetative stages of pea; soil samples were taken from 0-90 cm soil layers in order to determinate the soil inorganic nitrogen at the end of winter

The inhibitory effects of nitrate on SNF are well known (Minchin et al., 1989). Voisin et al. (2002) have shown inhibition effects of nitrate on SNF of a sole pea crop in field experiments by varying the availability of soil mineral nitrogen at sowing. Fujikake et al. (2003) and Fujikake et al. (2002) have demonstrated that, during the first three weeks of soybean growth, the inhibition of SNF by nitrate was reversible. But there is a lack of knowledge about the effects of N application at different stages on SNF of field pea. Moreover the quantity of N_2 fixed in intercrops may vary according to the date of N application due to its effect on crop growth and N-demand.
In sole cropped cereals it is well known that the rate and timing of nitrogen application have a big effect on crop growth, grain yield and grain protein content (Jeuffroy and Bouchard, 1999; Limaux et al., 1999; Recous et al., 1988). Grain protein content (GPC) of cereal was shown to be only slightly increased by N fertilization applied at sowing on intercrops (Ghaley et al., 2005; Jensen, 1996). However, it is not known if late N applications could increase GPC of intercropped wheat, as happens in sole crops.

The main objective of this study was thus to analyse the influence of timing of nitrogen fertilization in pea-wheat intercrops (1) on the dynamics of nitrogen acquisition of each species, (2) on the inhibition and recovery of SNF after N application and (3) on grain protein content.

2. Materials and Methods

2.1. Experimental data

General design and crop management

Field experiments were carried out in 2006-2007 and 2007-2008 at two locations in France (Table 1). Details of each experiment were given in a previous paper (Naudin et al., 2009).
Crops were sole crops (SC) and intercrops (IC) of winter pea and wheat. Intercrops were grown in a substitutive design, each species being sown at half its optimal density in the corresponding sole crops, both species being mixed within the rows. Details of soil, weather data, sowing densities, cultivar and pest management were also given previously (Naudin et al., 2009).

Crop N management

Inorganic soil N was measured at the end of winter (February) and varied from 55 to 69 kg N ha^{-1} in the 0-90 cm soil layer (Table 1). The contribution of wheat to the intercrop biomass observed on the same date varied from 50 (Exp A) to about 78 % (Exp B).
N was applied as NH_4NO_3 as liquid fertilizer on Exp A and B and in solid form on Exp C. Several dates of N application on intercrops were tested from wheat tillering to the end of wheat stem elongation. These are shown in Table 1, together with the application rates. On Exp A and Exp B, the fertilizer was enriched with ^{15}N ($\delta^{15}N$=200 ‰) in order to estimate the dynamics of the amount of nitrogen derived from air (Ndfa) and accumulated in pea shoots. Treatments, soil inorganic nitrogen at the end of winter, dates and rates of N fertilizer are shown in Table 1. Pea sole crops were always grown without applied N.

Plant sampling and analytical methods

For all experiments, plants were harvested separately several times during growth, from the end of winter to maturity, and more frequently (weekly) during the month after N applications on Exp A and B. The two intercrops A-IC8 and B-IC9 were only harvested during the month after N fertilization to contribute to the analysis of the ability of SNF to recover after N application.
Above-ground dry weight (DW) was determined after oven drying at 70 °C for 48 h. At harvest, grain and straw were weighed separately. All samples were ground and N content was measured using the Dumas procedure (Hansen, 1989) while ^{15}N enrichment was determined by mass spectrometry.
On all experiments, soil samples were taken from 0-90 cm soil layers in order to determine the soil inorganic nitrogen at the end of winter (Table 1). Nitrate and ammonium were measured after KCl extraction by standard colorimetric methods (Keeney and Wilson, 1989).

Table 2: Accumulated N at harvest according to the cropping and N-fertilizations effect and the date of fertilization effect (pooled data from all experiments)

	Nsh of IC (g m-²)		**Nsh of wheat** (g m-²)		**Nsh of pea** (g m-²)		**Ndfa** (g m-²)		**%Ndfa** (%)		**GPCw** (%)		**GPCp** (%)	
	mean ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD	**mean** ±SE	HSD
a) Cropping and N-fertilization effects														
SC			**20,5** ±0,5	a	**24,1** ±1,3	a	**18,4** ±1,0	a	**77** ±2,3	b	**10,8** ±0,3	a	**19,9** ±0,5	a
IC N0	**18,4** ±1,8	—	**6,6** ±0,4	c	**11,9** ±1,7	b	**10,8** ±1,4	b	**92** ±1,6	a	**9,9** ±0,3	ab	**18,5** ±0,3	b
IC N	**18,4** ±0,8	—	**8,7** ±0,3	b	**9,7** ±0,7	b	**8,2** ±0,6	c	**86** ±2,7	a	**9,8** ±0,2	b	**19,0** ±0,3	ab
b) Date effect														
IC N (wheat ZGS30)	**17,5** ±1,6	a	**8,5** ±0,4	—	**9,0** ±1,4	—	**7,9** ±1,2	—	**90** ±2,5	a	**9,2** ±0,3	b	**18,8** ±0,3	—
IC N (wheat stem elongation)	**18,9** ±0,9	b	**8,6** ±0,3	—	**10,3** ±0,8	—	**8,2** ±0,8	—	**81** ±5,2	b	**10,7** ±0,3	a	**18,8** ±0,5	—

SC: Sole crop of N-fertilized wheat or of no-fertilized pea; IC N0: no-fertilized intercrops, IC N: N-fertilized intercrops (whatever the time of N-fertilization); IC N (wheat ZGS30): intercrops N-fertilized when wheat ear inflorescence was at 1 cm above tillering node; IC N (wheat stem elongation): intercrops N-fertilized during wheat stem elongation; Nsh: accumulated N in shoot biomass (g m^{-2}); Ndfa: accumulated N in shoot biomass derived form air (g m^{-2}); %Ndfa: contribution of N derived form air to total accumulated N in shoot biomass (%); GPCw and GPCp: Grain protein content (%) of wheat and pea, respectively. Values are means of pooled data of all experiments ± SE. Analysis of variance (type III sum of squares, α=5 %) was carried out to test cropping and N-fertilization effects, and date effect: treatments with the same letter are not significantly different (Tukey's HSD test, α=5 %), "—" signifies that the analysis of variance indicates no significant difference between treatments.

2.2. Calculations and statistics

The amount of shoot N_2 fixed (Ndfa) was calculated as the product of pea shoot biomass, %N content and the proportion of plant N derived from N_2 fixation. The proportion of shoot N derived from N_2 fixation (%Ndfa) was determined using the ^{15}N natural abundance method for unfertilized treatments (Amarger et al., 1979).

$$\%Ndfa=100x((\delta^{15}N \text{ pea}-\delta^{15}N \text{ barley})/(\beta fix-\delta^{15}N \text{ barley})) \quad \text{(Eq 1)}$$

where βfix (-1) (Mariotti et al., 1980) is the isotopic fractionation factor associated with N_2 fixation. It corresponds to the ^{15}N enrichment of peas relying entirely on N_2 fixation.

The percentage of shoot N derived from N_2 fixation (%Ndfa) was determined using the ^{15}N dilution method for fertilized areas with ^{15}N enrichment (Rennie and Rennie, 1983). N-fertilized intercropped wheat was used as the reference crop for calculating N_2 fixation for intercropped peas:

$$\%Ndfa = (1– (A\%^{15}N \text{ excess pea}) / (A\%^{15}N \text{ excess wheat})) \times 100 \quad \text{(Eq 2)}$$

An unfertilized wheat sole crop (Wsc-N0) was used as the reference crop for calculating N_2 fixation in pea SC and intercropped wheat for intercropped pea.

Shoot N derived from soil and fertilizer (Ndfsf) of pea was estimated as the difference between total accumulated N and the amount of N_2 fixed. Shoot N derived from soil and fertilizer of wheat was assumed to be equal to shoot accumulated N (Nsh) of wheat.

The grain protein content was estimated from grain N content multiplied by 5.7 for wheat and by 6.25 for pea (Sosulski and Imafidon, 1990; Teller, 1932).

Normal distribution was tested with the Shapiro–Wilk test. Analyses of variance was done (type III sum of squares, α=5 %) and means were compared using Tukey's HSD tests (Honest Significant Differences, α=5 %) if a main effect or interaction was significant, using R software (R Development Core Team, 2009). When there were only two means to compare, Student t-tests (α=5 %) were used. Levels of significance of correlation coefficients calculated from linear regressions were tested using the table proposed by Fisher and Yates (1938).

3. Results

3.1. N partitioning at harvest

N fertilization applied to intercrops did not significantly increase N accumulation by the mixture (Table 2). Moreover, the amount of N accumulated by the mixture was significantly lower when N was applied during stem elongation than if it was applied earlier. N accumulated by unfertilized intercropped wheat was about one third of that of sole cropped wheat. N fertilizer increased N accumulation by intercropped wheat to a similar extent whenever it was applied. N accumulated by intercropped peas was about half that of sole cropped peas. N applications to intercrops did not affect the N accumulated by peas whatever the application dates. The date of N fertilization did not influence N accumulation by intercropped wheat and pea.

The amount of shoot N derived from air (Ndfa) by unfertilized intercropped peas was 41% less than that of sole cropped peas. N fertilization of the intercrop decreased the amount of N_2 fixed similarly for the two dates of N supply. The contribution of N derived from air (%Ndfa) in shoots of intercropped peas was increased by 17% in unfertilized intercrops compared to sole cropped pea. The contribution of Ndfa to N accumulation of intercropped pea (%Ndfa) was not

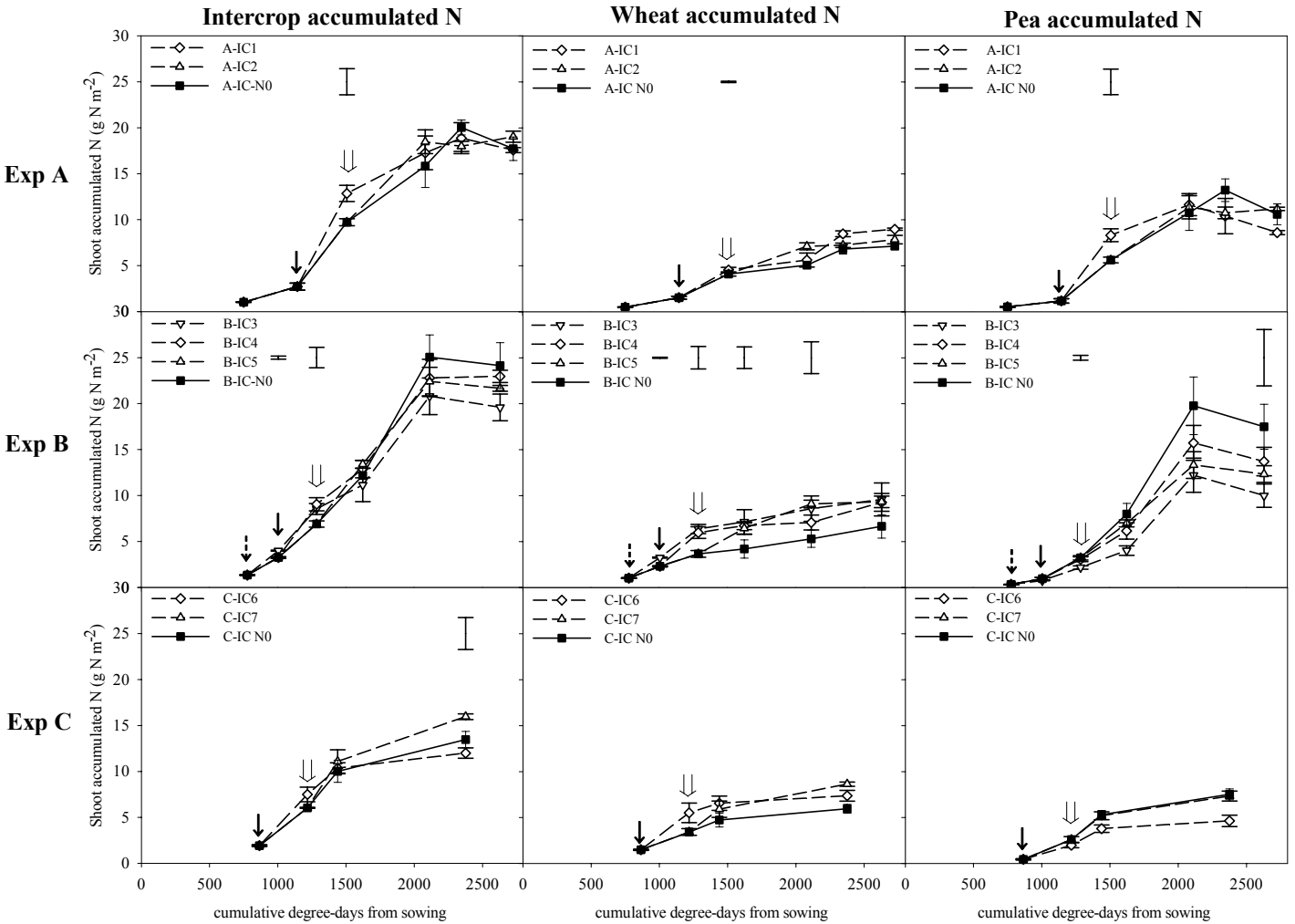

Fig. 1 N acquisition dynamics in an intercrop canopy, intercropped wheat and pea in unfertilized and N-fertilized conditions

Nsh: N accumulated in shoot (g N m^{-2}); A, B, and C refer to the three experiments. IC N0: unfertilized intercrop; ICx: N-fertilized intercrop. Values are means (n=3) ± SE (standard errors bars on plots), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5%) was carried out to compare unfertilized with N-fertilized total intercropped accumulated N, intercropped accumulated N of wheat, and intercropped accumulated N of pea: vertical bars represent Tukey's HSD (α=5%). ⇣: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

affected by N fertilization compared to unfertilized treatment. Late N application significantly decreased %Ndfa compared with earlier N application.

Grain Protein Content of wheat (GPCw) in sole crops (10.8 %) was no different from that of unfertilized intercropped wheat (9.9 %), but was higher than that of N-fertilized intercropped wheat (9.8 %). GPCw in intercrops was significantly increased by later N applications (10.7 %) compared with those made before wheat stem elongation (9.2 %). Grain Protein Content of pea (GPCp) in sole crops (19.9 %) did not differ from that of N-fertilized intercropped peas (19 %), but was higher than that of unfertilized ones (18.5%). GPCp in intercrops was not significantly affected by N fertilizer, whatever the date of application.

3.2. Effect of N fertilization regimes on N acquisition

Dynamics of N accumulation in intercrop components

For all experiments, total accumulated N in shoots (Nsh) of unfertilized intercrops increased gradually with thermal time during the vegetative phase until 2000 cumulative degree days from sowing (cdd) for Exp A and B, and until 1500 cdd for Exp C, and generally stopped increasing and became stable during the reproductive phase (Figure 1). N fertilization brought about a slight increase in total accumulated Nsh in the intercrop canopy during vegetative stages (Exp A and B), or at harvest (Exp C). The total Nsh of all intercrop canopies at harvest varied from 11.9 g m^{-2} (Exp C) to 24.1 g m^{-2} (Exp B). As previously noted by Naudin et al. (2009) about dry weight, in Exp A, the decrease in intercrop Nsh observed before harvest could be explained by the shedding of pea grains.

The dynamics of N accumulated by the canopy were similar with or without applied N. However N application influenced the dynamics of N acquisition of both species. In all experiments, N fertilization tended to increase the accumulated N of intercropped wheat and decrease that of intercropped pea. In Exp A and B, significant differences mainly appeared during several weeks after N application but were not observed at harvest (except for pea accumulated N of B-IC3 which was higher than those of other treatments of Exp B at harvest). In Exp C, N applications never resulted in significant differences in N acquisition of the intercrop components. Moreover, wheat and pea displayed differences in N acquisition over time. Pea accumulated on average 57 % (up to 80 %) (Exp B) of its total N acquisition after the beginning of pea flowering, whereas wheat accumulated only on average 37 % after it flowered.

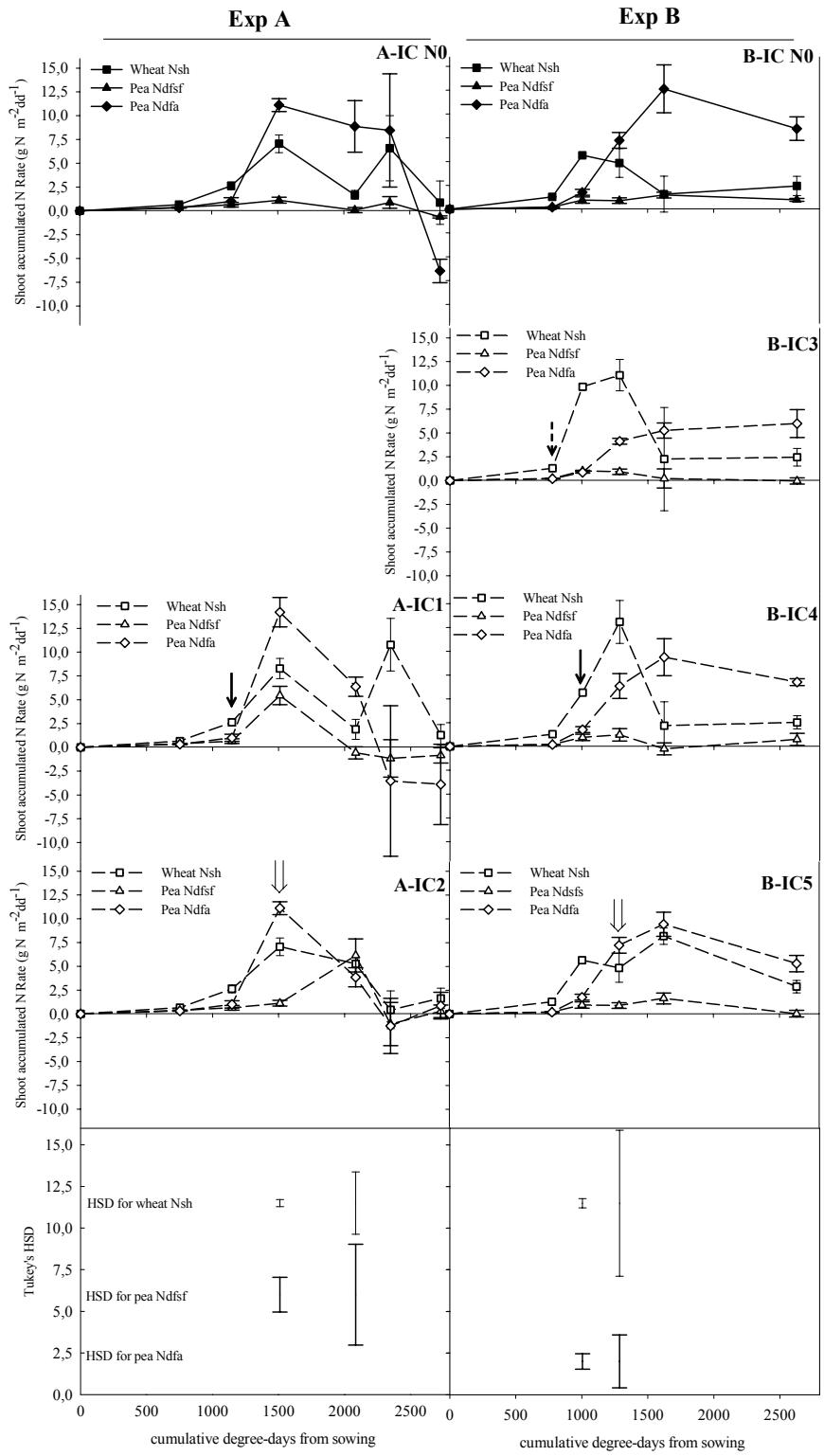

Fig. 2: N acquisition rate dynamics of intercropped wheat and pea in unfertilized and N-fertilized conditions
Wheat Nsh: N accumulated in wheat shoot corresponding to mineral N absorption; Pea Ndfsf: Nitrogen derived from soil and fertilizer; Pea Ndfa: Nitrogen derived from air; A, and B: references of experiments. IC N0: unfertilized intercrop; ICx: N-fertilized intercrop; dd: degree-day. Values are means (n=3) ± SE (Standard Errors bars), calculated for respective treatments. Analysis of variance (type III sum of squares, α=5 %) and Tukey's HSD test were carried out in each experiment to compare N acquisition rate on wheat (HSD for wheat Nsh) and on pea (HSD for pea Ndfsf, and HSD for pea Ndfa): vertical bars represent Tukey's HSD (α=5 %). ↓: N application during wheat tillering; ↓: N application when wheat ear was 1 cm above tillering node; ⇓: N application during wheat stem elongation.

Table 3: N-acquisition and partitioning after N-applications

Treatments	ΔNsh of wheat ($g\ m^{-2}$)	ΔNdfsf of pea ($g\ m^{-2}$)	Mineral N sharing (%)	ΔNdfa of pea ($g\ m^{-2}$)	%Ndfa (%)
	mean ±SE	**mean** ±SE	**mean** ±SE	**mean** ±SE	**mean** ±SE
A-IC $N0_{(A\text{-}IC1)}$	**2,6** ±0,3	**0,4** ±0,1	**86,7** ±1,7	**4,0** ±0,2	**91,0** ±2,0
A-IC1	**3,0** ±0,4	**2,0** ±0,3 *	**60,7** ±1,7 ***	**5,2** ±0,6	**72,6** ±1,6 **
A-IC $N0_{(A\text{-}IC8)}$	**3,0** ±0,6	**0,4** ±0,2	**90,2** ±4,9	**6,4** ±2,1	**95,5** ±2,2
A-IC8	**4,5** ±0,1	**1,8** ±0,6	**72,6** ±7,1	**5,6** ±0,5	**77,0** ±4,7 *
A-IC $N0_{(A\text{-}IC2)}$	**1,0** ±0,3	**0,1** ±0,1	**90,5** ±5,4	**5,1** ±1,6	**98,1** ±1,0
A-IC2	**3,0** ±0,2 **	**3,5** ±1,0 +	**48,0** ±5,5 **	**2,2** ±0,6	**39,1** ±4,6 ***
B-IC $N0_{(B\text{-}IC3)}$	**1,3** ±0,0	**0,2** ±0,1	**85,9** ±4,5	**0,4** ±0,1	**66,8** ±4,8
B-IC3	**2,2** ±0,1 ***	**0,2** ±0,0	**90,7** ±0,7	**0,2** ±0,0	**45,9** ±4,0 *
B-IC $N0_{(B\text{-}IC4)}$	**1,4** ±0,4	**0,3** ±0,1	**81,2** ±7,3	**2,0** ±0,2	**89,6** ±2,4
B-IC4	**3,7** ±0,6 *	**0,3** ±0,2	**92,3** ±2,9	**1,8** ±0,4	**84,6** ±8,0
B-IC $N0_{(B\text{-}IC5)}$	**1,6** ±0,5	**0,2** ±0,2	**81,3** ±15,9	**5,1** ±1,3	**96,8** ±2,2
B-IC5	**2,5** ±0,5	**0,6** ±0,2	**81,0** ±4,8	**6,8** ±1,7	**90,8** ±3,9
B-IC $N0_{(B\text{-}IC9)}$	**0,3** ±0,1	**2,0** ±0,4	**13,7** ±7,1	**9,7** ±1,7	**82,0** ±4,8
B-IC9	**1,4** ±0,7	**8,0** ±1,8 +	**13,7** ±6,9	**2,2** ±0,1 *	**22,8** ±3,6 ***

ΔNsh, ΔNdfa and ΔNdfsf: during the month (during 6 weeks for A-IC8) after N-applications, increments of total accumulated N, of N derived from air, and of N derived from soil and fertilizer, respectively. Mineral N-sharing: (ΔNsh of wheat / ΔNdfsf of intercrop) x 100; %Ndfa: (ΔNdfa / ΔNsh of pea) x 100; A and B: references of experiments. ICx: N-fertilized intercrop; IC $N0_{(ICx)}$: no-fertilized intercrop observed during the month after N-application on ICx. Values are means (n=3) ± SE (Standard Errors), calculated for respective treatments. Mean comparisons were carried out differences between no- and N-fertilized intercrops (Student t-test, α=5 %): "+": p<0.1; "*": p<0.05; "**": p<0.01; "***"; p<0.001.

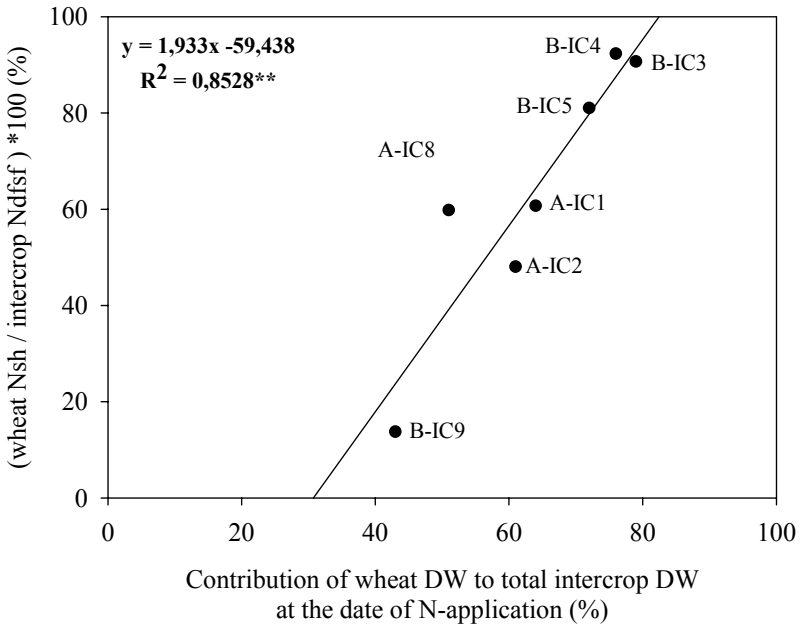

Fig. 3: Correlation between mineral N sharing during the month after N application and proportion of wheat in total intercrop biomass at the date of N application.
A and B refer to the two experiments. ICx: N-fertilized intercrop. DW: dry weight; Wheat Nsh: N accumulated in wheat shoot corresponding to mineral N absorption; intercrop Ndfsf: Nitrogen derived from soil and fertilizer accumulated in shoot of whole intercrop (wheat +pea). Values are means (n=3) calculated for respective treatments. "**"indicated that linear regression is significant at p<0.01

N acquisition rates of the intercrop components

Maximum N acquisition rates of wheat and of pea did not occur at the same time (Figure 2). For wheat they occurred during stem elongation (*i.e.* during the vegetative stage), whereas maximum pea N_2 fixation rate occurred during its reproductive stage. Except for A-IC2 and B-IC5, during pea reproductive stages, N_2 fixation rates of pea were higher than mineral N uptake rate of pea or wheat ($p<0.05$), whatever the N fertilization regime. From wheat ZGS30 to flowering, and except for A-IC2, mineral N uptake rates of wheat were always higher than those of pea ($p<0.05$) whatever the N fertilization regime. Thereafter, mineral N uptake rates of wheat and pea decreased at the end of growth and were not significantly different at harvest.

In Exp A, in comparison with unfertilized conditions and during the weeks following N application, N fertilization on A-IC1 significantly increased Ndfsf uptake rates of pea (+28 %), did not affect N_2 fixation rates of pea, and increased Ndfsf uptake rates of wheat (+17 %). At around wheat flowering, Ndfsf uptake rate of wheat and pea, and N_2 fixation rate, were not different from those of A-IC N0.
During the weeks following N application, N fertilization on A-IC2 increased Ndfsf uptake rate of peas (from 0.89 to 3.14 g N m^{-2} dd^{-1}), but did not affect the Ndfsf uptake rate of wheat or N_2 fixation rate of peas.

In Exp B, whatever the date of N fertilization, Ndfsf uptake rates of pea were never significantly different from those of N-fertilized intercropped peas. In comparison with the unfertilized intercrop, and during the weeks following N application, N fertilization tended to increase of Ndfsf rate of wheat and decrease N_2 fixation rates of peas. Significant effects were observed on B-IC3 (N application during wheat tillering): Ndfsf uptake rate of wheat increased by 74 % (5.66 and 9.85 g N m^{-2} dd^{-1}, observed at 1005 cdd on wheat of B-IC N0 and B-IC3, respectively) and N_2 fixation rate of peas decreased by 51 % (1.77 and 0.86 g N m^{-2} dd^{-1}, observed at 1005 cdd on wheat of B-IC N0 and B-IC3, respectively). Similar patterns were seen on the other fertilized treatments of Exp B, but these were not significant according to Tukey's HSD test.

3.3. Mineral N-sharing after N application

In unfertilized intercrops, wheat always took up much the larger share of mineral N (soil and fertilizer) (*i.e.* the ratio of the increment Nsh of wheat to that of Ndfsf of the intercrop during the month after N application). Indeed wheat accounted for more than 80 % of the mineral N accumulation except for B-IC9 (N application during wheat flowering and the beginning of pea seed filling) when wheat accumulated only 13 % of the soil N accumulated by the intercrop (Table 3).

In N-fertilized conditions, mineral N sharing during the month after N application was significantly correlated with the proportion of each intercropped components at the date of N application (Figure 3); whatever the date of N application.
In case of N application before beginning of pea seed filling (BSF), wheat acquired between 61 and 73 % in Exp A and between 81 and 92 % in Exp B of mineral N (from soil and fertilizer) acquired by intercrop during the month after N application. After N fertilization applied during pea flowering or near BSF, wheat contributed only to 48 % (A-IC2) and 14 % (B-IC9) to mineral N accumulation of intercrop.

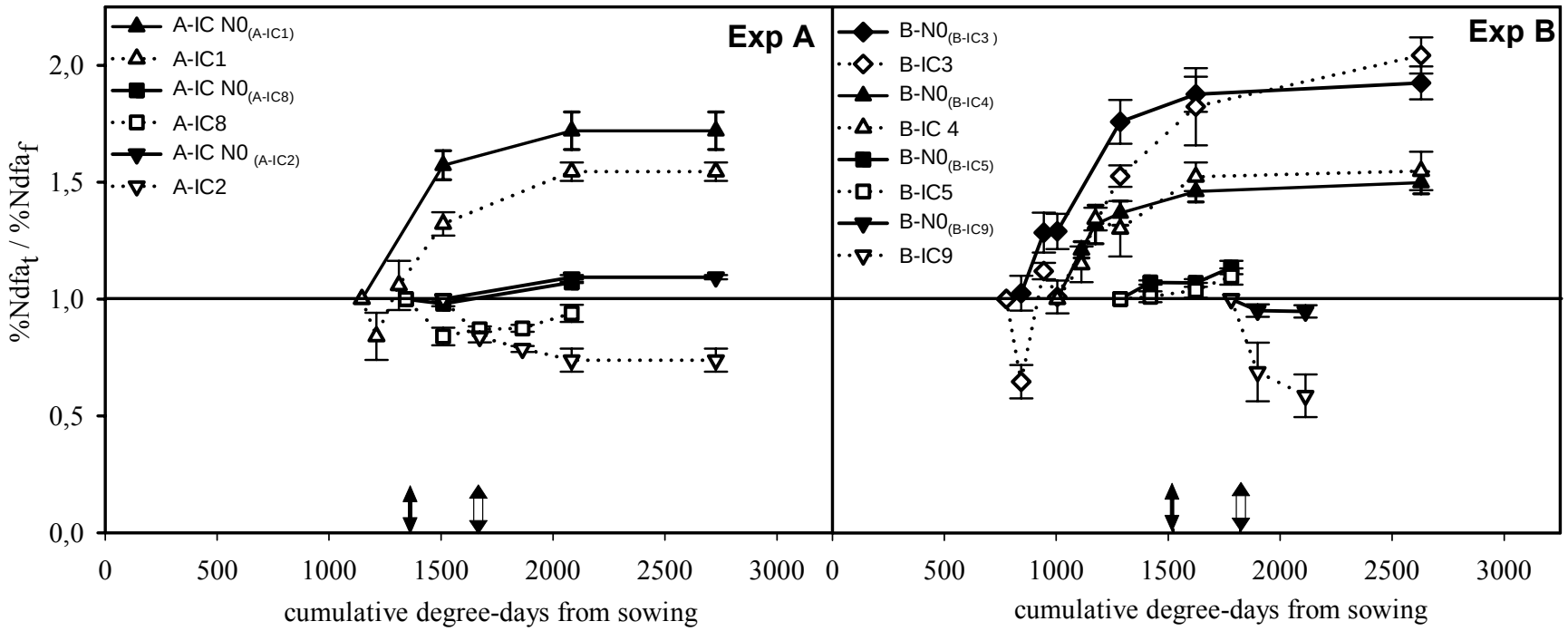

Fig. 4 Dynamic ratios between %Ndfa at the date t (%$Ndfa_t$) and %Ndfa observed at the date of N application (%$Ndfa_f$)
A and B refer to the two experiments. ICx: N-fertilized intercrop; IC $N0_{(ICx)}$: unfertilized intercrop in reference to the date of N application on ICx;.Values are means (n=3) calculated for respective treatments. ↕: beginning of pea flowering. ⇳: beginning of pea seed filling.

During the month after N application, increments of soil and fertilizer N accumulation (ΔNdfsf) in wheat shoots were increased by N application during wheat stem elongation in Exp A (A-IC2), and during tillering or at ZGS30 in Exp B (B-IC3 and B-IC4) (Table 3). Increments of soil and fertilizer N accumulation (ΔNdfsf) in pea shoot were significantly increased by early N application (B-IC3). Moreover, late N application near BSF (A-IC2 and B-IC9) greatly increased ΔNdfsf in pea shoot. However, these results were only significant at the statistical risk level α=10 %.

3.4. Effect of timing of N fertilization on the inhibition and recovery of N_2 fixation

N fixation by intercropped peas during the month after N application (ΔNdfa) was not affected by N fertilization, except for B-IC9 where ΔNdfa decreased (Table 3). The contribution of atmospheric N to total accumulated N in pea shoots during the month after N application (%Ndfa) was always decreased by N fertilization in Exp A (by 21, 19 and 60% for A-IC1, A-IC8 and A-IC2, respectively). In Exp B, N application had no effect on %Ndfa for peas of B-IC4 and B-IC5 but it decreased %Ndfa for early application (B-IC3) by 30 %, and for late application (B-IC9) by 72 %.

Figure 4 displays the dynamics of $\%Ndfa_t/\%Ndfa_f$ ratios, that is to say the dynamics of percentage of shoot N derived from air (%Ndfa) relative to its value at the time of N application in intercrops. For unfertilized intercropped peas, it shows that %Ndfa had different dynamics at the different dates of N application. When N was applied to A-IC1, B-IC3 and B-IC4, %Ndfa was increasing, whereas for A-IC8, A-IC2, B-IC5 and B-IC9 it was stable, indicating that SNF activity had reached its maximum or was on the decrease.

In Exp A, the decrease in $\%Ndfa_t/\%Ndfa_f$ ratios resulted from a brief inhibition of SNF during the first week after N applications on A-IC1 (Figure 4). At 1510 cdd, this led to significantly different $\%Ndfa_t/\%Ndfa_f$ ratios ($p=0.03694$) of those of A-IC1 to those of unfertilized intercropped peas. Thereafter the $\%Ndfa_t/\%Ndfa_f$ ratios from unfertilized situations were no different ($p=0.1497$ at 2084 and 2727 cdd).

Timing of N application on A-IC8 never led to different ratios of %Ndfa at a given time to its value on the date of N application in comparison with the corresponding ratios for unfertilized intercropped pea ($p=0.05615$, $p=0.06778$ and $p=0.06778$ at 1510, 2084 and 2727 cdd, respectively).

N applications on A-IC2 led to inhibition of SNF during the three following weeks, without possible later recovery, as shown by significant differences between $\%Ndfa_t/\%Ndfa_f$ ratios for A-IC2 and for unfertilized intercropped peas ($p=0.01628$ at 2084 and 2727 cdd).

The same patterns could be observed in Exp B. On B-IC3, N fertilization only led to a decrease in $\%Ndfa_t/\%Ndfa_f$ ratios at 844 cdd ($p=0.02143$). For B-IC4 and B-IC5, N application and its timing never affected $\%Ndfa_t/\%Ndfa_f$ ratios. N fertilization on B-IC9 led to a lasting decrease in %Ndfa relative to that observed at N application (Figure 4). Thus, at 2113 cdd, the %Ndfa ratio of B-IC9 was lower than that of unfertilized pea ($p=0.04903$).

4. Discussion

4.1. Effect of timing of N application on N acquisition dynamics

Whenever it was applied, N fertilizer did not affect the amount of N in shoots (Nsh) of the intercrop canopy, but did affect the source and the quantity of N accumulated in the shoots by each species, as previously shown by Ghaley et al. (2005) and Jensen (1996). It increased N accumulation by intercropped wheat and slightly decreased that of intercropped peas. Intercropped wheat is well-known to be more competitive for mineral soil N resources than intercropped pea (Andersen et al., 2005; Corre-Hellou et al., 2006; Hauggaard-Nielsen et al., 2009; Jensen, 1996). Except for N addition at around the beginning of pea seed filling (BSF), wheat acquired from 60 to 91 % of the mineral N accumulated by the intercrop canopy during the month after N application. Thus, intercropped wheat benefited most from N fertilization, which allows to become more competitive for light (Corre-Hellou et al., 2006). This modification of light competition then led to a decrease in pea growth and of symbiotic nitrogen fixation (SNF) potential. So N fertilization slightly decreased Nsh of intercropped peas because the total amount N accumulated N by intercropped pea was mainly the result of SNF activity and, in an intercropping situation, to light competition between the two components. Intercropping is often seen as a way to increase N_2 fixation in cropping systems. But this work demonstrates, as previously found in other legume-cereal intercrops such as peas and barley (Hauggaard-Nielsen et al., 2009), that intercropping increases the contribution of N_2 fixation compared to a pea sole crop but the amount of N_2 fixed is largely determined by pea growth and is dependent on the competitive strength of barley or wheat for light.

As shown for B-IC3, early N applications greatly benefited intercropped wheat to the detriment of pea. In this case, the competitive advantage of wheat for mineral soil N reduced pea growth (Naudin et al., 2009) and hence the potential amount of N_2 fixed by the pea shoots. Indeed, in Exp B at harvest, N fertilization decreased the amount of N derived from air accumulated in pea shoot of B-IC3 (9,31 g N m^{-2}) in comparison with that of B-IC N0 (15,36 g N m^{-2}), but differences were only significant at the 0,1 probability level ($p=0.0571$, data not shown). Late applications during the reproductive stages of pea (at around BSF) slightly disturbed crop growth but greatly modified N use by intercropped pea (Table 3). Therefore, early N applications could reduce the amount of Ndfa mainly by decreasing pea growth, whereas late N applications could reduce the amount of Ndfa by the decrease the contribution of Ndfa to total accumulated N in pea.

4.2. Crop growth and N demand at the time of N application greatly influenced responses of each component to N fertilization

N partitioning between each intercrop component was heavily influenced by the dynamics of their respective N demand. As shown in figure 3, whatever the date of fertilization, mineral N sharing during the month succeeding N application was highly correlated with the proportion of each species' biomass in the canopy, indicating that sharing of mineral N was dependent on growth and N demand of the components.

Wheat accounted for more than 80 % of soil N acquisition due to its faster root growth and higher N demand. This percentage decreased towards the end of growth because its demand declined earlier than that of peas. These results indicate that root traits, initial aerial growth and crop phenology, which may vary between cultivars, may greatly influence mineral N sharing and acquisition by each species. The cultivars were not the same in all experiments, which may explain some differences in the dynamics of N acquisition between sites. Moreover the differences in N demand between species may also vary with the weather conditions. Indeed, pea accounted for 40 % of the total biomass at the end of the winter in Exp A, whereas it only represented 20-25 % in Exp B and C (Table 1).

4.3. Recovery of Symbiotic Nitrogen Fixation depended on the stage of intercropped peas when N fertilization occurred

The effect of N fertilization on N use by intercropped peas depended greatly on its application date. The N demand of intercropped peas can be satisfied by N derived from soil and fertilizer (Ndfsf) or from air (Ndfa). N fertilization led to a peak of available soil nitrate which partly inhibited SNF activity by intercropped peas, as shown by Voisin et al. (2002). Just after N application, the N demand of intercropped peas was mainly satisfied by Ndfsf. But soil mineral N was rapidly absorbed due to the high competitive ability and high N demand of intercropped wheat, so that soil mineral N content rapidly decreased (data not shown) and SNF recovered, indicating that nitrate availability was not permanently detrimental to nodule activity. The lower competitive ability of wheat for soil N after the beginning of pea seed filling due to a shorter period of N accumulation of wheat compared to peas probably explains the bigger detrimental effect of late N application on SNF than that observed at earlier stages.

It is well known that the SNF activity of peas is at its maximum at the beginning of pea flowering (BF) (Voisin et al., 2002; Voisin et al., 2003) and decreases after the beginning of pea seed filling (BSF). Our experiments showed that recovery of SNF activity after N application was possible until peas flowered. N fertilization at about the BSF stage seems to bring forward the cessation of SNF. For such a late N fertilization, the high N demand of peas during its reproductive stages was more satisfied by absorbed mineral soil N. This is in accordance with previous observations of Jensen (1986) who, in an experiment on pot-grown peas as a sole crop, demonstrated that the recovery of N fertilizer was increased with late applications.

4.4. Buffer ability of SNF of IC peas and the positive effect of late N fertilization on grain protein content of IC wheat at harvest

N fertilization appears to be an interesting way to manage cereal-legume intercrops for specific agricultural production targets for which the proportions of the two components are important (Naudin et al., 2009). But grain quality factors, such as protein content, may also be important. Grain protein content of wheat was significantly increased by N applications at GS32 compared with earlier N additions. This is in accordance with results of Bedoussac and Justes (2009) and observations on sole crops where N applications at around flowering of wheat are known to greatly improve its grain protein content. Moreover, intercropped peas acted as a buffer when soil mineral N varied by adapting its N acquisition methods. In Exp A and B, soil mineral N at harvest was similar for intercropped and sole cropped wheat, whatever the N management, and was significantly lower than that of sole cropped pea (data not shown). Thus, intercropping seems to be a means to achieve better N fertilizer recovery in crops and could be a way of reducing the environmental risks associated with late N applications currently made to sole cropped wheat to ensure a target grain protein content.

5. Conclusion

The effects of N fertilization on pea-wheat intercrops are relevant to many complex objectives, such as the proportion of species, grain protein content and the efficient use of inputs. In order to better predict the effect of a strategy of fertilization on N acquisition in intercrops, this study reveals the need to take into account the conditions when the N is applied, in particular the growth stage of each species, which determines soil N sharing and not just the well-known inhibitory effect of nitrates on %Ndfa but also the recovery of N_2 fixation after N application, which varies considerably over time. These date effect of nitrate inhibition and recovery on SNF need to be confirmed and studied in controlled conditions by the analysis of nodule growth and activity in such conditions.

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References

Adu-Gyamfi, J., Ito, O., Yoneyama, T., Devi, G., Katayama, K., 1997. Timing of N fertilization on N2 fixation, N recovery and soil profile nitrate dynamics on sorghum/pigeonpea intercrops on Alfisols on the semi-arid tropics. Nutrient Cycling in Agroecosystems 48, 197-208.

Amarger, N., Mariotti, A., Mariotti, F., Durr, J.C., Bourguignon, C., Lagacherie, B., 1979. Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in 15N Natural abundance. Plant Soil 52, 269-280.

Andersen, M.K., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266, 273-287.

Anil, L., Park, J., Phipps, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53, 301-317.

Bedoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil. DOI 10.1007/s11104-009-0082-2.

Bellostas, N., Jensen, E.S., 2004. Intercropping of cereals and grain legumes in European organic farming systems.-INTERCROP. Grain Legumes 39, 14-15.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for Soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208;

Corre-Hellou, G., Brisson ,N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crop Res. 103, 76-85.

Fisher, R.A., Yates, F., 1938. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Edinburg.

Fujikake, H., Yashima, H., Sato, T., Ohtake, N., Sueyoshi, K., 2002. Rapid and reversible nitrate inhibition of nodule growth and N_2 fixation activity in soybean (*Glycine max* (L.) Merr.). Soil Sci. Plant Nutr. 48, 211-217.

Fujikake, H;, Yamazaki, A., Ohtake, N., Sueyoshi, K., Matsuhashi, S., Ito, T., Mizuniwa, C;, Kume, T., Hashimoto, S., Ishioka, N.S., Watanabe, S., Osa, A., Sekine, T., Uchida, H., Tsuji, A., Ohyama, T., 2003. Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. J. Exp. Bot. 54, 1379-1388.

Fujita, K., Ofosu-Budu K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant Soil 141, 155-175.

Ghaley, B.B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2005. Intercropping of wheat and pea as influenced by nitrogen fertilization. Nutr. Cycl. Agroecosys. 73, 201-212.

Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldhal. Acta Agr. Scan. 39, 113-118.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Reintroducing grain legume-cereal intercropping for increased protein production in European cropping systems. *In* 4th European conference on grain legumes, Cracow, (Eds) AEP, pp. 52-53.

Hauggaard-Nielsen, H., Jornsgaard, B., Kinane, J., Jensen, E.S., 2008. Grain-legume-cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agr. Food. Syst. 23, 3-12.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N_2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crop Res. 113, 64-71.

Jensen, E.S., 1986. The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (*Pisum sativum* L.). Fert. Res. 10, 193-202.

Jensen, E.S., 1996. Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil 182, 25-38.

Jeuffroy, M;H., Bouchard, C., 1999. Intensity and duration of nitrogen deficiency on wheat grain number. Crop Sci; 39, 1385-1393.

Keeney, D.R., Wilson, D.W., 1989. Nitrogen-inorganics forms. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis. Part II: Chemical and Microbiological properties, 2nd edn. American Society of Agronomy, Madison, Wisconsin.

Limaux, F., Recous, S., Meynard, J.M., Guckert, A., 1999. Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat. Plant Soil 214, 49-59.

Mariotti, A., Mariotti, F., Amarger, N., 1980. Fractionnements isotopiques de l'azote lors des processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plantes. Physiol. Veg. 18, 163-181.

Minchin, F.R., Becana, M., Sprent, J.I., 1989. Short-term inhibition of legume N2 fixation by nitrate. II Nitrate effects on nodule oxygen diffusion. Planta 180, 46-52.

Naudin, C., Corre-Hellou, G., Pineau, S., Crozat, Y., Jeuffroy, M.H., 2009. Timing effect of nitrogen fertilization on winter pea-wheat intercrops. I- Growth dynamics and grain yield. (submitted to Field Crops Res.).

Ofori, F., Stern, W.R., 1987. Cereal-legume intercropping systems. Adv. Agron. 41, 41-90.

R Development Core Team , 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rao, M.R., Rego, T.J., Willey, R.W., 1987. Response of cereals to nitrogen in sole cropping and intercropping with different legumes. Plant Soil 101, 167-177.

Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988, The fate of labelled ^{15}N urea and ammonium nitrate applied to a winter wheat crop. I. Nitrogen transformations in the soil. Plant Soil 112, 205-214.

Rennie, R.J., Rennie, D.A., 1983. Techniques for quantifying N2 fixation in association with non-legumes under field and greenhouse conditions. Can. J. Microbiol. 29, 1022–1035.

Sosulski, F.W., Imafidon, G.I., 1990.Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38, 1351-1356.

Teller, G.L., 1932. Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for protein in cereals and bread. Cereal Chem. 9, 261-274.

Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil 243, 31-42.

Voisin, A.S., Salon, C., Jeudy, C., Warembourg, F.R., 2003. Symbiotic N_2 fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. J. Exp. Bot. 54, 2733-2744.

Willey, R.W., 1979. Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. Field Crop Abstr. 32, 1-10.

Synthèse partielle

Cette partie basée sur des expérimentions de plein champ a permis de préciser des éléments de fonctionnements des associations céréales-légumineuses en lien avec différentes dynamiques de nutrition azotées. Elle démontre que la fertilisation azotée est un levier efficace pour orienter les performances finales notamment la proportion de chaque espèce dans le mélange, critère aujourd'hui mal maîtrisé. Un apport d'azote favorise la croissance de la céréale et pénalise celle de la légumineuse. La céréale apparaît plus compétitive que la légumineuse pour les ressources d'azote minéral pour une date d'apport intervenant avant Début du Remplissage des Grains du pois. Cependant l'intensité de la réponse à la date de fertilisation varie en fonction des écarts de dynamiques de croissance et de phénologie de chaque espèce avant l'apport, facteurs qui apparaissent déterminants dans le partage de l'N minéral et le comportement de la fixation symbiotique.

Chapitre 3 :

Inhibition et réversibilité de la fixation symbiotique du pois suite à différentes dates de courtes expositions aux nitrates et sous différents niveaux de disponibilité en carbone

Effet des nitrates sur la structure et la fonction de fixation des nodosités après une courte exposition à différents stades et étude des conditions de réversibilité de la fixation symbiotique en relation avec la nutrition carbonée.

Les expérimentations au champ explorant différentes stratégies de fertilisation azotée (Chapitre 2) ont mis en évidence que la fixation symbiotique du pois était réversible après une courte phase d'inhibition liée à la présence de nitrates mais que cette réversibilité n'est possible que si l'exposition aux nitrates intervient avant le stade de début du remplissage des grains.

Ce chapitre vise à approfondir les mécanismes en jeu dans une telle situation, à l'échelle de la plante de pois. L'effet inhibiteur des nitrates, à la fois sur la structure et sur l'activité de l'appareil fixateur a été étudié ainsi que les conditions de réversibilité suite à une courte exposition de l'appareil fixateur aux nitrates à différents stades phénologiques et pour différents niveaux de disponibilité en carbone.

Cette étude a été réalisée en conditions contrôlées (serre du LEVA – Angers) sur du pois (*Pisum sativum* L., cv Baccara) cultivé en hydroponie. Les parties racinaires des pois ont été exposées aux nitrates (5 meq N L^{-1}) pendant une courte période (8 jours) et pour trois stades différents (apparition des premières nodosités – floraison – remplissage des grains). Après retrait des nitrates, la réversibilité de la structure et de l'activité de l'appareil fixateur été étudiée sous deux conditions de disponibilité en rayonnement : lumière naturelle et ombrage (diminution du rayonnement photosynthétiquement actif de 65 %).

Cette expérimentation a été réalisée en étroite collaboration avec Christophe Salon et Anne-Sophie Voisin (UMR LEG – INRA de Dijon) et a bénéficié de leur expertise tant sur la culture en hydroponie que sur la physiologie des légumineuses.

EFFECT OF NITRATES ON STRUCTURE AND ACTIVITY OF PEA NODULES AFTER A SHORT-TERM EXPOSURE AT DIFFERENT CROP STAGES AND CONDITIONS OF RECOVERY OF SYMBIOTIC N_2 FIXATION IN RELATION TO C NUTRITION

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Running title: Ability of Symbiotic Nitrogen Fixation to recover after short-term inhibition by nitrates

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Abstract
Nitrates are well-known to bring about an inhibition of SNF by impairing nodule growth rather than fixation activity. Inhibition by nitrate and succeeding recovery of nodule growth and activity must be investigated in relation to plant phonological stage and C availability. Our main objectives were i) to study the inhibition effect of a short-term exposure to nitrate when applied at different crop stages on symbiotic structure and their activity and ii) to analyze the ability of SNF to recover after such exposure to nitrate in relation with C nutrition. Pea (*Pisum sativum* L. cv Baccara) was grown in a nutrient solution under greenhouse conditions and exposed to short-term of nitrate (5 meq NO_3^- L^{-1}) during early vegetative, flowering and seed filling stages. After nitrate removal, plants were grown under natural light or shaded conditions. Dry weight of plant, shoot, root, nodule, number of nodules and amount of N derived from air and nitrate were monitored from early vegetative stages to plant maturity. Nitrate reduced rate of nodule establishment when nodulated root were exposed to nitrate during vegetative phases while it entailed damage on existing nodules when applied during flowering and seed filling. Nitrate exposure always decreased specific activity of nodules. Moreover, nitrate exposure during vegetative stage brought about a starter effect by increasing plant growth and N-acquisition. Second, an extra wave of nodulation appeared for plant exposed to nitrate during vegetative and flowering stage. The ability to recover of SNF after nitrate removal is partly dependant on level of C availability to nodules. Thus, pea SNF can recover when a short-term inhibition by nitrate occurs before seed filling stages.

Keywords: *Pisum sativum* L.; Symbiotic N_2 Fixation, Nodules, Carbon, Nitrate.

† In tribute to Yves Crozat who had taken part in the first planning of this research project before deceased on June 20th, 2007.

1. Introduction

Base upon use of air dinitrogen, N_2 Symbiotic nitrogen fixation (SNF) in legumes could contribute to save non-renewable resources (by contrast with soil N uptake) and avoid environmental detrimental impacts related to the use of N fertilizers (Peoples *et al.*, 1995; Jensen and Hauggaard-Nielsen, 2003). Accordingly, a comprehensive understanding of SNF regulation is crucial so that it can be optimally taken into account in N fertilizer application management. SNF relies on both symbiotic structure and activity of legumes.

SNF is under the control of long- and short- distance regulation acting at the plant and at the organ levels as well as environmental factors, of which mineral soil N availability (Voisin *et al.*, 2002). Hence, numerous studies have established that nitrate ions can inhibit SNF (Streeter, 1985a, b). However, the way nitrate ions act on SNF is still under debate as several hypothesis are proposed. For instance, Streeter (1988) showed that nitrate inhibition affects structure rather than activity of nodules by impairing nodule growth. At the nodule level, modification of flavonoid production (Bandyopadhyay *et al.*, 1996) or infection blocking of root hairs by *Rhizobium* (Dazzo et Brill, 1978) may be involved in a decrease of nodule appearance. Moreover, nodule activity may also dramatically reduced when considering a putative negative feedback by products resulting from nitrate reduction (Bacanamwo and Harper,1997; Neo and Layzell, 1997) or a disturbance of the respiration of bacteroids through a decrease of the dioxygen diffusion into the nodule (Gordon *et al.*, 2002). At a whole plant level, a long-term inhibition may also play a major role through a limited photosynthate flows from shoot to root. (Francisco and Akao, 1993). As shown by Fujikake *et al.* (2003), a shortage of newly fixed carbon skeletons flow from shoot to root resulted in stopping cell expansion within nodules.

More importantly, the recovery of nodules growth and activity after inhibition due to short-term exposure to nitrate was not much investigated. Fujikake *et al.* (2002; 2003) have shown that nodule growth was immediately decreased by a short-term addition of nitrate (5 mMol L^{-1}), but rapidly recovered after nitrate removal. This inhibition effect was linked to photosynthate availability as adding 3 % (w/v) sucrose to the medium resulted in recovering initial nodule dry weight (Fujikake *et al.*, 2003; Raggio *et al.*, 1965). By contrast, N_2 fixing activity was enhanced by short-term exposure to nitrate at the beginning of the soybean cycle. Fujikake *et al.* (2002) hypothesized that early nitrate supply led to an increase of shoot dry weight, and thus to increase level of C supply to nodules when nitrate inhibition was removed. Indeed, activity of nodule largely relies on C nutrition (Kouchi *et al.*, 1986). Amounts of carbon skeletons supplied from shoot to nodules are highly variable according to species, age of root or nodule and plant phenology. Voisin *et al.* (2003a) demonstrated that carbohydrate supply to nodules decreases from 45 to 7 % of the net photosynthesis between early vegetative stages and seed filling. Thus, carbon is mainly allocated downward to nodulated roots during vegetative stages before being massively drain to newly appeared sinks for carbon and nitrogen such as reproductive tissues during seed filling stages (Voisin *et al.*, 2003b; Jeuffroy and Warembourg, 1991).

Nitrate inhibition of nodule growth depends on amount of nitrate supplied, period of nitrate supply and legume species (Davidson and Robson, 1986a). However, the studies on short-term inhibition induced by nitrate and succeeding recovery have mainly been carried out during the first weeks after seedling emergence and investigations at later stages are needed. Jensen (1986) have studied time effect by applying nitrates at sowing, beginning of pea flowering and flat pod stage in peas grown in pots. In comparison with no fertilized peas, the least decreasing effect of fertilizer nitrate (1.2 g N pot^{-1}) on the amount of N derived from air accumulated in pea plant was obtained by application at the flat stage pod. Moreover, contribution of N derived from air

Table 1: Composition of nutrient solutions

Nutrient salts	**Salt concentration (meq L^{-1})**	
	Control	**N**
KNO_3	0.0	1.5
K_2HPO_4	1.6	1.6
$Ca(NO_3)_2$	0.0	3.5
$MgSO_4$	2.0	2.0
$CaCl_2$	5.0	1.5
K_2SO_4	1.4	0.15
$NaNO_3$	0.0	0.0
NaCl	0.2	0.2
KH_2PO_4	0.0	0.0
$Mg(NO_3)_2$	0.0	0.0
mean observed atom ^{15}N (%)	0.0	2.79

in the total N accumulated in pea plant was more highly decreased by N-applications (2.4 g N pot^{-1}) at sowing than the same amount of fertilizer applied at flat pod stage. This confirms that period of exposure leads to different effect on plant N-acquisition and SNF functioning. However, these observations were realised at maturity and no information were given on symbiotic activity dynamics and on nodule growth dynamics.

Inhibition and succeeding recovery of nodule growth and activity may vary with crop stages according to N demand, allocation of C in different parts of the plant and age of nodules. Nitrates may affect N_2 fixation both by altering the structure of fixing nodules (number and dry weight) and limiting their specific activity. The effects of nitrates on the structure and the activity of nodules should be distinguished for a complete analysis of nitrates effects on SNF.

Our main objectives were i) to study the inhibition effect of a short-term exposure to nitrate applied at different crop stages on symbiotic structure and their activity and ii) to analyse the ability of SNF to recover after such exposure to nitrate with particular attention to C nutrition.

2. Material and Methods

2.1. Biological material and general growth conditions

1200 pea seeds (*Pisum sativum* L. cv Baccara) were first weighed and calibrated to 280-300 mg and then seeded for germination on moist filter paper in the dark at 17 °C. As soon as the radicle had reached 2-3 cm, 320 seedlings were selected for developmental homogeneity and transferred to hydroponics culture. The experiments were arranged in two randomised complete block designs (one for natural light, one for shading) with four blocs and two replicates per bloc. Plants were grown in 5.6 L pots covered with a lid bored with two holes so that each pot hosted two plants. In each pot, an inner wall separated the root systems of the two plants. The pots were covered with aluminium sheet to maintain the roots in the dark and to limit excessive heating of the nutrient solution by solar radiation.

Solution was continuously aerated, in each pot compartment, by a minimum airflow of 0.25 L min^{-1}. Plants were inoculated with *Rhizobium leguminosarum* bv *vicieae* (strain P221) so as to obtain a concentration of 10^8 Rhizobia per plant. pH of the nutrient solution was controlled and maintained between 6.5 and 7.5, which enables Rhizobia to survive freely in solution and to efficiently nodulate pea plants (Amarger, personal communication). Nutrient solution (Table 1) was supplied to compensate for plant consumption as necessary to keep optimal level in the pots. It was also totally renewed and re-inoculated at least every two weeks and each time its composition was changed. To avoid any deficiency, trace element and EDFS were added in nutrient solution. For fixation rate calculation, 5 meq L^{-1} nutrient solutions have been enriched with $K^{15}NO_3$ with a target of $\%A^{15}N=3$ %. This isotopic enrichment had been beforehand tested to be efficient to detect N flux in plant sampling.

Air and solution temperatures were monitored every minute by thermocouples and recorded in a data logger (CR10X, Campbell Scientific, Logan, UT, USA). Mean air temperature was 19.6 ± 1.9 °C, and mean solution temperature was 20.6 ± 1.9 °C. Outdoor incident cumulative Photosynthetically Active Radiation (PAR) above greenhouse was of 940 MJ m^{-2}. For calendar reason, natural photoperiod was decreasing. In order to reach flowering stages, it was artificially enlarged up to 16 hours by the use of electric light (neon were used to avoid interference on photosynthesis). Light availability for shaded pea was decreased of 65 % in comparison of that for pea grown natural light.

Table 2: Experimental treatments

Reference	Date of NO_3^- exposition (DAE)	Dose of NO_3^- exposition ($meq.L^{-1}$)	Growth conditions after NO_3^- exposition
Control	---	0	natural light
N_{Veg} L+	from 14 to 22	5	natural light
N_{Veg} L-		5	shaded
N_{Flo} L+	from 49 to 57	5	natural light
N_{Flo} L-		5	shaded
N_{SF} L+	from 56 to 64	5	natural light
N_{SF} L-		5	shaded

DAS: Days After seedling Emergence; Veg: Vegetative stage; Flo: Flowering; SF: Seed Filling; L+ and L-: growth under natural light and under shading, respectively.

Germination started with imbibition on 11/08/08 and first nodules appeared 10 Days After seedling Emergence (DAE) (on 23/08/08). Beginning of flowering and beginning of seed filling occurred 42 DAE (on 24/09/08) and 56 DAE (on 08/10/08), respectively. Last harvest was at 78 DAE (on 30/10/08) with 40.5 % ± 2.59 of dry mater in grains. During the experiment pests and diseases were controlled with pesticide applications when required.

2.2. Treatments, sampling and analytical method

First treatment (Control) was never exposed to nitrates and never shaded (Table 2). Other treatments were exposed only once during crop cycle for seven days to a nutrient solution containing nitrates. Three different dates of nitrates exposure were tested, during vegetative stage (N_{Veg}) (after firsts nodules appearance), during Flowering (N_{Flo}) and at beginning of Seed Filling (N_{SF}). Nitrates exposure has been realised at the concentration of 5 meq L^{-1}. After the week of nitrates exposure, half of the plants was grown under natural light (L+) and other half was grown under shade until harvest (L-).

Each treatment was sampled several times during crop cycle: before nitrates exposure, at the end of nitrates exposure and every two weeks until final harvest. Plants were divided in different organs: initial seed, nodules, roots, shoot and reproductive organs. Before drying, nodules were separated from roots and placed on a plate to be pictured with Canon EOS 350D digital (lens: SIGMA 50mm F2.8 DG). Pictures were analysed with ImageJ version 1.40g freeware (National Institute of Health, USA; http://rsb.info.nih.gov/ij/) in order to obtain the number of nodules. Dry weight of each organ was determined after oven drying at 70 °C for 48 h. Roots and nodules were ground together, as well as aerial parts and N content were measured according to Dumas procedure (Hansen, 1989). ^{15}N enrichment was determined by mass spectrometry on initial seed samples, before nitrates exposure samples, end of nitrates exposure samples and as well on nutrient solution samples.

2.3. Calculations and statistical analysis

From the date t_a to the date t_b, the quantity of N derived from air ($Ndfa_{a\rightarrow b}$) was calculated as the difference between increments of total accumulated N ($Nplte_{a\rightarrow b}$) and of N derived from nitrate absorption during nitrate exposure ($Ndfn_{a\rightarrow b}$):

$$Ndfa_{a\rightarrow b} = Nplte_{a\rightarrow b} - Ndfn_{a\rightarrow b} \qquad (g\ plte^{-1}) \qquad \text{Eq 1}$$

N derived from seed was not taken into account because contribution of seed to total N accumulated by plant was not significant from the date of the first sampling (14 days after seedling emergence).

The contribution of N derived from air to total accumulated N in plant was calculated as the ratio between the quantity of N derived from air and the sum of N derived from absorption and fixation. Thus, from the date t_a to the date t_b, the contribution of N derived from air to total accumulated N in plant ($\%Ndfa_{a\rightarrow b}$) was calculated as follows:

$$\%Ndfa_{a\rightarrow b} = (Ndfa_{a\rightarrow b} / (Ndfa_{a\rightarrow b} + Ndfn_{a\rightarrow b})*100 \qquad (\%) \qquad \text{Eq 2}$$

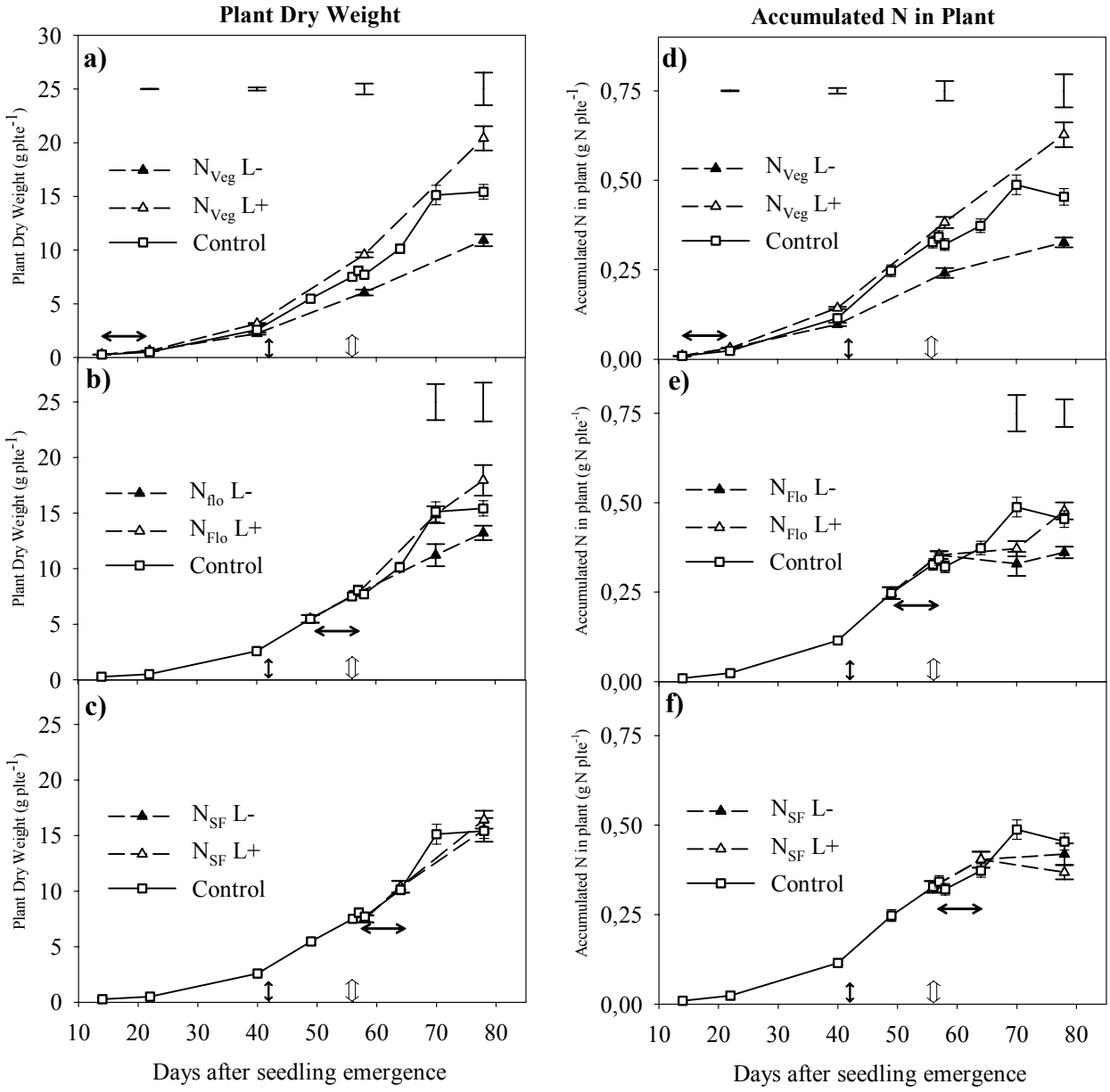

Figure 1: Growth and N acquisition dynamics of pea plants

Control: pea plant never exposed to NO_3^- and grown under natural light. N_{Veg}, N_{Flo}, and N_{SF}: pea plant exposed to NO_3^- (5 meq NO_3^- L^{-1}) during Vegetative stage, Flowering or Seed Filling, respectively. Values are means (n=8) ± SE (standard errors bars on plots). Analysis of variance (type III sum of squares, α= 5%) was carried out to compare treatments at respective date of observation: vertical bars represent Tukey's HSD (α=5 %). ↔: exposure to nitrates ↕: beginning of pea flowering. ⇕: beginning of pea seed filling.

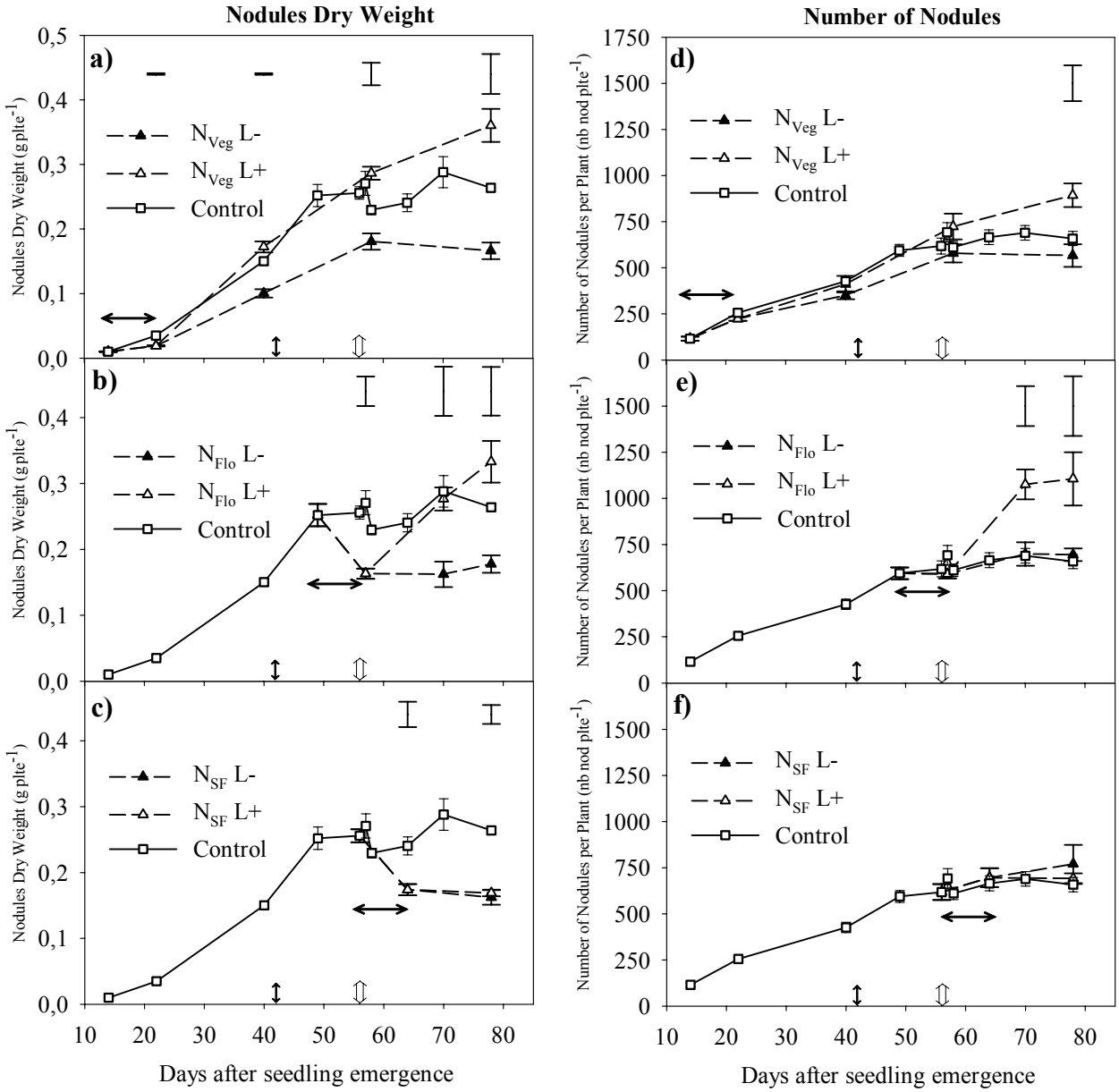

Figure 2: Dynamics of nodules dry weight accumulation and of number of nodules per pea plants

Control: pea plant never exposed to NO_3^- and grown under natural light. N_{Veg}, N_{Flo}, and N_{SF}: pea plant exposed to NO_3^- (5 meq NO_3^- L^{-1}) during Vegetative stage, Flowering or Seed Filling, respectively. L+ and L-: growth conditions after NO_3^- exposure (under natural light, and shade until harvest, respectively). Values are means (n=8) ± SE (standard errors bars on plots). Analysis of variance (type III sum of squares, α= 5%) was carried out to compare treatments at respective date of observation: vertical bars represent Tukey's HSD (α= 5%). ↔: exposure to nitrates ↕: beginning of pea flowering. ⇕: beginning of pea seed filling.

Table 3: Pea growth; N-acquisition rate; nodules growth rate, appearance rate and activity; and biomass partitioning during the two weeks following the removal of nitrate exposure

Treatments		Plant growth rate (mg DW $d^{-1}plte^{-1}$)			Plant N-acquisition rate (mg N $d^{-1}plte^{-1}$)			Nodules growth rate (mg DW $d^{-1}plte^{-1}$)			Appearance rate of nodules (nb nod $d^{-1}plte^{-1}$)			%Ndfa (%)			Specific activity of nodules (g N [g nod DW]$^{-1}$ d^{-1})			(nodulated root) / plant (on DW basis)			nodules / (nodulated root) (on DW basis)		
Time	Reference	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD
14-22 DAE	Control	**28.50**	±0.968	b	**1.81**	±0.059	b	**3.11**	±0.108	a	**17.53**	±1.557	a	**100**	±0.000	a	**0.0797**	±0.0025	a	**0.36**	±0.005	a	**0.20**	±0.005	a
	N_{veg} L+	**41.32**	±1.622	a	**2.72**	±0.113	a	**1.10**	±0.098	b	**13.52**	±0.966	b	**11**	±2.875	b	**0.0215**	±0.0055	b	**0.32**	±0.004	b	**0.10**	±0.004	b
49-57 DAE	Control	**324.29**	±50.782	---	**11.80**	±2.158	---	**2.34**	±2.792	a	**12.21**	±7.247	---	**100**	±0.000	a	**0.0458**	±0.0083	a	**0.15**	±0.005	---	**0.23**	±0.007	a
	N_{Flo} L+	**291.68**	±34.761	---	**13.26**	±1.714	---	**-11.10**	±1.906	b	**-0.53**	±5.123	---	**12**	±4.731	b	**0.0114**	±0.0049	b	**0.14**	±0.004	---	**0.15**	±0.009	b
56-64 DAE	Control	**326.61**	±68.272	---	**5.69**	±2.640	---	**-1.92**	±2.553	a	**5.95**	±6.204	---	**100**	±0.000	a	**0.0301**	±0.0070	a	**0.11**	±0.003	---	**0.22**	±0.009	a
	N_{SF} L+	**360.58**	±85.214	---	**9.48**	±3.643	---	**-10.23**	±1.152	b	**9.78**	±9.546	---	**2**	±1.382	b	**0.0016**	±0.0013	b	**0.11**	±0.003	---	**0.16**	±0.009	b

DAE: Days After seedling Emergence; Control: pea plant never exposed to NO_3^- and grown under natural light. N_{Veg}, N_{Flo}, and N_{SF}: pea plant exposed to NO_3^- (5 meq NO_3^- L^{-1}) during Vegetative stage, Flowering or Seed Filling, respectively d: day; DW: Dry Weight; nb nod: number of nodules. %Ndfa is the calculated as the ratio between N derived from air and the sum of N derived from air and N derived from root absorption. "Specific activity of nodules" is calculated as the ratio between the quantity of fixed N_2 and the accumulation rate of nodules dry weight. Values are means (n=8) ± SE (standard errors bars on plots). Analysis of variance (type III sum of squares, α=5 %) was carried out and treatments with the same letter are not significantly different (Tukey's HSD test, α=5 %), whereas "---" means that the analysis of variance indicates no significant difference between treatments.

Table 4: Pea growth; N-acquisition rate; nodules growth rate, appearance rate and activity; and biomass partitioning during the two weeks following the removal of nitrate exposure

Treatments		Plant growth rate (mg DW $d^{-1}plte^{-1}$)			Plant N-acquisition rate (mg N $d^{-1}plte^{-1}$)			Nodule growth rate (mg DW $d^{-1}plte^{-1}$)			Appearance rate of nodules (nb nod $d^{-1}plte^{-1}$)			Specific activity of nodules (g N [g nod DW]$^{-1}$ d^{-1})			(nodulated root) / plant (on DW basis)			nodules / (nodulated root) (on DW basis)		
Time	Reference	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD	**mean**	SE	HSD
22-40 DAE	Control	**115.47**	±4.659	b	**5.06**	±0.246	b	**6.41**	±0.356	b	**9.48**	±1.791	---	**0.0546**	±0.0014	---	**0.22**	±0.003	a	**0.27**	±0.007	a
	N_{veg} L+	**141.17**	±4.044	a	**6.24**	±0.189	a	**8.53**	±0.472	a	**10.42**	±2.035	---	**0.0663**	±0.0035	---	**0.22**	±0.006	ab	**0.25**	±0.008	ab
	N_{veg} L-	**90.44**	±5.314	c	**3.69**	±0.263	c	**4.53**	±0.360	c	**7.06**	±1.187	---	**0.0627**	±0.0049	---	**0.20**	±0.004	b	**0.22**	±0.012	b
57-70 DAE	Control	**543.04**	±64.934	a	**11.22**	±2.370	a	**1.34**	±2.683	b	**-0.20**	±3.919	b	**0.0398**	±0.0085	a	**0.08**	±0.004	---	**0.23**	±0.007	a
	N_{Flo} L+	**542.55**	±61.933	a	**1.35**	±1.856	b	**8.73**	±1.486	a	**37.37**	±6.424	a	**0.0122**	±0.0061	b	**0.09**	±0.004	---	**0.21**	±0.011	a
	N_{Flo} L-	**262.45**	±61.963	b	**-1.89**	±2.062	b	**-0.07**	±1.600	b	**8.38**	±6.175	b	**0.0079**	±0.0037	b	**0.09**	±0.006	---	**0.15**	±0.010	b
64-78 DAE	Control	**378.84**	±66.376	---	**5.78**	±2.171	---	**1.67**	±1.260	---	**-0.51**	±3.385	---	**0.0243**	±0.0086	---	**0.07**	±0.003	---	**0.25**	±0.006	a
	N_{SF} L+	**431.64**	±71.096	---	**-2.50**	±1.974	---	**-0.36**	±0.674	---	**-0.37**	±2.599	---	**0.0078**	±0.0063	---	**0.07**	±0.003	---	**0.15**	±0.005	b
	N_{SF} L-	**366.45**	±86.209	---	**1.11**	±2.611	---	**-0.84**	±0.933	---	**5.30**	±9.071	---	**0.1991**	±0.0089	---	**0.08**	±0.001	---	**0.14**	±0.007	b

DAE: Days After seedling Emergence; Control: pea plant never exposed to NO_3^- and grown under natural light. N_{Veg}, N_{Flo}, and N_{SF}: pea plant exposed to NO_3^- (5 meq NO_3^- L^{-1}) during Vegetative stage, Flowering or Seed Filling, respectively. L+ and L-: growth conditions after NO_3^- exposition (under natural light, and shade until harvest, respectively). d: day; DW: Dry Weight; nb nod: number of nodules. "Specific activity of nodules" is calculated as the ratio between the amount of fixed N_2 and the accumulation rate of nodules dry weight. Values are means (n=8) ± SE (standard errors bars on plots). Analysis of variance (type III sum of squares, α=5 %) was carried out and treatments with the same letter are not significantly different (Tukey's HSD test, α=5 %), whereas "---" means that the analysis of variance indicates no significant difference between treatments.

From the date t_a to the date t_b and during period of nitrate exposure, the amount of absorbed nitrate ($Ndfn_{a\rightarrow b}$) was calculated as the product of pea dry weight with %N content and the proportion of plant N derived from nitrate absorption ($\%Ndfn_{a\rightarrow b}$). $\%Ndfn_{a\rightarrow b}$ was calculated as follows (Rennie and Rennie, 1983):

$$\%Ndfn_{a\rightarrow b} = \frac{\%A^{15}N\ excess_N - \%A^{15}N\ excess_{N0}}{\%A^{15}N\ excess_{Sol} - \%A^{15}N\ excess_{N0}} * 100 \quad (\%) \qquad \text{Eq 3}$$

Where:

$\%A^{15}N\ excess_N$ = isotopic ^{15}N excess of pea exposed to nitrate, at the date (b)
$\%A^{15}N\ excess_{N0}$ = isotopic ^{15}N excess of pea never exposed to nitrate, at the date (b)
$\%A^{15}N\ excess_{sol}$ = isotopic ^{15}N excess of the nutrient solution with nitrate, at the date (b)

From the date t_a to the date t_b, efficiency of nodule for N_2 fixation (ε) is defined as the ratio between the quantity of fixed N_2 ($Ndfa_{a\rightarrow b}$) and the accumulation rate of nodules dry weight (DW_{nod}). ε was calculated as follows (Voisin *et al.*, 2007):

$$\varepsilon = \frac{Ndfa_{a\rightarrow b}}{\int_{t_a}^{t_b} DW_{nod}.dt} \quad (g\ N\ [g\ nod\ d^{-1}]^{-1}) \qquad \text{Eq 4}$$

Where:

$$\int_{t_a}^{t_b} DW_{nod}.dt = \sum_{i=a}^{b} (DW_{nod(i)} + DW_{nod(i+1)}) x (t_{(i+1)} - t_{(i)}) / 2 \qquad \text{Eq 5}$$

(trapezoid sum approximation)

Normal distribution was tested with the Pearson chi-square test (α=5 %). Analyses of variance were performed (type III sum of squares, α=5 %) and means were compared using Tukey's HSD test (Honest Significant Differences, α=5 %), if a main effect or interaction was significant, using R software (R Development Core Team, 2009).

3. Results

3.1. Growth, N acquisition, structure and activity of nodules without exposure of nitrate

Dry weight (DW) of pea grown without nitrates regularly increased and reached 15 g pl^{-1} 70 days after imbibition, then remained constant. A similar N accumulation pattern was monitored until the harvest (0.5 g pl^{-1} at maturity; Figure 1). The number and dry weight of nodules increased up to 50 DAE and then remained constant (Figure 2). The ratio of nodulated root DW / plant DW decreased during the crop cycle from 0.36 to 0.07. The ratio of nodule DW / nodulated root DW varied from 0.20 to 0.27 (Table 3 and 4). The activity of nodules decreased during the crop cycle from 0.08 to 0.02g N [g nod $d^{-1}]^{-1}$ (Table 3 and 4).

3.2. Effect of nitrates on N acquisition, growth, structure and activity of nodules during exposure

3.2.1. N acquisition and growth during nodulated root exposure to nitrates

Exposing peas to nitrate at a vegetative stage results in a higher pea growth rate (+45 %; .Table 3). N acquisition rate also increased by 50 % compared to unfertilized treatment. On the

contrary, pea growth and N accumulation patterns were similar when peas was exposed to nitrates during flowering and seed filling compared to unfertilized treatment. Contribution of N derived from air to total N-acquisition (%Ndfa) during the exposure to nitrates was very low (from 2 to 12 %) whatever the date of exposure (Table 3).

3.2.2. Structure and activity of nodules during nodulated root exposure to nitrates

Concerning the nodule structure, the number and dry weight of nodules increased with a lower rate when nitrates are applied at the vegetative stage whereas a root exposure to nitrate during the flowering and seed filling stages hardly changed the number of nodules and decreased nodule growth (Table 3).

Nitrates dramatically decreased the specific activity whatever the stage of exposure to nitrate (Table 3). The decrease was higher with an exposure during seed filling (-93 %) than during vegetative and flowering stages (-73 %).

3.3. Pea N acquisition and growth, structure and activity of nodules after nodulated root exposure to nitrates

3.3.1. Under natural light conditions

Pea growth and N acquisition

Growth and N acquisition dynamics were monitored after removing nitrate (Figure 1). Results clearly show that the period of nitrate exposure has a massive effect on growth and N acquisition. Hence, after removal of nitrate applied during the vegetative period, pea plants exhibit both higher growth and N accumulation rates (+20 %) than control plants (*ie* not exposed to nitrate; Table 4). At harvest, dry weight and N accumulated were increased by 32 and 40 %, respectively, compared to control plants (Figure 1). By contrast, removal of nitrate after exposing root to nitrate during the flowering period results in a similar growth compared to control treatment and a reduced N accumulation rate, yet a recovery of N accumulation is monitored between 70 and 78 DAE (Figure 1). Finally, removing nitrate after root exposure during seed filling stopped growth and N accumulation from then until harvest: no recovery was recorded (Figure 1).

Structure and activity of nodules

The period of root exposure to nitrate has a massive effect on nodule dry weight and, to a much lesser extent, on the number of nodules patterns after the removal of nitrate (Figure 2). Results point out that nodule dry weight can recover to a control level at harvest during the period following the removal of nitrate when nitrate is applied before seed filling (*i.e.* during vegetative and flowering stages). For instance, dry weight of nodules increased with a higher rate (+33 % compared to control) after removal of nitrate applied during the vegetative stages, entailing a similar nodule dry weight during 30 days and a larger dry weight and number of nodules at the final harvest (+36 % for both dry weight and number compared to Control at 78 DAE) (Figure 2). In the same line, nodule dry weight recovered from exposure to nitrate at flowering. Hence, nodule dry weight and the nodules number kept on increasing from the removal of nitrate to the final, harvest to reach 0.35 g $plte^{-1}$ and 1106 nodules $plte^{-1}$, respectively, whereas the dry weight and the of nodules remained constant for the control (0.26 g $plte^{-1}$ and 658 nodules $plte^{-1}$, respectively) (Figure 2). However, no modification about the growth or number of nodules was observed after the removal of nitrate applied to root during the seed filling stages compared to control (Table 4): the nodule dry weight remained constant (Figure 2).

Specific activity pattern, defined as the ratio between the amount of fixed N_2 and the accumulation rate of nodules dry weight, was also monitored and is presented in Table 4.

Similar specific activity values were calculated following removal of nitrate during either vegetative period (from 0.0546 to 0.063 g N [g nod d^{-1}]$^{-1}$) or seed filling period (from 0.0078 to 0.0243 g N [g nod d^{-1}]$^{-1}$) compared to control. However, the specific activity was reduced after removing nitrates applied during the flowering period (0.0398, 0.0122, and 0.0079 g N [g nod d^{-1}]$^{-1}$ for Control, N_{Flo} L+ and N_{Flo} N6, respectively) (Table 4).

3.3.2. Under low light conditions

Both pea growth and N acquisition rates under low light conditions after the removal of nitrates were reduced by 36 %, 51 % and 14 % at vegetative phase, flowering and seed filling, respectively (Table 4). At the final harvest (78 days after seedling emergence), dry weight and N accumulation were divided by 2 compared to plants under natural light conditions (Figure 2). Shading pea resulted in a reduced ability of pea to counteract the effect of root exposure to nitrate. Indeed, the increase of nodule dry weight after an exposure to nitrate during vegetative stages and the flowering period was lower and null for shaded plants, respectively. At 78 DAE, the number of nodules of N_{Flo} L- was reduced by 37 % and the dry weight of nodules by 47 % compared to N_{Flo} L+ (Figure 2). There was no modification of the number and growth of nodules after an exposure to nitrates during seed filling stages for shaded plants compared to plants never exposed to nitrates as observed for plants grow under natural light (Table 4 and figure 2).

3.4. Pattern of C allocation during and after nodulated root exposure to nitrate

The ratio of nodulated root DW / plant DW decreased when roots were exposed to nitrates during the vegetative stages but this ratio value was not modified when nitrate was applied during the flowering and the seed filling period (Table 3). The ratio of nodule DW / nodulated root DW was reduced by 50% during root exposure to nitrate, whatever the stage (Table 3).

After the removal of nitrates, the ratio values of nodulated root DW /plant DW were similar to those ones under control conditions, but for the shaded treatment. Indeed, the nodulated root DW-to-plant DW ratio value of shaded plant exposed to nitrate during vegetative stage was slightly decreased (Table 4). The ratio of nodule DW/nodulated root DW increased after the removal of nitrates: it was similar to the control except for shaded plants and plants exposed at seed filling whatever light conditions (Table 4).

4. Discussion

4.1. Nitrate affects both structure and activity of nodules

An exposure to nitrates had different effects on the structure of fixing nodules according to the stage of pea. As shown by Fujikake *et al.* (2002), Pate and Dart (1961) and Daimon *et al.* (1999) dry weight of nodules was always decreased during nitrate exposure in comparison with that of plants never exposed to nitrates, whatever the period of N exposure (Table 3). Numbers of nodules were only decreased by nitrate exposure during vegetative stage. Thus, our results complete previous observations by clearly demonstrating that nitrates reduced the speed of nodule establishment during vegetative phases whereas it entailed damage on existing nodules during flowering and seed filling.

SNF was almost totally replaced by nitrate absorption, whatever the stage of exposure: nitrates heavily limited SNF and specific activity of nodule was divided by 2.

4.2. Starter effect on plant growth by early exposure to nitrate

Plant growth was significantly enhanced during nitrate exposure at the early vegetative stages whereas for later stages (during flowering and seed filling), crop growth was similar with or without nitrate assimilation (Figure 1). This is in accordance with Jensen (1986) who has demonstrated that low N application at sowing (before nodules appearance) acted as a starter supply by increasing dry weight production of pea plant. Moreover, during vegetative stages, root DW / plant DW ratio were increased by 50 % by nitrate exposure indicating an unsatisfied N demand, as suggested by the "functional equilibrium" theory of Brouwer (1962) and as previously observed (Davidson and Robson, 1986b; Voisin et al., 2003c). Thus, at early vegetative stages, plant growth seemed to be less limited by nitrate absorption by than N_2 fixation which seemed not to be able to totally satisfy N demand. Two hypotheses could be proposed to explain that pea can not respond to a high N demand linked to a high growth: time needed for the establishment of nodules or time needed for starting fixation activity.

4.3. Recovery of SNF is highly dependant on C nutrition

Nodule growth dynamics after nitrate removal (Figure 2) indicated that recovery did not occur for pea grown under low light, of for pea exposed to nitrate during seed filling. Same patterns could be observed with nodules DW / nodulated root DW ratio. It decreased by 27 to 50 % during nitrate exposure, whatever the stage of pea. Then, it increased after nitrates removal to become not significantly different from that observed on plant never exposed to nitrates, except for pea which were grown under low light and for pea exposed during seed filling. This suggests that the ability to recover of SNF is dependant on level of C nutrition to nodule and confirmed conclusions of Fujikake *et al.* (2002; 2003).

In low light growth conditions and after nitrate exposure at early vegetative stage, plant growth, C allocation to nodules and nodules growth were lower than those observed on plant never exposed to nitrate. This argues for a systemic regulation of SNF which adjust itself to plant growth in conditions of limited photosynthesis (due to low light availability) (Francisco and Akao, 1993).
After exposure during flowering stages, specific activity of nodules remained at the low level observed during nitrate exposure. In these conditions, the low availability of photosynthates due to shaded conditions did not allow SNF recovery, and the damage on nodule growth during exposure to nitrates was not compensated by new nodules due to a low carbon allocation to nodules entailing an early stop of N accumulation.
After exposure during seed filling stage, C allocation to root and nodule is known to be in competition with seed filling (Voisin *et al.*, 2003b; Jeuffroy and Warembourg, 1991) and recovery did not occur.

On the contrary, a second wave of nodule appearance was observed for pea exposed during vegetative and flowering stages, and grown under natural light conditions (Figure 2). At early vegetative stage, nitrates increased plant growth and plant N-acquisition during nitrate exposure and after removal. Then, the growth of nodules which was affected during exposure to nitrates was rapidly recovered due to a high C availability and proved plasticity traits.

After the two weeks succeeding an exposure to nitrates during flowering, N accumulation remained at the level observed during nitrate exposure due to the delay between nodules establishment and their activity is too long and new nodules did not benefit to plant N nutrition. But, at the end of the plant cycle, N accumulation in plant reached the level of the control.

5. Conclusion

The results presented here demonstrate that inhibition due to short-term inhibition to nitrate differently affect structure and activity of nodules among stage of exposure. Moreover succeeding recovery was shown as dependant on stage of exposure and C nutrition. Second wave of nodulation appeared for plant exposed to nitrate during vegetative and flowering stage. Such ability could be interesting to increase yield of field pea. However for pea exposed to nitrate during flowering, new nodules were not able to enhance N nutrition of plants because of delay for establishing nodule and fixing activity or because of C competition with seed filling.

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References

Bacanamwo M, Harper JE. 1997. The feedback mechanism of nitrate inhibition of nitrogenase activity in soybean may involve asparagines and/or products of its metabolism. Physiologia Plantarum 100, 371-377.

Bandyopadhyay AK, Veena J, Nainawatee HS, 1996. Nitrate alters the flavonoid profile and nodulation in pea (Pisum sativum L.). Biol. Ferti. Soils, 21, 189-192.

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth. J. Agric. Sci. 10, 399-408.

Daimon H, Hori K, Shimizu A, Nakagawa M. 1999. Nitrate-induced inhibition of root nodule formation and nitrogenase activity in the peanut (Arachis hypogaeg L.). Plant Proc. Sci. 2, 81-86.

Davidson IA, Robson MJ. 1986a. Effect of contrasting patterns of nitrate application on the nitrate uptake, N2 fixation, nodulation and growth of white clover. Annals of Botany 57, 331-338.

Davidson I, Robson M. 1986b. Interactions between nitrate uptake and N2 fixation in white clover. Plant and Soil 91, 401-404.

Dazzo FB, Brill WJ, 1978. Regulation by fixed nitrogen of host-symbiont recognition in the Rhizobium-clover symbiosis. Plant Physiol., 62, 18-21.

Francisco PB, Akao S, 1996. Autoregulation and Nitrate Inhibition of Nodule Formation in Soybean cv. Enrei and its Nodulation Mutants. J. Exp. Bot. 44, 547-553.

Fujikake H, Yamazaki A, Ohtake N, Sueyoshi K, Matsuhashi S, Ito T, Mizuniwa C, Kume T, Hashimoto S, Ishioka N-S, Watanabe S, Osa A, Sekine T, Uchida H, Tsuji A, Ohyama T. 2003. Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. J. Exp. Bot. 54, 1379-1388.

Fujikake H, Yashima H, Sato T, Ohtake N, Sueyoshi K. 2002. Rapid and reversible nitrate inhibition of nodule growth and N2 fixation activity in soybean (Glycine max (L.) Merr.). Soil Sci. Plant Nutr. 48, 211-217.

Gordon AJ, Skot L, James CL, Minchin FR. 2002. Short-term metabolic responses of soybean root nodules to nitrate. J. Exp. Bot. 53, 423-428.

Hansen B. 1989. Determination of nitrogen as elementary N, an alternative to Kjeldhal. Acta Agric.Scan. 39, 113-118.

Jensen ES. 1986. The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (Pisum sativum L.). Fert. Res. 10, 193-202.

Jensen ES, Hauggaard-Nielsen H. 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? Plant and Soil 252, 177-186.

Jeuffroy, M.-H., Warembourg, F.R., 1991. Carbon transfer and partitioning between vegetative and reproductive organs in Pisum sativum L. Plant Physiol. 97, 440-448.

Kouchi H, Akao S and Yoneyama T 1986 Respiratory utilization of 13C-labelled photosynthate in nodulated root systems of soybean plants. J. Exp. Bot. 37, 985–993.

Neo HH, Layzell DB. 1997. Phloem glutamine and the regulation of O_2 diffusion in legume nodules. Physiologia Plantarum 113, 259-267.

Pate JS, Dart PJ. 1961. Nodulation studies in legumes. Plant and Soil 15, 329-346.

Peoples MB, Herridge DF, Ladha JK. 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant and Soil 174, 3-28.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raggio M, Raggio N, Torrey JG. 1965. The Interaction of Nitrate and Carbohydrates in Rhizobial Root Nodule Formation. Plant Physiology 40, 601-606.

Rennie RJ, Rennie DA, 1983. Techniques for quantifying N_2 fixation in association with non-legumes under field and greenhouse conditions. Can J Microbiol 29, 1022–1035.

Streeter JG. 1985a. Nitrate inhibition of legume nodule growth and activity. I. Long term studies with a continuous supply of nitrate. Plant Physiol. 77, 321-324.

Streeter JG. 1985b. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Plant Physiol. 77, 325-328.

Streeter JG. 1988. Inhibition of legume nodule formation and N_2-fixation by nitrate. CRC Critical Reviews in Plant Science 7, 1-23.

Voisin A-S, Bourion V, Duc G, Salon C. 2007. Using an Ecophysiological Analysis to Dissect Genetic Variability and to Propose an Ideotype for Nitrogen Nutrition in Pea. Ann Bot, 1-12.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003a. Seasonal patterns of 13C partitioning between shoots and nodulated roots of N2- or nitrate-fed Pisum sativum L. Ana. Bot.-London 91, 539-546.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003b. Symbiotic N2 fixation activity in relation to C economy of Pisum sativum L. as a function of plant phenology. J. Exp. Bot. 54, 2733-2744.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003c. Root and Nodule Growth in Pisum sativum L. in Relation to Photosynthesis: Analysis Using 13C-labelling. Ann Bot 92, 557-563.

Voisin A-S, Salon C, Munier-Jolain NG, Ney B. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (Pisum sativum L.). Plant and Soil 243, 31-42.

Synthèse partielle

Cette partie basée sur des expérimentations en conditions contrôlées a permis d'éclairer l'effet inhibiteur des nitrates et la réversibilité de la fixation chez le pois en analysant séparément l'impact sur la structure et sur l'activité de l'appareil fixateur en fonction du stade phénologique et de la disponibilité en carbone.
Elle démontre qu'en tout début de cycle, l'inhibition de l'appareil fixateur en lien avec une courte exposition aux nitrates se caractérise par un ralentissement de la croissance des nodosités, de la vitesse d'apparition des nodosités et une diminution de l'activité spécifique des nodules. Pendant les stades reproducteurs, l'inhibition se caractérise par une diminution de la croissance des nodosités et de l'activité spécifique. De plus, il a été démontré que la réversibilité de la fixation symbiotique après courte exposition aux nitrates était fonction de l'allocation carbonée aux nodosités. La réversibilité de la fixation symbiotique est possible chez le pois si une courte inhibition due aux nitrates survient avant les stades de remplissage du grain. Ainsi, la réversibilité de l'appareil fixateur ne s'est observée que dans les cas où le ratio nodules / racines nodulées augmentait à nouveau après l'exposition aux nitrates.

Chapitre 4 :
Modélisation et propositions de stratégies de conduites azotées

Simulation de la réponse d'association pois blé d'hiverà des régimes de fertilisation contrastés : développement, évaluation et utilisations d'AZODYN-IC

Ce chapitre vise à faire la synthèse des connaissances acquises sur le fonctionnement des associations pois-blé (partage de la lumière, interaction entre le partage de l'azote et de la lumière, *etc.*) dans le développement d'un modèle de culture AZODYN-IC adapté à ces cultures et rendant compte de l'impact de différentes dynamiques de disponibilité en azote minéral. AZODYN-IC a été construit par couplage de deux modèles de cultures pures existants : AZODYN pour le blé (Jeuffroy and Recous, 1999) et AFISOL pour le pois (Vocanson, 2006 ; Biarnès et al., 2009). La description du modèle et son évaluation à partir des résultats expérimentaux exposés au chapitre 2 font l'objet du premier article ci-dessous Partie I).

Ce chapitre vise aussi à utiliser AZODYN-IC afin de caractériser la réponse des performances (rendement total, proportion de blé dans le rendement, taux de protéines des grains de blé et de pois) de plusieurs associations (P70W30, P50W50, P30W70) à de larges gammes de disponibilité en azote minéral, et pour 26 années climatiques dans le contexte pédologique de La Jaillière. Ceci permet de prolonger la démarche expérimentale présentée dans le chapitre 2. L'objectif final de cette étude est la proposition de pistes pour la définition de règles de décision pour des conduites azotées adaptées à différents objectifs de production (article ci-dessous Partie II).

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PARTIE I:

SIMULATION OF THE RESPONSE OF A WINTER PEA-WHEAT INTERCROP TO CONTRASTED N-FERTILIZER APPLICATIONS: DESIGN AND ASSESSMENT OF AZODYN-IC MODEL.

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Running title: Simulated pea-wheat intercrop growth under contrasted N fertilizer applications

Abstract
Grain legume-cereal intercrops have gained higher interest under temperate regions as they allow a better use of available resources (nitrogen, water and light) together with lower inputs into a sustainable development framework. Modeling is a powerful tool to explore a wide range of practice combinations under various soil and climate conditions within a limited time-scale to optimize intercrop management. Our work aimed at providing a dynamic operational decision oriented intercrop growth model (AZODYN-IC) relying on updated knowledge about annual intercrop functioning in term of (i) resource sharing between species along the growth cycle (light and belowground resources, such as nitrogen and water), (ii) light-nitrogen acquisition interrelation into the intercrop, (iii) response to soil N availability. The presented model is based upon sole-crop growth models for wheat (AZODYN) and pea (AFISOL) well known to satisfactorily simulate sole-cropped wheat and pea growths under fluctuating soil N availabilities. Parameters were directly derived from the two sole crop models. Rules of resource sharing rely on nitrogen and water demand of each species. When the soil supply is limiting, water and N acquisition is limited by root exploration, soil nutrient supply and below-ground resources taken up by the companion species. Leaf area expansion is under the control of the satisfaction of N demand, it-self computed through an adapted version of N dilution curve to intercrop growth. Light sharing depends on LAI growth and leaf properties of each species. Dynamic pattern of simulated total nitrogen accumulation, LAI, dry matter accumulation of each species and soil inorganic nitrogen were confronted to an independent dataset from experiments conducted in 2007 and 2008 in La Jaillière (France) with contrasted N fertilizer applications (date and rate of N fertilizer applications). Yield, productivity and resource sharing were assessed with extra datasets from Grignon (France, 2007) and La Jaillière (2006, 2008). It results that AZODYN-IC can well simulate below-ground resource taken up, light capturing by intercrop as well as resource sharing between species along the growth cycle leading to realistic yields for the various combinations "applied N fertilizer rates × locations". Such a model will be further used to predict effects of different crop management combinations (plant density, date of sowing, date and rate of N fertilizers, for instance) on intercrop growths and yields. It can then be usefully handled as a valuable tool for guiding decision rules depending on targeting yields and species proportions at harvest.

Keywords*:* intercrop, competition, facilitation, light, nitrogen, model

1. Introduction

Intercropping consists of growing simultaneously two or more species on the same piece of land. This practice results in a more efficient use of available resources (such as soil water and nitrogen) than sole crops (see Malézieux et al., 2008 for a review). This result has largely been demonstrated through the calculation of a commonly used index known as the Land Equivalent Ratio often higher than 1 in spring pea-barley (Jensen, 1996a; Hauggaard-Nielsen and Jensen, 2001; Corre-Hellou et al., 2006) and spring pea-wheat (Ghaley et al., 2005) intercrops. This advantage can be partly explained by both differences in root exploration and nitrogen source (N derived from soil N uptake or from N_2 fixation) between the species. Cereals have an earlier date of emergence and a faster root penetration than pea (Corre-Hellou and Crozat, 2005a; Hauggaard-Nielsen et al., 2001). Accordingly they have a competitive advantage for the uptake of below-ground resources (water and nitrogen) early in the growth cycle. This advantage is strengthened by the fact that (i) cereal plant density is half of sole-cropped cereals into substitutive intercropping design resulting in higher available inorganic soil nitrogen amount per plant for wheat and (ii) intercropped grain legumes rely more on N derived from atmospheric N_2 fixation than sole-cropped legumes due to higher competition for soil nitrogen with the companion cereals (Corre-Hellou et al., 2006). Indeed, during the first growth stages, N demand by pea is satisfied by seed N remobilization. Then N demand is massively fulfilled through soil inorganic nitrogen root uptake before nodule establishment and start of atmospheric N_2 fixation activity, which accounts for the highest proportion of N removed by pea during the end of the crop cycle. Overall, species intercropping clearly results in increased yields along with no and low nitrogen inputs (Jensen, 1996a; Ghaley, et al., 2005; Corre-Hellou et al., 2006; Bedoussac et Justes, 2009).

Numerous studies have investigated the effect of inorganic soil nitrogen availability on intercrop growth, yield and species proportions at harvest varying the stand plant density (Bulson et al., 1997; Neumann et al., 2007), relative frequency (*i.e.* proportion of each species at sowing within an intercrop) (Hauggaard-Nielsen et al., 2006), rate of N fertilizer applications (Jensen, 1996a; Hauggaard-Nielsen et Jensen, 2001; Ghaley et al., 2005; Corre-Hellou et al., 2006, 2007). It has been found that intercrop yield does not increase along with available soil inorganic N but the proportion of cereals into intercrop increases at harvest. To make intercrops successful and widely used by farmers under temperate lands, the challenge is to determine to what extent N fertilization (rate and timing) can drive yields and proportions of each intercropped species as well as quality parameter values (protein contents in wheat grains in particular) within a highly variable climate and soil environment and accounting for different crop management practices. As stated earlier, most of the previous experiments focussed on a single factor and very few strived to study the combined effect of practices which is time-consuming.

Modelling can be helpful to screen all combinations and refine variety×crop practices ones that are the most suited to reach production goals. To date, most intercrop models have mainly focussed on competition for light by comparing different formalisms for light partitioning (maize-bean, Tsubo and Walker, 2002; maize-sorghum, Ozier-Lafontaine et al., 1997; millet-groundnut, Marshall and Willey, 1983; maize-sugarcane, Wallace et al., 1990; perennial ryegrass-white clover, Faurie et al., 1996). However, it has been demonstrated that crop growth, yield and grain quality, depend on both acquisition and species sharing of light as well as water and nitrogen under disease-free conditions. Indeed, below-ground resource acquisition and light partitioning are interrelated within cereal- legumes intercrops (Corre-Hellou et al., 2006). Accounting for nitrogen dynamics in intercrops is crucial to drive N fertilizer applications. Existing sole crop models can provide a solid ground to develop intercrop model. Hence, FASSET (Jacobsen et al., 1998; Berntsen et al., 2004) or STICS (Brisson et al., 2004; Corre-

Hellou et al., 2007; Corre-Hellou et al., 2009) have been extended for simulating spring pea-barley intercrop growth. In such models, light sharing depends on the simulation of the heights and LAI of each species. LAI is modelled on a thermal time basis in STICS (Corre-Hellou et al., 2009) and FASSET (Olesen et al., 2002). Height is either linked to LAI in STICS or a parameter in FASSET. LAI production is limited by crop nitrogen content and dry matter accumulation in both models. Moreover, in the intercropped version of STICS (Corre-Hellou et al., 2009), light sharing is based upon a detailed description of the canopy structure (shape of the canopy, thickness/length ratio of the shape, dominant and understorey canopies, shaded and lit parts) requiring numerous parameters to be estimated. Such a high complexity in those models (leaf area distribution along the canopy profile, height of the canopy) requires an estimate of numerous parameters and a step of calibration is needed to adapt the model to other intercrops. Moreover, there is no straight link between light sharing (*i.e.* LAI) and N acquisition in each species. However, numerous studies have demonstrated that leaf area expansion is linked to N demand satisfaction (through the N dilution curve and nitrogen nutrition index) (Corre-Hellou et al., 2006; Lemaire et al., 2007, 2008). Corre-Hellou et al. (2009) underlined the fact that light sharing for the development of an intercrop model designed to support a decision oriented-tool can be modelled through a basic operational description of LAI and leaf properties of each species for species with similar heights as usually observed in pea-cereal intercrops.

The proposed daily time step pea-wheat intercrop model (AZODYN-IC) is derived from the wheat AZODYN model and pea AFISOL model. AZODYN was basically designed to simulate nitrogen deficiency occurrence and subsequent effects on grain yield and nitrogen content through soil N availability, N uptake and crop growth processes under disease-free field conditions for sole wheat (*Triticum aestivum* L.) crops (Jeuffroy and Recous, 1999). The model was then successfully assessed against independent datasets from different wheat genotypes grown under a wide range of soil N availabilities, climate conditions and farming practices (David et al., 2004; David et al., 2005; Barbottin et al., 2006; David and Jeuffroy, 2009). It can now be used as a valuable and operational decision-making tool to (i) trigger real-time N fertilizer applications and (ii) rank best cultivars relative to yield, grain protein content and nitrogen losses through environment (Meynard et al., 2002; Barbottin et al., 2006). A crop model based on the same formalisms as AZODYN was also built for pea (*Pisum sativum* L.) growth. In this model, two ways of N acquisition by pea (nitrate root uptake and atmospheric N_2 fixation, namely) were included as described below (Biarnès et al., 2009; Vocanson, 2006). Due to their ability to respond to N availability, AZODYN (Jeuffroy and Recous, 1999) and AFISOL (Vocanson, 2006) were then chosen to build a model (AZODYN IC) simulating the response of intercrop growth and yield to contrasted N fertilizer applications. The final outcome is to provide a decision oriented-tool for managing N fertilizer application on intercrop depending on production goals.

This paper aims at designing and assessing a pea-wheat intercrop model, from the coupling of the two sole-crop models relying only on below-ground resources sharing interrelating with light interception through LAI growth, and without parameterization step of each sole crop model. The assessment of the model's ability to account for competition and complementarity for nitrogen and radiation was based on the availability of datasets from experiments conducted under various rates and dates of N fertilizer application in different locations.

2. Model description

AZODYN soil and plant processes have been extensively described previously (Jeuffroy and Recous, 1999; David et al., 2004; David et al., 2005). So was AFISOL model (Vocanson, 2006; Biarnès et al., 2009). For both species, crop growth is defined according to Monteith's equation (Monteith, 1977):

$$DM(d) = R_g(d) \cdot \varepsilon_c \cdot \varepsilon_i \cdot \varepsilon_b \quad (1)$$

where DM(d) is the dry matter at day d (kg ha^{-1}) ; Rg(d) is the incoming global radiation at day d (MJ m^{-2}) ; ε_c is the climatic efficiency (0.48) ; ε_i is the light interception efficiency by each species (%) ; ε_b is the radiation use efficiency (kg DM MJ^{-1}). Radiation use efficiency is under the control of (i) crop N status, (ii) temperature, (iii) water deficit and (iv) species phenology. The model description section focuses on equations and underlying assumptions chosen for below-ground (nitrogen, water) and light resource sharing between species along the life cycle.

2.1. Competition for light

From a general viewpoint, competition for light within any intercrop relies on (i) stem growth rate (*i.e.* height of each species), (ii) leaf area distribution along the stem profile, (iii) leaf area index dynamics (green and senesced leaves) during the growth cycle and (iv) leaf properties (light reflectance, leaf angle) of each species. The weight of each pre-cited variable on light sharing depends on architectural features of the studied intercrop. In order to decide whether a specific module of leaf area pattern for each species should be included in the model, the leaf area along each species canopy profile was monitored every 10 cm at a vegetative stage for both species, on one field experiment. These data were then fit to an equation derived from Berntsen et al. (2004). The comparison of parameter values from each species showed no significant difference between the two species (data not shown). Hence, for this legume-cereal intercrop, leaf area in each species was assumed similarly distributed along the stem. In the model, potential leaf production is linearly correlated to crop critical nitrogen contents for each species (Lemaire et al., 2007):

$$LAI_d = D \cdot QNc_d \quad (2)$$

where D is the slope (m^2 leaf. m^{-2}soil. g^{-1}N) for wheat (0.028) and pea (0.033). Wheat green leaf dynamics is controlled by (i) NNI (expressing N deficiency occurrence during the vegetative period) and (ii) net leaf N mobilization to grains starting from the anthesis. TTime-course of green leaf area was monitored and is expressed as the percentage of total leaf area as follows (Eq. 3):

$$\%GL = -0.00005 \cdot t^2 + 0.1243 \cdot t + 21.48 \quad (3)$$

where t is the thermal time (°Cd) from sowing. %GL was multiplied to the potential leaf production when the leaf senescence started in pea.

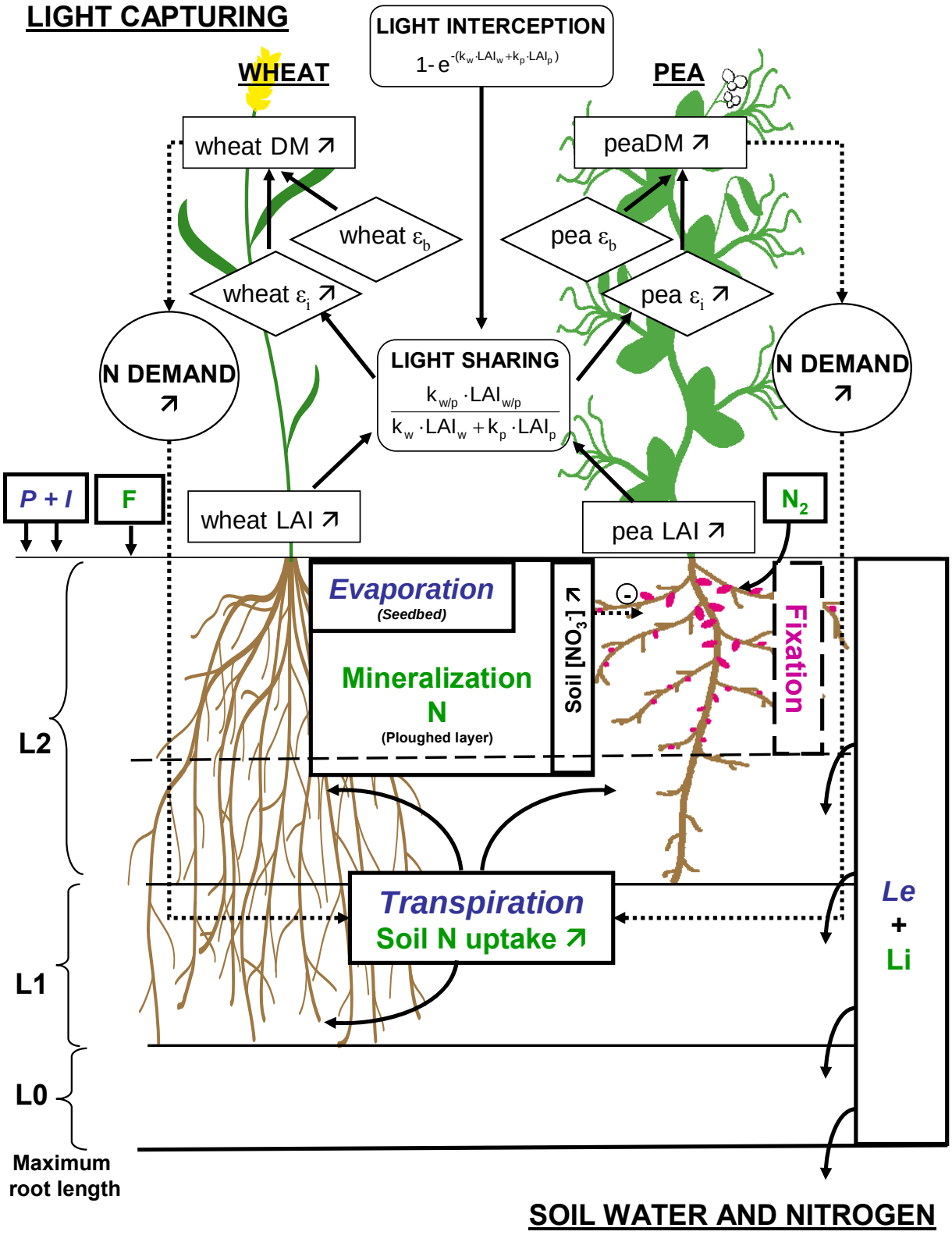

Figure 1: Conceptual diagram of nitrogen, water and light sharing into AZODYN. L2, L1 and L0 are soil layers colonized by two, one or none species roots, respectively. Organic N mineralization and evaporation only occur along the ploughed layer profile and into the seedbed, respectively. P and I are precipitations (mm) and irrigations (mm). F is applied inorganic N fertilizer (kg N ha^{-1}). Soil water and nitrogen are distributed among all layers through water leaching (Le) and N lixiviation (Li). LAI and k are leaf area index (m^2 leaf. m^{-2} soil) and extinction coefficient (unitless) for each species. ε_i and ε_b are light interception (%) and radiation use (kg DM MJ^{-1}) efficiencies. DM is crop dry matter (kg ha^{-1}). W and P underscripts refer to wheat and pea, respectively.

Given contrasted leaf properties between both species, specific extinction coefficient values (k) were included into the light sharing equation. Probability that incoming PAR is not intercepted by intercrop canopy (P_0) is defined by the below equation:

$$P_0 = e^{-(k_w \cdot LAI_w + k_p \cdot LAI_p)} \quad (4)$$

where k is the extinction coefficient (unitless) for wheat (w; k_w =0.72; Gosse et al., 1986) or pea (p; k_p =0.57; Ney, 1994) and LAI is the leaf area index (leaf m^2 soil m^{-2}) of each species. Interception efficiency of each species is then calculated according to Keating and Carberry (1993) and Tsubo and Walker (2002) as follows:

$$\varepsilon_{iw/p} = \varepsilon_{imax\ w/p} \cdot (1 - P_0) \cdot R \quad (5)$$

$$R = \frac{k_{w/p} \cdot LAI_{w/p}}{k_w \cdot LAI_w + k_p \cdot LAI_p} \quad (6)$$

where $\varepsilon_{imax\ w/p}$ is the maximum interception efficiency for wheat (w, 0.96; Gosse et al., 1986) or pea (p, 0.983 for winter pea; Brun, 2002).

2.2. Competition for water and nitrogen

Access to water and nitrogen (*i.e.* depletion rate and partitioning of below-ground resources along the soil profile between crops) is of great importance within an intercrop along the life cycle. According to Corre-Hellou et al. (2006), competition for below-ground resource uptake between species is primarily driven by each species demand for water and nitrogen. However when N deficiency or water shortage occurs, water and nitrogen acquisition by each species is also restricted by root exploration, soil nutrient supply and water and nitrogen taken up by the companion crop (Fig. 1).

Here a "functional" soil layer concept is proposed based on root dynamics (Fig. 1). Root growth kinetic is therefore parameterized with root penetration rate and maximum root length for each species. Values were obtained from Vocanson et al. (2006a and b) for pea. Vocanson (2006) established a close and linear correlation between the root growth rate and incoming photosynthetic active radiations (0.009 mm. MJ^{-1} PAR). Similarly, a linear relationship between root penetration rate and the thermal time established by Corre-Hellou et al. (2005a) for intercropped barley was introduced into the model (0.14 cm °Cd^{-1}) for the wheat root growth, assuming cereals have a close root elongation rate. For both species, root growth is stopped when (i) temperature is below 0°C or (ii) date is beyond the date of flowering in pea and wheat or (iii) roots reach the maximal root depth defined as the maximal soil depth or a species characteristic.

Three soil layers are then defined as roots of each species elongate along the soil profile: one layer contains elongating roots from both crops (L2) whereas the below layer is only colonized by roots of one or another species (L1). As long as intercrop roots do not reach the maximum root depth, a third layer containing no root is also considered (L0), between the L1 layer and the maximal root depth. This approach allows competition to be simulated for below-ground resources when both crop roots colonized the same soil layer under resource deficiency. The species with the faster root growth rate can take nitrogen or water up to match its requirements from L1 when water or nitrogen demand is not satisfied with L2 availability. Thus, it is assumed that this species benefits from a faster root penetration rate so that it can fulfill its water or nitrogen requirement. Accordingly the model accounts for the competitive advantage towards the

fast root growth rate species. In turn it reduces the water or nitrogen stress effect on shoot growth, which would jeopardize competition for light and ultimately species yield.

AFISOL derived soil water balance module was introduced (i) to estimate water leaching and subsequent N lixiviation out of the plant-soil system and (ii) to account for water stress on organic nitrogen mineralization rates, evaporation and pea growth (based on the Fraction of Transpirable Soil Water, FTSW, Lecoeur and Sinclair, 1996). Soil water stock is fed by daily precipitations and irrigations, if any. If water amounts provided by both precipitations and irrigation are under a threshold, the day is considered as a day with no water input (dnow). Summing dnow is then introduced in the equation 2 (see below) to limit evaporation (E). Water flows out of the soil system through evaporation (E), crop transpiration (T) and leaching (Le). Evaporation only occurs into the seed bed (30 mm) whereas crop transpiration is taken into account in the rooted layers (L2 and L1). Neither evaporation nor crop transpiration occurs into the unrooted layer (L0). For each layer (L2, L1 and L0), the Available Water Reserve (AWR, mm) at day d results from equation (7):

$$AWR_d = P_d + I_d + Le_{d-1} - (E_d + T_{d-1}) \quad (7)$$

where P_d are precipitations (mm), I_d is irrigation (mm), Le_d^{-1} is water leaching from the above layer at day d-1 (mm), T_{d-1} is transpiration at day d-1 (mm). Evaporation (E) and potential transpiration (T_{pot}) are derived from Lecoeur and Sinclair (1996), respectively.

If cumulated potential transpiration of both species in L2 does not exceed AWR in the corresponding layer then water partitioning between both species is only driven by potential transpiration ($T = T_{pot}$). However if potential transpiration of intercrop (wheat + pea) is larger than AWR in L2, transpiration by each species also depends on respective potential transpiration of the companion crop as it concurrently depletes water in L2 as:

$$T_{w/p} = \frac{T_{pot\,w/p}}{T_{pot\,w} + T_{pot\,p}} \quad (8)$$

The species with the longest root system can also pull water up from L1 to match crop water demand as long as both T_{pot} in L1 and extra water demand due to unsatisfied water supply in L2 do not exceed AWR in L1. Water leaching (Le) is calculated as the default component in the soil-crop water balance as it corresponds to the remaining water, if any, into each layer after intercrop transpiration occurred. Water leaching out of soil layers L2, L1 and L0 allows to compute daily water amount (i) pouring in L1 and L0 and (ii) leaking out of the whole soil-plant system (below L0). These values are then used to calculate N lixiviation.

Soil nitrogen balance module is detailed in Jeuffroy and Recous (1999). Soil N budget relies on the N sheet balance method (Machet et al., 1990). For each layer (L2, L1 and L0), the Available Soil Nitrogen (ASN; kg N ha^{-1}) at day d is calculated as follows:

$$ASN_d = Mr_d + Mw_d + Mh_d + F_d + Li_{d-1} - U_d \quad (9)$$

where Mr, Mw and Mh are mineralized N derived from crop residues, organic waste and humus (kg. ha^{-1}), respectively, F is inorganic N fertilizer derived-input (kg ha^{-1}), Li is N lixiviated out of the above layer at day d^{-1} (kg ha^{-1}) in the layer, U is N taken up by the intercrop (kg ha^{-1}). Mineralization only occurs within ploughed layer (30 cm). Mineralization rates defined for each nitrogen source are temperature- and soil water content-controlled processes, with mineralization of humus also depending on soil clay and carbonate contents. N outputs are (i) N taken up by

intercrop and (ii) N lixiviation during the growth cycle. N lixiviation is estimated on the basis of water leaching (as explained above) according to Burns equation (Burns et al., 1974). Unavailable soil N amount for crop N uptake is also set to 20 kg N ha^{-1} along the whole soil profile and is homogeneously partitioned among soil layers. Under non limiting soil N conditions, N taken up by each species is driven by N demand. Maximal N dilution curves determined for wheat (Justes et al., 1994) and pea (Ney et al., 1997) are used to define N demand along the life cycle. Maximal N dilution curves were used to account for maximal N accumulation into crops. As suggested by Cruz and Soussana (1997), nitrogen content based on the N dilution curve (expressed as a nitrogen percentage of shoot dry matter (%N)) in each species is determined by accounting for both pea and wheat dry matters (DM) when each species is sown at half density (compared to a sole crop set-up) in a substitutive set-up:

$$\%N = a \cdot (DM_w + DM_p)^{-b} \quad (10)$$

where a= 8.3 and 9.28 for wheat and pea, respectively, b= 0.44 and 0.55 for wheat (Justes et al., 1994) and pea (Ney et al., 1997) for the maximum N dilution curve, respectively. If soil N content is not large enough to match N requirement in both species then available soil N is partitioned as explained above for water. For wheat, N uptake is only governed by N transport system activity, associated regulations and soil N concentrations. By contrast N acquired by pea mainly derived from atmospheric N (Ndfa). For pea, N taken up by roots from soil N pool is therefore calculated as the difference between N demand (from N dilution curve) and N accumulated from N_2 fixation. As shown by Voisin et al. (2002), N amounts derived from air decreased when root nodules are surrounded by large inorganic nitrogen amounts. Equations derived from Voisin et al. (2002) were then included into the model. Corre-Hellou et al. (2006) demonstrated that the efficiency of instantaneous N_2 fixation also depends on shoot-to-root carbon allocation (*i.e.* providing carbon skeletons to fuel nodule activity and growth) so that maximum daily N amount derived from fixation (kg ha^{-1}) is directly linked to daily crop growth as described in Eq. 11:

$$\text{Maximum } N_2 \text{ amount}_d = A \times DM_d \quad (11)$$

where A is the slope (0.028 kg N kg^{-1}DW), DM_d is the crop dry matter at day d (kg ha^{-1}). Fixation also depends on the development stage of the crop (Voisin et al., 2002). Water shortage occurring after flowering can also alter N_2 fixation through a slower nodule activity and formation. Accordingly it was assumed that N derived from air is null if the water shortage duration (*i.e.* water stock under the threshold of one third of AWR) exceeds 7 days after the final stage in seed abortion of the crop. Based upon a study carried out by Vocanson (2006), variation of soil compaction was also accounted for simulating how deep nodule production can occur along the soil profile. Maximum nodule production depths ranged from 30 (high level of soil compaction) to 150 mm (no compaction).

Similarly to the water balance module, available soil N content is shared between species as a part of N taken up by each other species in the L2 soil layer under N deficiency. Nitrogen uptake can occur in L1 by the species with the longest root to take up extra nitrogen as long as both N demand in L1 and extra demand due to N starvation in L2 are not larger than available N pool in this layer.

Parameters needed to run AZODYN-IC are directly derived from the two basic sole crop models AZODYN and AFISOL: no experiment was conducted to determine new values of parameters under an intercrop design and no specific parameterization step was necessary.

Table 1: Sowing densities (seeds. m^{-2}), N fertilizer rates (kg N ha^{-1}) and application dates (calendar days) for field experiments conducted at La Jaillière (2006, 2007, 2008; LJ) and Grignon (2007; G). Wheat and pea genotypes are "Cézanne" and "Lucy" at La Jaillière, respectively. Wheat and pea genotypes are "Trocadéro" and "Cartouche" at Grignon, respectively. EA: early application; SA: standard application; LA: late application of N fertilizer.

Year (location)	Species	Sowing density	N input	Treatments	Date of application
2006 (LJ)	**W/P**	125/40	0	1	-
			40	6	12-avr
2007 (LJ)	**W/P**	125/40	0	2	-
			44	7	14-mars
			44 (LA)	8	18-avr
			30; 30	12	06/04; 29/05
2007 (G)	**W/P**	120/40	0	4	-
2008 (LJ)	**W**	260	0	-	
			45	-	07/03 (SA)
			80; 65; 40	-	07/03; 20/03; 15/05
	P	80	0	-	
	W/P	130/40	0	3	-
			45	9, 10, 11	07/02 (EA) or 07/03 (SA) or 10/04 (LA)
			30 or 60	5, 13	01-avr
			90	14, 15, 16	07/02 (EA) or 07/03 (SA) or 10/04 (LA)

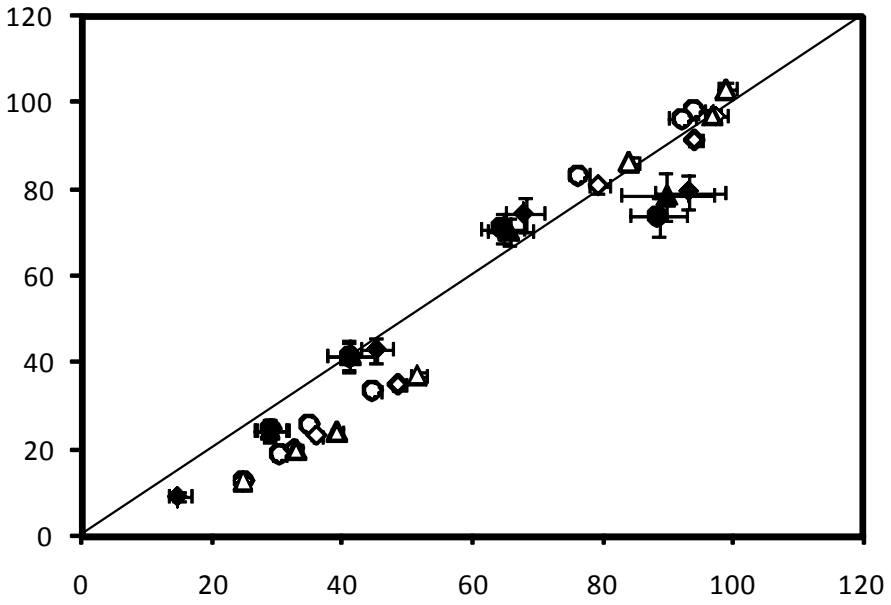

Figure 2: Pea *vs* wheat canopy heights (cm) monitored along the 2006-2007 (filled symbols) and 2007-2008 (empty symbols) experiments (n=15) within an intercrop for different nitrogen rates applied. (2008: 0, 45 and 90 kg N ha^{-1}; 2007: 0, 44, 60 kg N ha^{-1}). Application dates are detailed into table I. Bars are standard deviations when larger than the symbol.

3. Materials and methods

3.1. Experimental design

Data used for model assessment were obtained from field experiments carried out in France at La Jaillière (47°26'N, 0°57'W; 2006, 2007 and 2008) and at Grignon (48°85'N, 1°92'W; 2007). For each site, winter pea and wheat were either sole- or intercropped sown according to a replacement design (*i.e.* in the intercrop, each species was sown half the density of sole crop).

A range of N fertilizer rates (from 0 to 185 kg N. ha^{-1}) was applied at different times (from wheat tillering stage (GS26) to end of flowering stage of wheat (GS69) as liquid (La Jaillière experiments) or solid (Grignon experiments) ammonium nitrate. Plant densities, rates and dates of N fertilizer application are detailed in Table 1.

Weeds, pests and diseases were optimally kept under control. No irrigation was provided. In La Jaillière experiments, a dynamic assessment of the amount of nitrogen derived from air (Ndfa) in pea plants required the use of ^{15}N-labelled fertilizer ($NH_4{}^{15}NO_3$) in fertilized areas. All experiments were arranged in a randomized complete block design with three replicates.

3.2. Soil and plant measurements and analytical procedures

All input variables and crop characteristics required to run the model AZODYN-IC were recorded along the crop cycle. Inputs and parameters are precisely detailed in Recous and Jeuffroy (1999). Briefly, regular climate data (minimal, maximal and mean temperature, rainfall, evaporation and global incoming radiations) were daily recorded near the experimental sites. Crop management and soil analysis were also provided. Appearance of key-stages for each crop was accurately determined along the growth cycle for each experiment.

For experiments conducted in both sites, soil nitrogen contents, crop dry matters, yields and crop N contents were measured at harvest. Above-ground dry matter was determined after oven drying at 80°C for 48 h. At harvest, grains and straws were separately weighed. All samples were then grounded and N contents were measured according to the Dumas procedure (Hansen, 1989).

For experiments carried out at La Jaillière, soil nitrogen contents, leaf area index, crop nitrogen and crop above-ground dry matter of each species were also monitored monthly along the growth cycle (except between sowing and the end of the winter). Green leaves were separated from other parts of the plant for each species. Green leaf area was determined using a LI3100 area meter (LI-COR Inc., NE, USA). The heights of both intercropped species under increasing N fertilizer rates (0, 45, 60, 90 kg N ha^{-1}; Fig. 2) were fortnightly measured from the end of winter (start of stem elongation for wheat) to harvest during experiments conducted at La Jaillière in 2007 and 2008).

At La Jaillière and Grignon sites, soil samples were taken from 0–30, 30–60 and 60–90 cm soil layers from the harvested plot. Soil nitrate and ammonium contents were measured after KCl extraction by standard colorimetric methods (Keeney and Wilson, 1989). Soil and crop measurements provide observed data.

3.3. Calculations of N_2 fixation, Land Equivalent Ratio and RMSEP

The amount of N_2 fixed was calculated as the product of pea biomass, %N content and the proportion of plant N derived from N_2 fixation.

For non-fertilized treatments and fertilized areas without ^{15}N enrichment (Grignon experiments), the percentage of plant N derived from N_2 fixation (%Ndfa) was determined using the ^{15}N natural abundance method (Amarger et al., 1979). Unfertilized sole cropped (SC) wheat was used as reference crop for calculating N_2 fixation in SC pea and intercropped (IC) wheat for IC pea:

$$\%\text{Ndfa} = \frac{\delta^{15}\text{Npea} - \delta^{15}\text{Nwheat}}{\beta\text{fix} - \delta^{15}\text{Nwheat}} \cdot 100 \qquad (12)$$

where βfix (-1) (Mariotti et al., 1980) is the isotopic fractionation factor associated with N_2 fixation processes. It corresponds to the ^{15}N enrichment of pea relying only on N_2 fixation.

For fertilized areas with ^{15}N enrichment, the percentage of shoot N derived from N_2 fixation (%Ndfa) was determined using the ^{15}N dilution method for fertilized areas with ^{15}N enrichment (Rennie and Rennie, 1983). N-fertilized intercropped wheat was used as the reference crop for calculating N2 fixation for intercropped peas:

$$\%\text{Ndfa} = \left(1 - \frac{\text{A}\,\%^{15}\text{N excess pea}}{\text{A}\,\%^{15}\text{N excess wheat}}\right) \cdot 100 \qquad (13)$$

Grain protein content is derived from grain N content which is multiplied by 5.7 or 6.25 for wheat or pea, respectively (Teller, 1932; Sosulski and Imafidon, 1990).
LER is the Land Equivalent Ratio calculated for dry matter, yield and crop total nitrogen content as follows:

$$\text{LER} = \frac{P_{IC}}{P_{SC}} + \frac{W_{IC}}{W_{SC}} \qquad (14)$$

where P and W are the assessed variables for pea and wheat. IC and SC correspond to inter- and sole-cropped design, respectively. The reference treatment for the sole crop was the high N level.
RMSEP (Root Mean Squared Error of Prediction) values were computed for evaluating the accuracy of AZODYN-IC to simulate gain of productivity (LER) and resources partitioning (carbon and nitrogen) between pea and wheat (wheat to pea ratio) at harvest. The calculation is as follows:

$$\text{RMSEP}_j = \sqrt{\frac{\sum_{i+1}^{n} (Y_{i\,obs} - Y_{i\,sim})^2}{n_j}} \qquad (15)$$

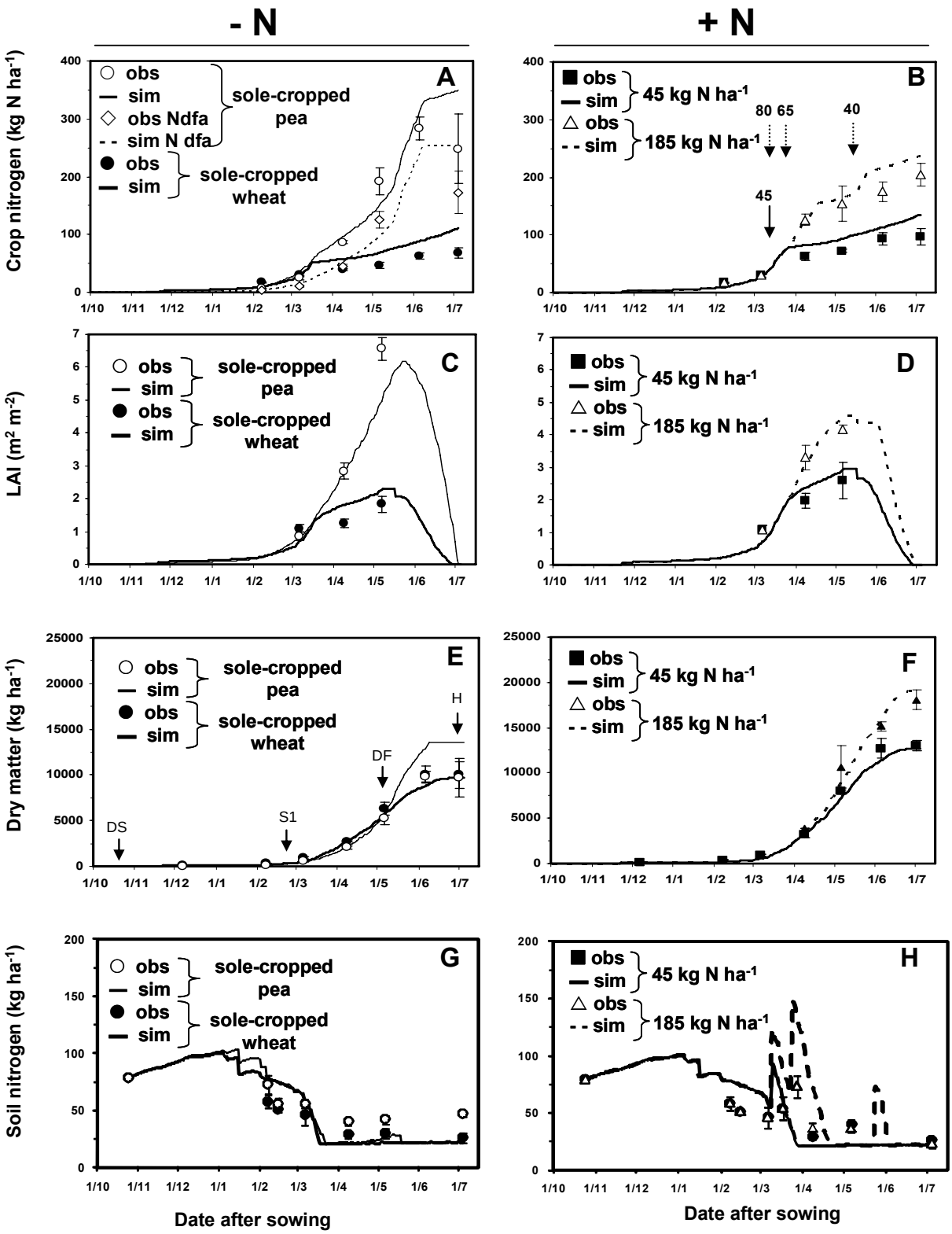

Figure 3: Time-course of observed (obs, symbol) and simulated (sim, line) crop nitrogen taken up (A, B; kg N ha^{-1}), leaf area index (C, D; LAI, m^2. m^{-2}), crop dry matter (E, F; kg ha^{-1}) and soil N (G, H; kg ha^{-1}) during the growth cycle in sole-cropped wheat and pea under various N fertilizer levels (0 kg N ha^{-1} (-N; filled and empty circles for wheat and pea, respectively); 45 kg N ha^{-1} (filled squares) and 185 kg N ha^{-1} (empty triangles)). Arrows correspond to N fertilizer application dates. N fertilizer rates are mentioned above arrows when required. N derived from fixation in pea is presented into plot A (empty diamond-shaped symbol; dashed line). Bars are standard deviations when larger than the symbol. Key-stages are presented into plot E. DS: dater of sowing; S1: spike 1 cm stage; DF, date of flowering; H: harvest.

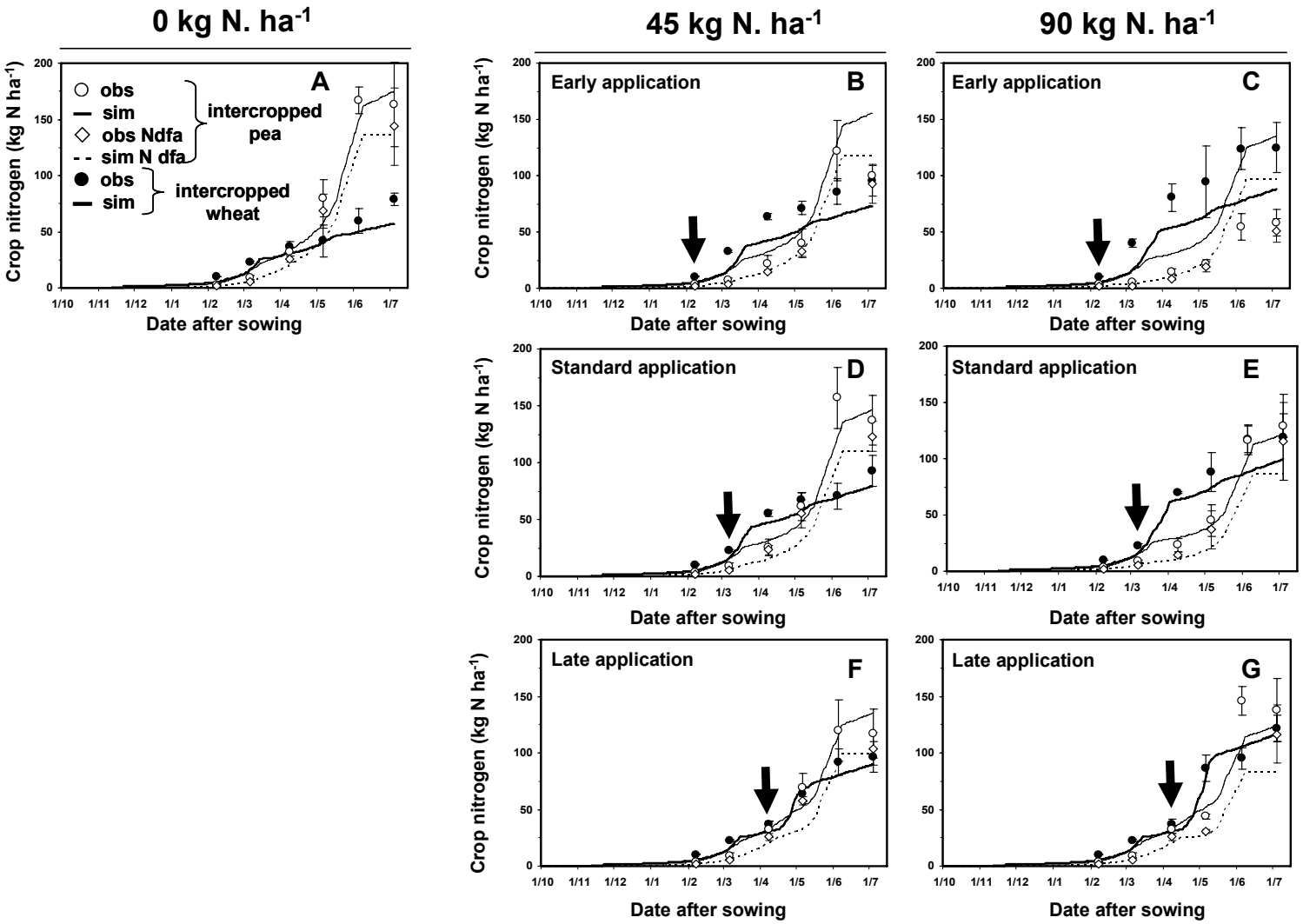

Figure 4: Time-course of measured (circle) and simulated (line) crop nitrogen taken up (kg N ha^{-1}), N derived from N_2 fixation in pea (kg N ha^{-1}; empty diamond-shaped symbols) during the growth cycle in inter-cropped wheat (filled circle; bold line) and pea (empty circle; plain line) under various N fertilizer levels (0 kg N ha^{-1}; 45 kg N ha^{-1} and 90 kg N ha^{-1}). Arrows correspond to N fertilizer application dates. Bars are standard deviations when larger than the symbol.

4. Results

4.1. Heights of each intercropped species under various N fertilizer applications

All data together show that wheat crop is slightly taller than pea crop: 6.5 ± 0.35 cm and 1 ± 0.73 cm gaps were monitored during the vegetative and the grain filling periods, respectively (Fig. 2). Despite a faster stem growth rate of wheat, it cannot be concluded that it overwhelmingly overtops pea crop under such an intercrop during the experiment. Accordingly, stem growth rates of both species were assumed as equivalent and height difference of each species was not included into the model.

4.2. Dynamics of N taken up, LAI, crop growth and soil N amounts for the sole crops

When no fertilizer is added, model outputs show good agreement with observed data along the growth cycle (Fig. 3A, C, E, G) from the end-of-winter (mid-February) until harvest. Simulated wheat N uptake is slightly overestimated at mid-March (Fig. 3A), concomitantly to a fast depletion of simulated soil inorganic N amounts (Fig. 3G). This explains the larger N amount accumulated by wheat at harvest as simulated N accumulation slope parallels observed accumulated N after March. For pea, taking into account both ways of N acquisition in pea allows to keenly simulate N amount accumulation along the growth cycle (Fig. 3A). N derived from symbiotic fixation (SNF) is in good agreement with observed data, except at harvest. However, observed N accumulated by pea at harvest decreases due to observed grain losses.

LAI growth simulated by AZODYN-IC matches well measured data during the vegetative period (Fig. 3C). It thus underlines the relationship between the LAI expansion and the satisfaction of N demand (through NNI) is robust. During the grain filling, no LAI was monitored so that it is difficult to assess the accuracy of the model to simulate LAI during this phase. However, crop growth was satisfactorily simulated for both species, even though pea dry matter was overestimated at the end of the cycle (Fig. 3E). When N fertilizers are applied, AZODYN-IC gives a good account of all observed variables in wheat sole-crops along the growth cycle (Fig. 3B, D, F, H).

4.3. Dynamics of N into the soil-plant system under an intercrop design

For the unfertilized treatment, the total N amount accumulated by winter pea-wheat intercrop (241.9 ± 43 kg N ha^{-1}; Fig. 4A) is close to that accumulated by sole-cropped pea (248 ± 60.4 kg N ha^{-1}; Fig. 3A) and much higher than N taken up by sole-cropped wheat (67.8 ± 9.3 kg N ha^{-1}) when grown under the same N input level. Partial nitrogen land equivalent ratios for wheat and pea were 1.06 and 0.59, respectively. It demonstrates that substitutive intercropped design allows wheat and pea crops to reach up to twice and 18% larger N contents in each respective species than corresponding sole-cropped design does. Model outputs show that AZODYN-IC can satisfactorily simulate N accumulation in both species during the growth cycle, yet simulated N taken up by wheat was slightly underestimated during the end of the grain filling for the early and standard applications (Fig. 3B-G).

SNF in intercropped pea accounts for a larger proportion of N accumulated than in sole cropped pea (57% in sole-cropped pea *vs* 88% in intercropped pea; Fig. 3A and 4A) at harvest. Model can accurately predict N derived from atmospheric N_2 fixation until harvest. As soil N taken up by pea root is defined as the default component into the model (*i.e.* resulting from the difference between the crop N demand (N dilution curve) and Ndfa), an under-accumulation of SNF by the end of the growth cycle would result in a higher soil inorganic N taken up ultimately a higher competition towards the companion wheat species for soil inorganic nitrogen.

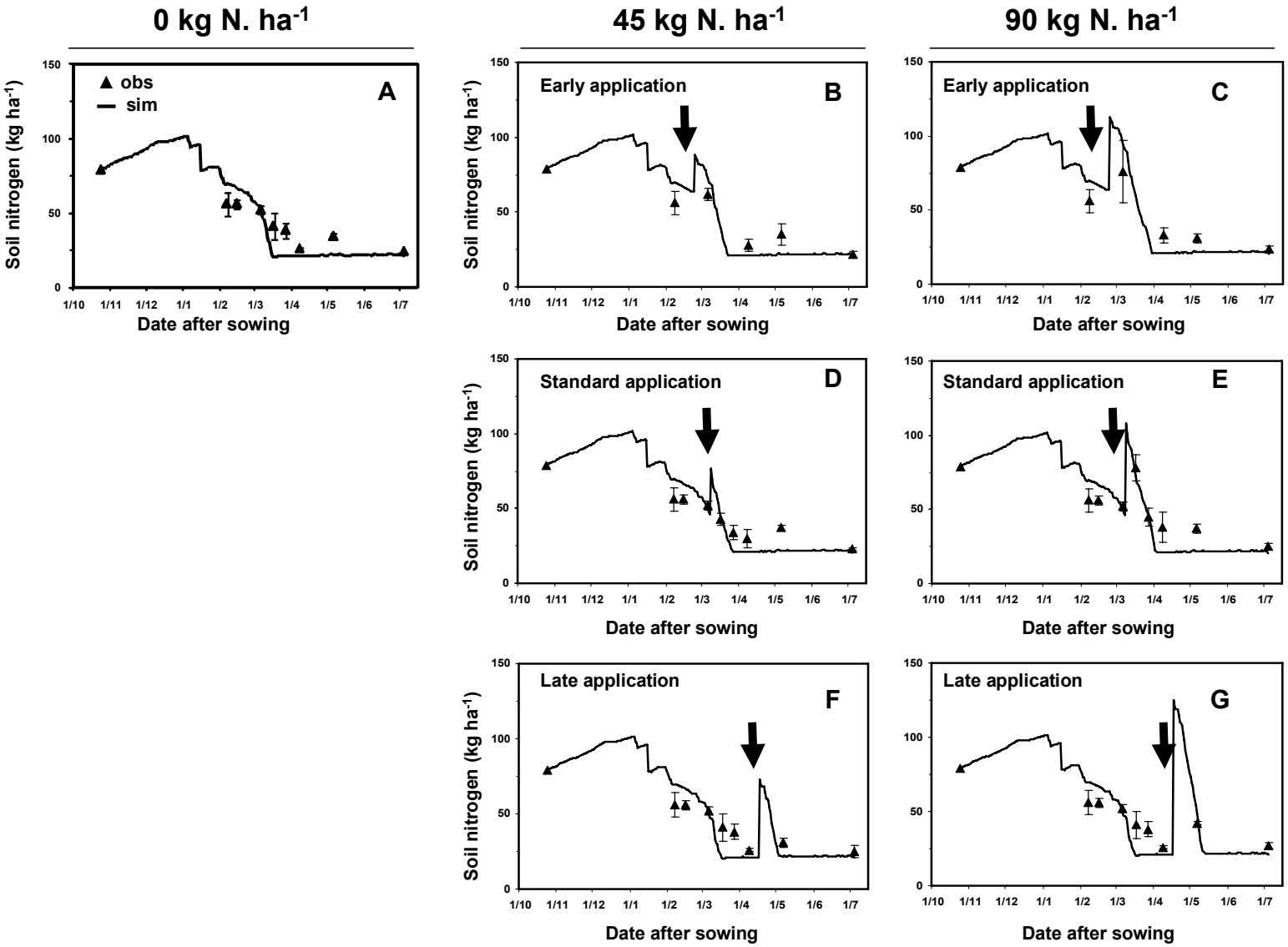

Figure 5: Time-course of measured (symbols) and simulated (line) soil nitrogen amounts in intercrops under various N fertilizer levels (0 kg N ha^{-1}, 45 kg N ha^{-1} and 90 kg N ha^{-1}). Arrows correspond to N fertilizer application dates. Bars are standard deviations when larger than the symbol

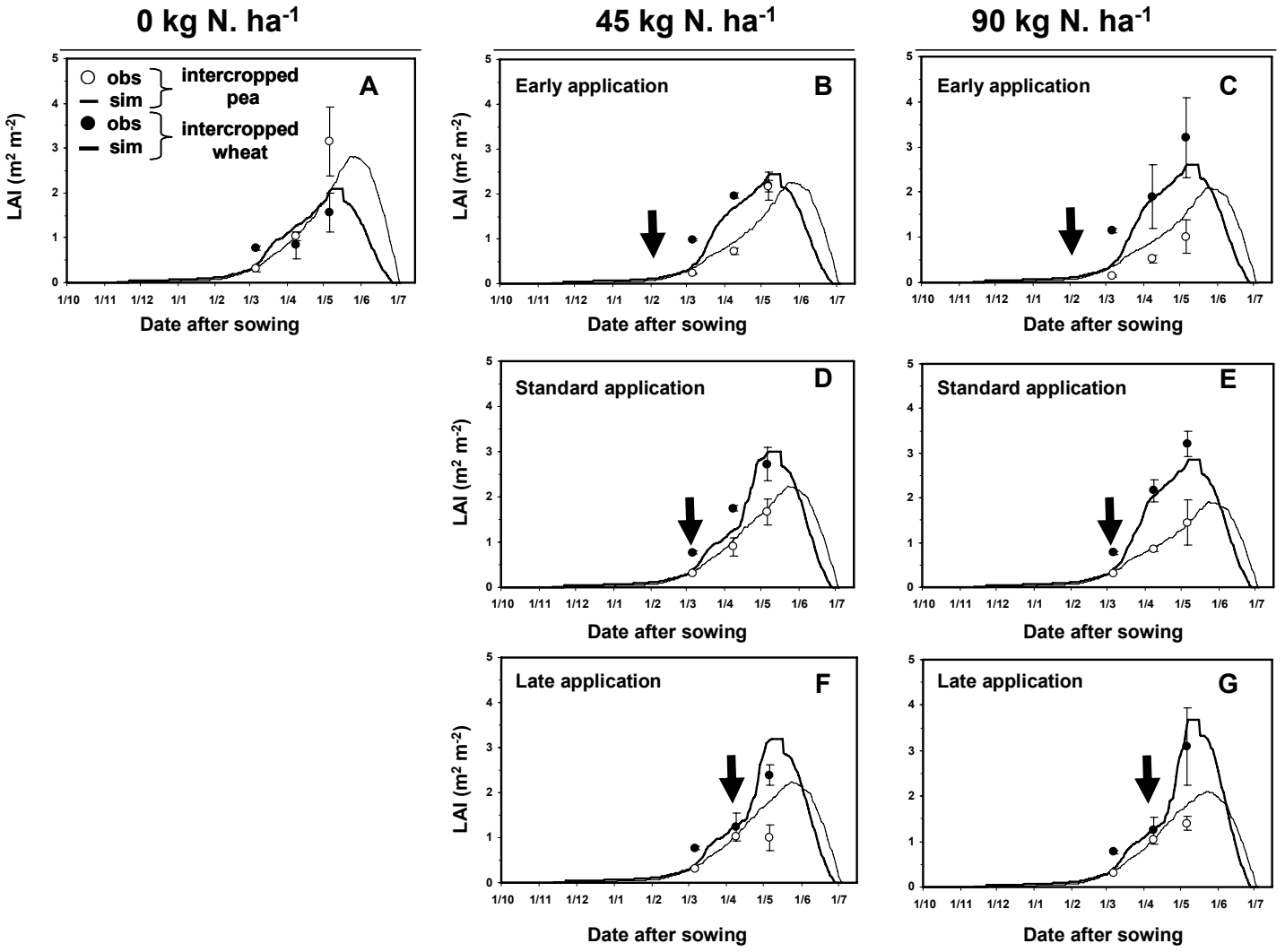

Figure 6: Time-course of measured (symbols) and simulated (line) leaf area index (LAI) (m^2. m^{-2}) during the growth cycle in inter-cropped wheat (filled circle; bold line) and pea (empty circle; plain line) under various N fertilizer levels (0 kg N ha^{-1}; 45 kg N ha^{-1} and 90 kg N ha^{-1}). Arrows correspond to N fertilizer application dates. Bars are standard deviations when larger than the symbol.

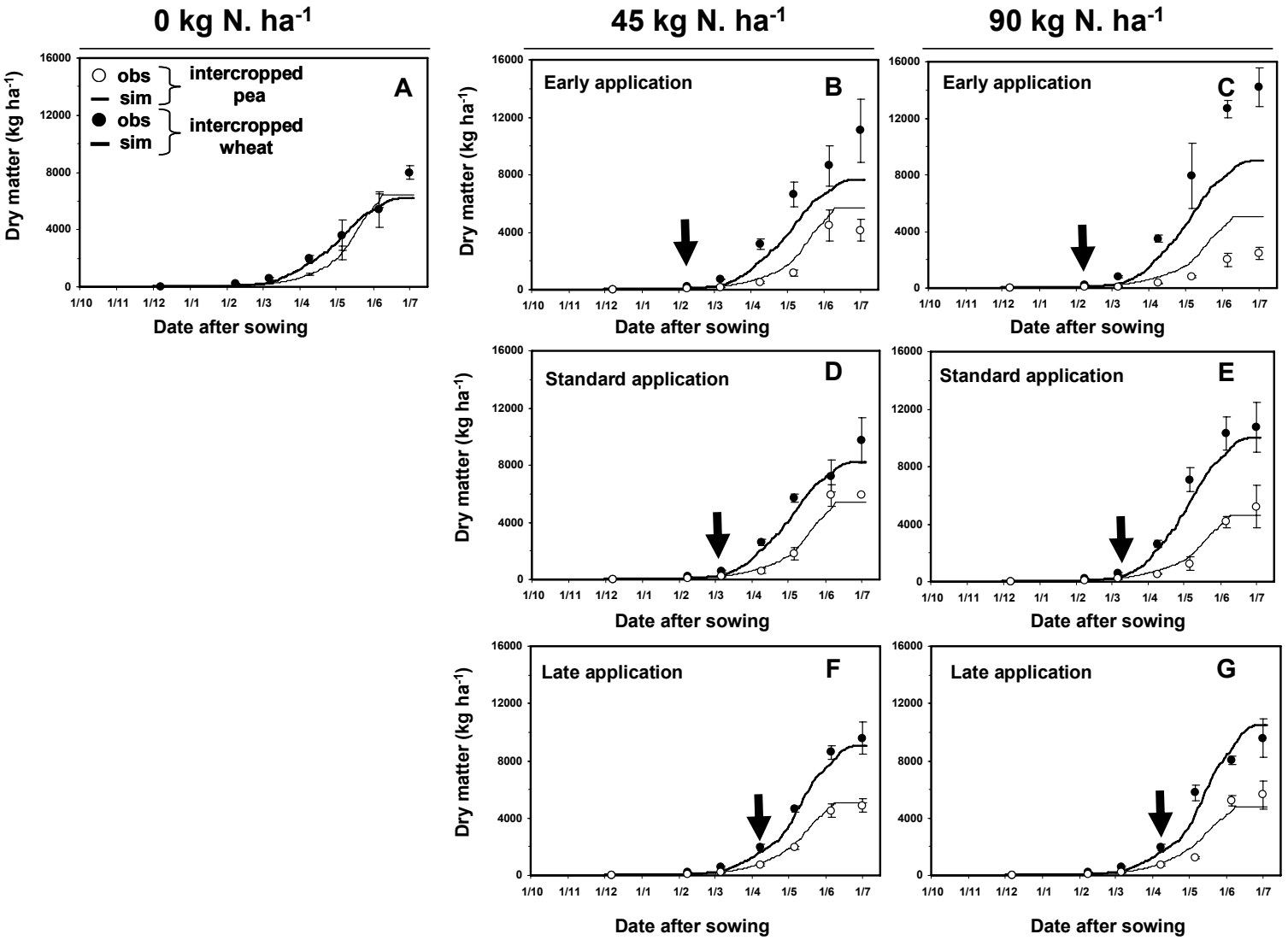

Figure 7: Time-course of measured (symbols) and simulated (line) dry matters (DM) (kg. ha^{-1}) during the growth cycle in inter-cropped wheat (filled circle; bold line) and pea (empty circle; plain line) under various N fertilizer levels (0 kg N ha^{-1}; 45 kg N ha^{-1} and 90 kg N ha^{-1}). Arrows correspond to N fertilizer application dates. Bars are standard deviations when larger than the symbol.

Adding N fertilizers does not significantly change observed N contents by intercrop when compared to unfertilized treatment (241.9 ± 4 kg N ha^{-1}). Indeed, intercrop N content values range from to 195.8 ± 11.8 kg N ha^{-1} for early application of 45 kg N ha^{-1} to 259.2 ± 9.6 kg N ha^{-1} for the late application of 90 kg N ha^{-1} (Fig. 4B, C, D, E, F, G). However, observed and simulated N partitioning between each species within the intercrop at harvest was largely modified by either the rate or the date of application of inorganic N fertilizers. For all treatments taken together, N amounts accumulated in wheat and pea at harvest average 105.9 ± 5.2 kg N ha^{-1} and 118.3 ± 9.3 kg ha^{-1}, respectively. N input application increases proportion of nitrogen accumulated in intercropped wheat (33 % of N accumulated in intercrop without N fertilizer *vs* 50 % with N fertilizer). Proportion of observed N accumulated in intercropped wheat is all the larger that N fertilizer is applied early during the growth cycle. This trend is strengthened when applied N fertilizer rate levels increase. Hence, for the highest level of N fertilization (90 kg N ha^{-1}), proportions of N accumulated in wheat are 47, 49 and 68 % for the late, standard and early applications, respectively (Fig. 4C, E, G). This is due to both a slight increase of N uptake by wheat (from 121.3 ± 6.7 to 124.9 ± 12.8 kg N ha^{-1} for the late and early applications of 90 kg N ha^{-1}, respectively) and a decrease of N accumulation in intercropped pea (from 137.9 ± 16.1 to 58.2 ± 6.8 kg N ha^{-1} for the late and early applications, respectively) (Fig. 4C, E, G). Under a fertilized plot, N accumulation by each intercropped species is satisfactorily simulated during the growth cycle and at harvest. AZODYN-IC is responsive to inorganic N input whenever it is applied between early February and early April. However, N uptake by wheat was underestimated from the end of the winter to harvest for early N applications. For these plots a low value of apparent N use efficiency (data not shown) was predicted by AZODYN-IC.

Overall, predicted SNF are in agreement with measured data at harvest for all the fertilized treatments. However, confronting observed *vs* simulated data along the growth cycle points out simulated N derived from symbiotic fixation is overwhelmingly shut down for the late application of 90 kg N ha^{-1} (Fig. 4G). In this case, the inhibitory effect of inorganic soil nitrogen leads to an underestimation of N amounts from air during the pea pod filling (Fig. 4 G).

Soil inorganic N amounts available for intercrops are well simulated by AZODYN-IC (Fig. 5A, B, C, D, E, F, G). Applied N fertilizer is available as soon as it is applied, except for the early application (Fig. 5 B and C). Soil N after harvest is realistically predicted for all treatments (averaging 20 kg N ha^{-1} for all N input levels).

4.4. GLAI dynamics for intercropped species

The patterns of the green leaf area index (GLAI) of each intercropped species are displayed in Fig. 6. For the unfertilized treatment, observed pea and wheat LAI exhibit close values, except at the beginning of flowering when pea LAI value is larger than wheat one (Fig. 6A). Applying N fertilizers leads to a decrease of pea LAI and a concomitant increase of wheat LAI, all the larger that N input levels increase (Fig. 6 B, C, D, E, F, G). Dates and rates of applied N fertilizers are satisfactorily taken into account by the model as model outputs fit well observed data. Although pea GLAI values at flowering are overestimated when N fertilizer is applied lately (Fig. 6 F and G), the wheat to pea GLAI ratio is well predicted all along the vegetative period (*i.e.* from March 3rd to May 5th). Hence, simulated efficiencies of light interception by intercrop (*i.e.* through the GLAI of both species) show a good agreement to measured ones for all N input levels (data not shown). Otherwise, it is not possible to assess the ability of the model to simulate properly the GLAI time-course over the grain filling in each species as only yellow to green leaf dry matters ratio were monitored.

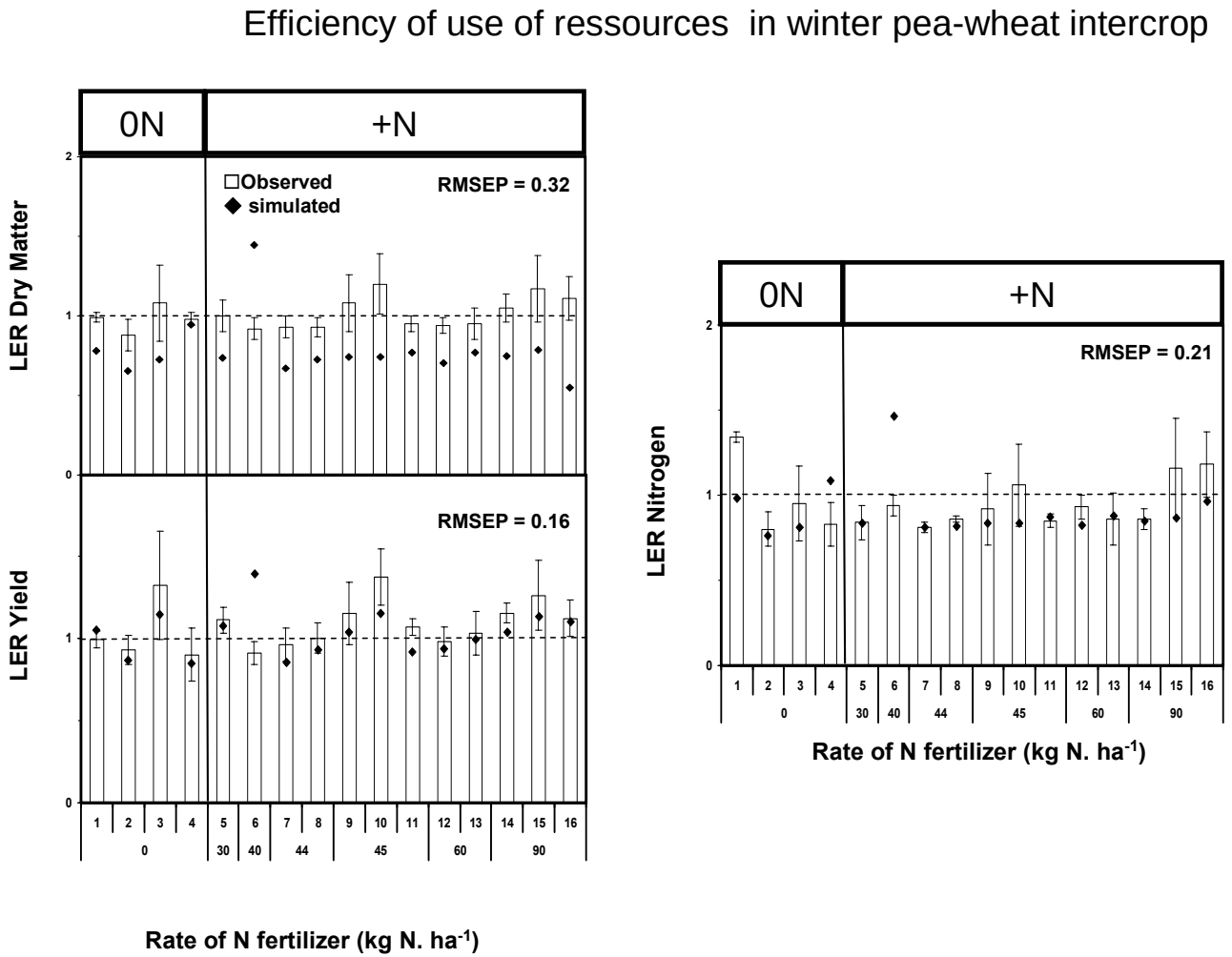

Figure 8: Observed (columns) and simulated (filled diamond-shaped symbols) dry matter (A), yield (B) and N content (C) LER at harvest when increasing rate of N fertilizers are applied in two different locations (La Jaillière in 2006, 2007, 2008 and Grignon in 2008). Applied N amounts are written along the x-axis for each plot into the figure. See Table 1 for detailed description of each treatment. Horizontal dashed line corresponds to ratio equal to 1. Bars are standard deviations.

Carbon and Nitrogen partitioning between species at harvest

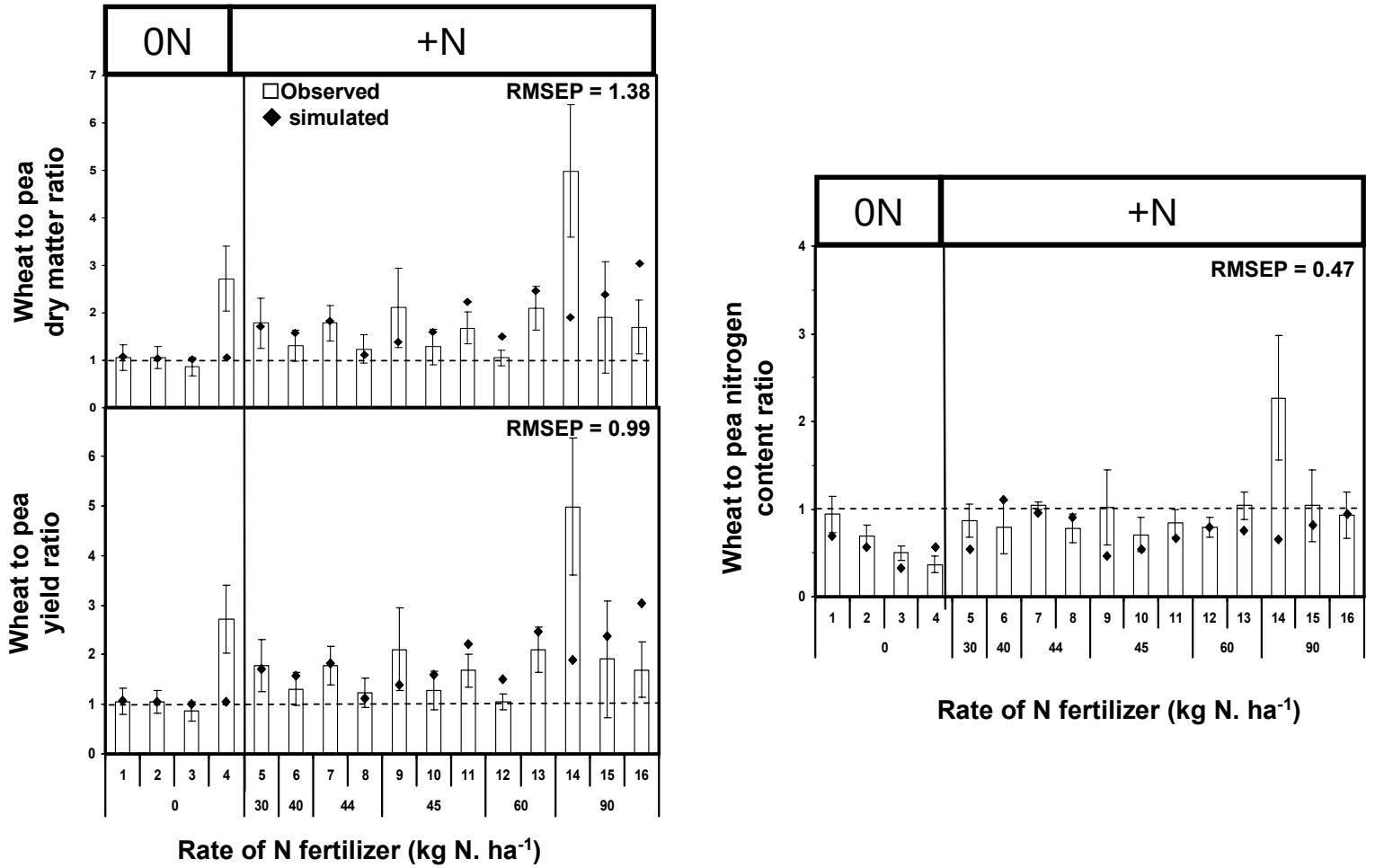

Figure 9: Simulated (filled diamond-shaped symbols) and observed (empty histograms) wheat to pea dry matter (A), yield (B) and nitrogen content (C) ratio at harvest when increasing rates of N fertilizers are applied in two different locations (La Jaillière in 2006, 2007, 2008 and Grignon in 2008). Applied N amounts are written along the x-axis for each plot into the figure. Horizontal dashed line corresponds to ratio equal to 1. Bars are standard deviations.

4.5. Growth of intercropped species

The pattern of intercrop growth is well simulated by AZODYN-IC (data not shown) over all N fertilizer treatments (averaging 14534 ± 2784 kg ha^{-1}). Applying N inputs on intercrop does not enhance intercrop growth leading to a similar dry matter amount at harvest when compared to plots with no N input. Wheat and pea growths parallel when no N fertilizer was added leading to similar dry matter accumulations in both species at harvest (Fig. 7A). Model satisfactorily simulates growth patterns, even though wheat growth was slightly underestimated during the grain filling from June 1st and then wheat to pea dry matter ratio was inversed at harvest (Fig. 7A).

Application of N fertilizers benefited to wheat growth to various extents whereas pea growth was decreased when compared to unfertilized plots (Fig. 7B, C, D, E, F, G). The model can dynamically account for the effect of N application on dry matter partitioning between wheat and pea when N fertilizers are applied beyond March 7th, whatever the N input levels (Fig. 7D, E, F, G). Growth simulated by AZODYN-IC is the responsive to various timing of N application. However, model outputs also show that wheat and pea growths were under- and overestimated, respectively, for an early N fertilization (Feb 7th; Fig. 7B and C), linked to the prediction in N accumulated in each crop. This is emphasized along with the applied N amount (*i.e.* application of 90 kg ha^{-1}; Fig. 7C).

4.6. Final performances of intercrop simulated by AZODYN-IC

After assessing dynamics of nitrogen and crop growth into the soil-plant system for either sole- or intercropped species from the experiments conducted at La Jaillière in 2008, the robustness of the model was tested against harvest datasets from La Jaillière (2006, 2007 and 2008) and Grignon (2007) (see Table 1 for treatments).

Simulated wheat yield into intercrop is overestimated by AZODYN-IC (3725 ± 225kg ha^{-1} for the observed yield *vs* 4221 ± 251 kg ha^{-1} for the simulated yield, averaged over all the treatments; RMSEP for intercropped wheat yield = 1044 kg ha^{-1}). By contrast, simulated intercropped pea yields are in the range of the observed pea yields (2584 ± 157 kg ha^{-1} for the observed yield *vs* 2386 ± 124 kg ha^{-1} for the simulated yield, averaged over all the treatments; RMSEP for intercropped pea yield = 787 kg ha^{-1}).

Plotting measured LER for DM, yield and crop nitrogen *vs* applied rates of nitrogen fertilizer points out that values vary around 1, independently of the nitrogen fertilization rate (Fig. 8). Altogether, these data show that AZODYN-IC can satisfactorily predict the efficiency of use of resources by intercrop in term of yields and nitrogen accumulations (RMSEP = 0.16 and 0.21 for yield and total nitrogen accumulated by intercrop, respectively), yet AZODYN-IC outputs were less accurate for dry matter accumulation (RMSEP = 0.32). Indeed, simulated LER values for dry matter accumulation were underestimated whereas predicted values for intercrop yield and total nitrogen accumulation were within the range of biological variability (Fig. 8). Regarding carbon and nitrogen partitioning between the two species (Fig. 9), simulated wheat to pea ratio values match well measured data for dry matter, yield and crop nitrogen, except for treatment 14 corresponding to early application of N fertilizers in La Jaillière (2008).

5. Discussion

The aim of this study was to design an innovative intercrop growth model (i) based upon coupling two sole-crop growth models (AZODYN and AFISOL for wheat and pea, respectively) and (ii) relying on knowledge about rules of resources sharing as described in the literature (Corre-Hellou *et al.*, 2005b; 2006; 2007) to simulate the development and growth of an annual intercrop with no new parameter values to estimate.

5.1. N dynamics at the crop and plant levels within an intercrop

Availability of soil inorganic nitrogen is of major importance as it drives intercrop growth, performances and species proportions at harvest (Hauggaard-Nielsen, 2006). It is then essential to simulate accurately N accumulated by intercrop as well as N sharing between the species. Model outputs clearly show that N taken up and partitioned between pea and wheat crops was realistically predicted by AZODYN-IC for a broad range of N fertilizer applications (amount and rate) and different locations (La Jaillière and Grignon).

What makes AZODYN-IC original is its ability to simulate the effect of contrasted N fertilization strategies (dates and rates of N applications) on intercropped species growth through a strong and explicit interrelation between N dynamics (including the pea N_2 fixation component) and light interception. At the crop level, this result confirms that partitioning of soil inorganic N according to N demand from each species drives the accumulation of soil inorganic nitrogen by each species within a cereal-legume intercrop. However, for early N fertilizer applications, simulated N amount accumulated in pea and wheat were over- or underestimated, respectively. From an experimental viewpoint, this is explained by the fact that an early application of a large amount of liquid N fertilizer (90 kg N ha^{-1}) on the canopy led to a dramatic reduction of pea leaf area along with a larger wheat growth into the plot explaining the discrepancy between simulated outputs and observed data.

On the one hand, the structure of AZODYN-IC is designed to include the complementarity for N acquisition along the growth cycle within a limited space: root inorganic soil N uptake for both species and atmospheric N_2 fixation for pea only. Model outputs point out that N amount derived from atmospheric N_2 fixation was better simulated when no N fertilizer was added. It raises the question to what extent nodules activity and/or numbers are reduced and can recover after a N application in a period during which N fixation is highly active. So far, N_2 fixation pattern is defined as proposed by Voisin et al. (2002) and Corre-Hellou et al. (2006). Confronting model outputs *vs* observed data suggests that this might be due to an overwhelmingly inhibitory effect of soil inorganic nitrogen on nodule activity. Extra experiments are necessary to examine to what extent symbiotic N_2 fixation can recover after N input application at different crop stages during the growth cycle. Indeed, predicting daily contribution of N derived from fixation to total N accumulation in pea is critical as it determines the sink force for inorganic soil N towards the wheat. Moreover, it is noteworthy AZODYN-IC doesn't account for some plant-plant mechanisms through which extra N can be provided to wheat. For example, Jensen (1996b) reported that N deposited by pea through root and nodules turn-over and root exudates (ammonium and amino acids) can be transferred to barley under controlled conditions. This transfer can represent up to 19% of N taken up by barley 70 days after sowing, even though these processes and their intensity within a life cycle into an annual intercrop are difficult to monitor under field conditions. However, the good agreement between simulated and observed data suggest that plant-plant N transfer may represent a minor contribution to N acquisition by intercropped wheat during the growth cycle, even though experimental data are necessary to support this conclusion.

On the other hand, Corre-Hellou et al. (2007) showed in spring pea-barley intercrop that soil inorganic N sharing between species depends on the levels of N fertilization, *i.e.* N partitioning is mainly under the control of the root system architecture for low N input levels whereas N partitioning is driven by N demand competition for high N input levels. Here, the original dynamic soil layers growing up along with the root elongation of each species was chosen to take into account the advantage of the species with the faster root elongation rate to fulfill its nutrient requirement.

5.2. GLAI expansion and light capturing by and within the intercrop under the control of N acquisition

GLAI is a key variable for simulating light interception and crop growth under sole crop design. GLAI is also involved in light sharing between each species under an intercrop design. A satisfactory simulation of GLAI (resulting from the balance between LAI production and leaf senescence) is then required for each species to predict intercrop growth as well as proportions of each species at harvest.

To date, existing cereals-legumes intercrop models are derived from sole-crop growth models. For instance, FASSET (Jacobsen et al., 1998) and STICS (Brisson et al., 1998) have been extended to simulate spring pea-barley intercrop growth (Berntsen et al., 2004; Brisson et al., 2004). In such models, LAI production is modeled on either a thermal time basis (FASSET, Jacobsen et al., 1998; SIRIUS, Jamieson et Semenov, 2000; STICS, Brisson et al., 1998), or both thermal time basis and daily crop growth (DAISY, Hansen et al., 1991). The effect of N deficiency on LAI, if any, is indirectly introduced through different formalisms describing effect of NNI (Olesen et al., 2002). Confronting simulated *vs* observed data pointed out the time-course of simulated GLAI in pea does not match the observed data when N fertilizer is applied (Corre-Hellou et al., 2009).

In AZODYN-IC, LAI production directly (i) depends on the critical nitrogen amounts and (ii) is under the control of NNI in each species. By contrast to precited models, this formalism allows to interrelate below-ground resources and light interception and sharing between winter pea and wheat crops. Use of this formalism is innovative as it has not been included in any intercrop growth model to our knowledge. LAI values simulated by AZODYN-IC show they are responsive to N application as AZODYN-IC simulates realistic wheat to pea LAI ratio (*i.e.* competition for light interception between wheat and pea) during the vegetative period leading to inversion of LAI values to the benefit of wheat. In the same line, a straightforward formalism was introduced into the model for light capturing by and within the pea-wheat intercrop: light interception depends on LAI values and leaf architectural features (k values) from each species. Whereas it is hardly possible to validate light sharing between each species through an experimental set-up, simulated light interception efficiencies match measurements of light interception efficiencies within the intercrop (data not shown). Such equation can be used because winter pea and wheat have similar height rates and leaf area distribution pattern along the canopy profile (data not shown). Indeed, results from sensitivity analysis with FASSET (Berntsen et al., 2004) suggested that leaf area distribution along the canopy profile may be of great importance for describing the competition for light between species.

6. Conclusion

The strength of our approach is to take full advantage of both (i) tighting the links between below-ground resources and light sharing through the LAI component under the control of N acquisition and crop nitrogen status within cereals-legumes intercrops and (ii) describing intercrop functioning with easily measurable parameters. Actually, no parameterization step was required to build AZODYN-IC so that intercrop growth only relies on trophic competition. As the model mainly relies on N dynamics within the soil-plant system, it also emphasizes that this element is critical. AZODYN-IC is operational and can then be usefully handled as a valuable tool to manage intercrops depending on targeted yields. Accordingly, AZODYN-IC will be used to simulate the response of intercrop growth and yield production under a large crop management combination (sowing density, date of sowing, rate of N fertilizer and date of application, for instance) and eventually provide decision rules regarding N availability to optimize intercrop management.

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References

Amarger, N., Mariotti, A., Mariotti, F., Dürr, J.C., Bourguignon, C., Lagacherie, A., 1979. Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in 15N natural abundance. Plant soil. 52, 269-280.

Barbottin, A., Le Bail, M., Jeuffroy, M.-H., 2006. The AZODYN crop model as a decision support tool for choosing cultivars. Agron. Sustain. Dev. 26, 107-115.

Bédoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil. in press (doi: 10.1007/s11104-009-0082-2)

Berntsen, J., Haugaard-Neilsen, H., Olesen, J.E., Petersen, B.M., Jensen, E.S., Thomsen, A., 2004. Modelling dry matter production and resource use in intercrops of pea and barley. Field Crop Res. 88, 69-83.

Biarnès, V., Lecoeur, J., Jeuffroy, M.-H., Vocanson, A., Ney, B., 2009. A model which integrates knowledge on pea crop physiology and agronomic diagnosis. In: Physiology of the pea crop. Science Publishers, Enfield, USA, pp 197-208. in press.

Brisson, N., Mary, B., Ropoche, D., Jeuffroy, M.-H., Ruget, F., Nicoullaud, B., Gate, P., Devienne-Barret, F., Antonioletti, R., Durr, C., 1998. STICS: a generic model for the simulation of crops and their water and nitrogen balances: I. Theory and parameterization applied to wheat and corn. Agronomie 18, 311–346.

Brisson, N., Bussière, F., Ozier-Lafontaine, H., Tournebize, R., Sinoquet, H., 2004. Adaptation of the crop model STICS to intercropping. Theoretical basis and parameterization Agrononomie 24, 409-421.

Brun, D., 2002. Développement du pois d'hiver en zone Nord. Master report, ENSAIA, Nancy, France.

Bulson, H.A.J., Snaydon, R.W., Stopes, C.E., 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. J. Agric. Sci. 128, 59-71.

Burns, I.G., 1974. A model for predicting the redistribution of salts applied to fallow soils after excess rainfall or evaporation. J. Soil Sci. 25, 165-178.

Corre-Hellou, G., Crozat, Y., 2005a. Assessment of root system dynamics of species grown in mixtures under field conditions using herbicide injection and ^{15}N natural abundance methods: A case study with pea, barley and mustard. Plant Soil. 276, 177-192.

Corre-Hellou, G., Crozat, Y., 2005b. N_2 fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems as affected by weeds and peaweevil (*Sitona lineatus* L.). Field Crops Res. 22, 449-458.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific Competition for soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208.

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crop Res. 103, 76-85.

Corre-Hellou, G., Faure, M., Launay, M., Brisson, N., Crozat, Y., 2009. Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea-barley intercrops. Field Crops Res. 113, 72-81.

Cruz P., Soussana, J.-F. Mixed crops. 1997. In: Lemaire, G. (ed.), Diagnosis of the Nitrogen Status in Crops. Springer Verlag, Berlin, Heidelberg, pp. 131-144.

David, C., Jeuffroy, M.-H., Recous, S., Dorsainvil, F., 2004. Adaptation and assessment of the AZODYN model for managing the nitrogen fertilization of organic winter wheat. Eur. J. Agron. 21, 249-266.

David, C., Jeuffroy, M.-H., Laurent, M., Mangin, M., Meynard, J.M. 2005. The assessment of AZODYN-ORG model for managing nitrogen fertilization of organic winter wheat. Eur. J. Agron. 23, 225-242.

David, C., Jeuffroy, M.-H., 2009. A sequential approach for improving Azodyn crop model under conventional and low-input conditions. Eur. J. Agron. in press.

Faurie, O., Soussana, j.-F., Sinoquet, H. 1996. Radiation interception, partitioning and use in Grass-Clover mixtures. Ann. Bot. 77, 35-45.

Ghaley, B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E. 2005. Intercropping of Wheat and Pea as Influenced by Nitrogen Fertilization. Nutr. Cycl. Agroecosyst. 73, 201-212.

Gosse, G., Varlet-Grancher, C., Bonhomme, R., Chartier, M., Allirand, J.M., Lemaire, G., 1986. Production maximale de matière sèche et rayonnement solaire intercepté par un couvert végétal. Agron. 6, 47–56.

Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldhal. Acta Agr. Scan. 39:113-118.

Hansen, S., Jensen, H.E., Nielsen, N.E., Svenden, H., 1991. Simulation of nitrogen dynamics and biomass production in winter using the Danish simulation model DAISY. Fert. Res. 27, 245-259.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E. S., 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res. 70, 101-109.

Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. Field Crop Res. 95, 256-267.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.

Jacobsen, B.H., Petersen, B.M., Berntsen, J., Boye, C, Sorensen, C.G., Sogaard, H.T., Hansen, J.P., 1998. An integrated economic and environmental farm simulation model (FASSET). Report No. 102. Danish Institute of Agricultural and Fisheries Economics, Copenhagen, Denmark.

Jamieson, P.D., Semenov, M.A., 2000. Modelling nitrogen uptake and redistribution in wheat. Field Crops Res. 68, 21-29.

Jensen, E.S., 1996a. Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil. 182, 25-38.

Jensen, E.S., 1996b. Barley uptake of N deposited in the rhizosphere of associated field pea. Soil Biol. Biochem. 28, 159-168.

Jeuffroy, M.-H., Recous, S., 1999. AZODYN: a simple model simulating the date of nitrogen deficiency for decision support in wheat fertilization. Eur. J. Agron. 10,129-144.

Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huché, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74, 397-407.

Keating, B. A., Carberry, P. S., 1993. Resource capture and use in intercropping: solar radiation. Field Crops Res. 34, 273-301.

Keeney, D. R., Wilson, D. W., 1989. Nitrogen-inorganic forms. In: Page, A. L., Miller, R. H., Keeney, D. R. (Eds), Methods of Soil Analysis. Part II Chemical and 207 Microbiological Properties. Second edition. American Society of Agronomy, Madsiob, WI, USA.

Lecoeur, J., Sinclair, T.R., 1996. Field pea (*Pisum sativum* L.) transpiration and leaf growth in response to soil water deficits. Crop Sci. 36, 331-335.

Lemaire, G., Oosterom, E., Sheehy, J., Jeuffroy, M.-H., Massignam, A., Rossato, L., 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? Field Crops Res. 105, 253-265.

Lemaire, G., van Oosterom, E., Jeuffroy, M.-H., Gastal, F., Massignam, A., 2008. Crop species present different qualitative types of response to N deficiency during their vegetative growth. Field Crops Res. 105, 253-265.

Machet, J.M., Dubrulle, P., Louis, P., 1990. AZOBIL: a computer program for fertilizer N recommandations based on a predictive balance sheet method. In: Scaife, A. (Ed.), Proc. 1st Congress of the European Society for Agronomy, Paris pp. 21–22.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S., Valantin-Morison, M. 2008. Mixing plant species in cropping systems: concepts, tools and models. A review. Agron. Sustain. Dev. 28, 1-20.

Mariotti, A., Mariotti, F., Amarger, N., 1980. Fractionnements isotopiques de l'azote lors de processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plantes. Physiol . Veg. 18, 163-181.

Marshall, B., Willey, R. W., 1983. Radiation interception and growth in an intercrop of pearl millet/groundnut. Field Crop Res. 7, 141-160.

Meynard, J.M., Cerf, M., Guichard, L., Jeuffroy, M.H., Makowski, D., 2002. Nitrogen, Decision Support and Environmental Management. Agronomie 22, 817-829.

Monteith, J.-L., 1977. Climate and efficiency of crop production in Britain. Phil. Trans. R. Soc. Lond. 281, 277-294.

Neumann, A., Schmidtke, K., Rauber, R. 2007. Effects of crop density and tillage system on grain yield and N uptake from soil and atmosphere of sole and intercropped pea and oat. Field Crop Res., 100, 285-293.

Ney, B., 1994. Modélisation de la croissance aérienne d'un peuplement de pois, *In* UNIP-ITCF-INRA, ed. Agrophysiologie du pois protéagineux.

Ney, B., Doré, T., Sagan, M. 1997. Grain legumes. In: Lemaire, G., (Ed.), Diagnosis of the nitrogen status in crops. Springer, pp. 107-118.

Olesen, J.E., Petersen, B.M., Bernsten, J., Hansen, S., Jamieson, P.D., Thomsen, A.G., 2002. Comparison of methods for simulating effects of nitrogen on green leaf area index and dry matter growth in winter wheat. Field Crop Res. 74, 131-149.

Ozier-Lafontaine, H., Vercambre, G., Tournebize, R., 1997. Radiation and transpiration partitioning in a maize-sorghum intercrop: Test and evaluation of two models. Field Crop Res. 49, 127-145.

Rennie, R.J., Rennie, D.A., 1983. Techniques for quantifying N_2 fixation in association with non-legumes under field and greenhouse conditions. Can. J. Microbiol. 29, 1022–1035.

Sosulski, F.W., Imafidon, G.I., 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38, 1351–1356.

Teller, G.L., 1932. Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for protein in cereals and bread. Cereal Chem. 9, 261-274.

Tsubo, M., Walker, S., 2002. A model of radiation interception and use by a maize-bean intercrop canopy. Agric. Meteorol. 110, 203-215.

Vocanson, V., 2006. Evaluation ex ante d'innovations variétales en pois d'hiver (*Pisum sativum* L.) : approche par modélisation au niveau de la parcelle et de l'exploitation agricole. Ph. D. manuscript, Institut National Agronomique Paris-Grignon, 293 p.

Vocanson, A., Estrade, J.-R., Boizard, H., Jeuffroy, M.-H., 2006a. Effects of soil structure on pea (*Pisum sativum* L.) root development according to sowing date and cultivar. Plant Soil. 281, 121-135.

Vocanson, A., Jeuffroy, M.-H., Estrade, J.-R., 2006b. Effect of sowing date and cultivar on root system development in Pea (*Pisum sativum* L.). Plant Soil. 283, 339-352.

Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil. 243, 31-42.

Wallace, J.S., Batchelor, C.H., Dabeesing, D.N., Soopramanien, G.C., 1990. The partitioning of light and water in drip irrigated plant cane with a maize intercrop. Agr. Water Manage. 17, 235-256.

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PARTIE II :

EXPLORING CROP N-MANAGEMENT STRATEGIES FOR WINTER PEA-WHEAT INTERCROPS USING A MODELING APPROACH.

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Running title: N-fertilization management in pea-wheat intercrops

Abstract

Annual intercrops such as winter pea-wheat are gaining increasing interest in Europe as they provide higher productivity along with lower inputs, of which nitrogen fertilizers. Beyond the environmental benefit, it is of great importance to be able to drive intercrop towards targeted production goals so that such design may be successfully adopted by farmers. It is now well established that mineral soil nitrogen plays a major role in intercrop performances through N and light sharing. However, no studies could provide any decision rule for guiding farmers to reach optimized intercrop performances. Aim of this study was to extend field experimentations by simulating wider range of N-management strategies (sowing densities x N-supply rates x N-supply dates) for a range of soil mineral N contents at sowing, and using a 26 climatic year-dataset. These simulations were obtained through AZODYN-IC, which was previously designed to satisfactorily simulate response of such intercrop performances to various soil mineral N availability dynamics. This approach allowed investigating the response of intercrops performance (total yield, % of each species, grain protein content) to various dynamics of soil N availabilities. It highlighted the interactions between rates, date of N-supply and sowing densities on performance of intercrops. Eventually, this work gives an opportunity to propose simplified linear models useful to test decision rules for N-management of intercrop according to soil N mineral content at the end of winter, contribution of wheat in intercrop biomass at the end of winter, estimation of N-mineralization form end of winter to harvest, and rate and date of N-application.

Keywords: intercrop, N fertilization, species proportions, AZODYN-IC, simulations, decision rules.

1. Introduction

Intercropping consists of growing simultaneously two or more species on the same piece of land (Willey, 1979). This practice is of great interest as intercropped species can better use available resources (such as soil water, nitrogen and light) than sole-cropped species as shown in spring pea-barley (Jensen, 1996; Hauggaard-Nielsen and Jensen, 2001; Corre-Hellou et al., 2006) and spring pea-wheat (Ghaley et al., 2005) intercrops (see also Malézieux et al., 2008 for a review). This is mainly explained by exploring different ecological niches (root exploration, nitrogen sources) in space and in time for both intercropped species. Such a design has been successfully adopted by organic farmers.

Nonetheless, this way of cultivation may also be interesting under a conventional agriculture when considering a reduction of fossil input spread over the land along with an increase of yield productivity and an improvement of grain quality within the sustainable agriculture framework. Moreover, such annual intercrops can provide diverse outcomes depending on production goals. Hence, it can be produced for silage (high levels of protein rich-biomass), for wheat protein-rich grain along with low N input or growing leguminous with reduced occurrence of commonly issues endured when sole-cropped (weeds, lodging, soil nitrogen leaching). Depending on targeted production goal, species proportions at harvest must be kept under control. To make annual intercropping successful under a conventional agriculture, it is important to provide reliable decision rules guiding farmers practices for specific production goals.

Availability of soil mineral nitrogen has been shown as a critical element influencing species proportions at harvest. Hence, numerous studies have investigated the effect of mineral soil nitrogen availability on intercrop growth, yield and species proportions at harvest varying the relative frequency (*i.e.* proportion of each species at sowing within an intercrop) (Bulson et al., 1997; Neumann et al., 2007; Hauggaard-Nielsen et al., 2006), rate or date of N fertilizer applications (Jensen, 1996; Hauggaard-Nielsen and Jensen, 2001; Ghaley et al., 2005; Corre-Hellou et al., 2006; Naudin et al., 2009a, b). It has been demonstrated that intercrop yield slightly increases along with increased available soil mineral N but the proportion of cereals into intercrop largely increases at harvest. To make intercrops successful and widely used by farmers under temperate lands, the challenge is to drive intercrops (i) accounting for initial conditions (sowing density, soil mineral nitrogen and the potential soil N mineralization during the growth cycle) at sowing and (ii) determining to what extent N fertilization application during spring (rate and timing) can be used to dynamically bend each intercropped species growth toward yields and proportions as well as quality parameter values (protein contents in wheat grains in particular) at harvest within a highly variable climate.

As stated earlier, most of the previous field experiments about cereal-legume intercrops focussed on a single factor and very few strived to study the combined effect of practices which is time-consuming and expensive. Accordingly, modelling can be helpful to i) screen crossed combinations of crop managements such as sowing density and N fertilization applications (rates and dates) by accounting for soil N availability (ii) to propose N-strategies for further testing under field conditions (Bergez et al., 2009) To that purpose, AZODYN-IC (Malagoli et al., 2009) was designed to simulate winter pea-wheat intercrop from sowing to harvest taking into account interrelation between N and light sharing within intercrop. It has been successfully validated for a wide range of contrasted N fertilization strategies in two locations in France (Malagoli et al., 2009).

The aim of this paper is first to assess the performance of wheat-pea intercrops (total intercropped grain yields, contribution of wheat to total grain yield, grain protein content in wheat and pea, their inter annual variability) without N supply and the effect of two factors which may largely contribute to this variability: soil mineral N availability and the proportion of wheat in intercrop stand after winter to include the dominance occurring shortly at the beginning of the crop cycle (Bellostas et al., 2003). Secondly, the effect of N input applications for a wide range of rate * date combinations was tested to see how each combination shaped the final variables at harvest. Finally, linear models was proposed to simulate the most critical performances (proportion of wheat in intercrop yield and grain protein content in wheat) as a function of soil mineral N, date and rate of N inputs and proportion of wheat in intercrop biomass after winter.

2. Materials and methods

2.1. AZODYN-IC model description

Description of AZODYN-IC has been detailed in Malagoli et al. (2009). Briefly, this daily time step intercrop model (Azodyn) is derived from Azodyn (Jeuffroy and Recous, 1999) and Afisol (Vocanson, 2006; Biarnès et al., 2009) models. For both species, crop growth is defined according to Monteith's equation (Monteith, 1977). Radiation use efficiency is under the control of (i) crop N status, (ii) temperature, (iii) water deficit and (iv) species phenology.
Leaf area index, when no nitrogen deficiency occurs, is linearly correlated to crop critical nitrogen contents for each species:

$$LAI_d = D \times QNc_d \quad (1)$$

where LAI_d is the leaf area index (m^2 leaf area/m^2 soil surface) ; D is the slope (m^2 leaf area/kg N) ; QNc is the critical nitrogen content at day d calculated through the N dilution curve for each species. Soil nitrogen balance module is detailed in Jeuffroy and Recous (1999). Available Soil Nitrogen (ASN; kg/ha) at day d is calculated from (i) mineralized N derived from crop residues, organic waste and humus, (ii) mineral N fertilizer derived-input, (iii) N lixiviated to the observed layer from the above layer at day d-1, (iiii) N taken up by the crops. Mineralization only occurs within ploughed layer (30 cm). Soil N outputs are (i) N taken up by crops and (ii) N lixiviation during the growth cycle. N supply also relies on the root elongation rate by each species. Thus, three soil layers were defined as roots of each species elongate along the soil profile: one layer contains elongating roots from both crops (L2) whereas the below layer is only colonized by roots of one or another species (L1). As long as intercrop roots do not reach the maximum root length a third layer containing no root (L0) is also considered. This approach allows simulating competition for below-ground resources when both crop roots colonize the same soil layer under resource deficiency. The species with the faster root growth rate can take nitrogen or water up to match its requirements from L1 when water or nitrogen demand is not satisfied in L2. Thus it assumes this species takes benefit of a faster root penetration rate so that it can fulfill its water or nitrogen requirement. Accordingly, the model accounts for the competitive advantage towards the fast root growth rate species.

Under non limiting soil N conditions N taken up by each species is driven by N demand. Maximal N dilution curves determined for wheat (Justes *et al.*, 1994) and pea (Ney *et al.*, 1997) were used to account for maximal N accumulation into crops. As suggested by Cruz and Soussana (1997), critical nitrogen content (expressed as a nitrogen percentage of shoot dry matter (%N) in each species is determined by accounting for both pea and wheat dry matters (DM) when each species is sown at half density (compared to a sole crop set-up) in a replacement set-up:

$$\%Nc = a \cdot (DM_w + DM_p)^{-b} \quad (2)$$

where a (kg/ha) and b (unitless) are parameters of the N dilution curves for each species. However N uptake is limited by a maximum N uptake rate for each species. For wheat, N uptake is only governed by N transport system activity, associated regulations and soil N concentrations. By contrast, N acquired by pea mainly derived from atmospheric N (Ndfa). For pea, N taken up by roots from soil N pool is therefore calculated as the difference between N demand (from N dilution curve) and N accumulated from N_2 fixation. As shown by Voisin *et al.* (2002) and Corre-Hellou *et al.* (2006), N amounts derived from air depend on (i) mineral nitrogen amounts surrounding root nodules, (ii) water deficit and (iii) shoot-to-root carbon allocation. All these regulations are included into the model.

Given contrasted leaf properties, specific extinction coefficient values (k) for wheat (w) and pea (p) were included into the light sharing equation. Probability that incoming PAR is not intercepted by intercrop canopy (P_0) is defined by the below equation:

$$P_0 = e^{-(k_w \cdot LAI_w + k_p \cdot LAI_p)} \quad (3)$$

where k is the extinction coefficient (unitless) for each species and LAI is the leaf area index (m^2 leaf area/m^2 soil surface). Interception efficiency of each species is then calculated according to Tsubo and Walker (2002) as follows:

$$\varepsilon_{iw/p} = \varepsilon_{imax\ w/p} \cdot (1 - P_0) \cdot R \quad (4)$$

$$\text{with } R = \frac{k_{w/p} \cdot LAI_{w/p}}{k_w \cdot LAI_w + k_p \cdot LAI_p} \quad (5)$$

where $\varepsilon_{imax\ w/p}$ is the maximum interception efficiency for either wheat (w) or (p).

The model has been validated under contrasted N fertilizer strategies in two locations in France (Malagoli et al., 2009).

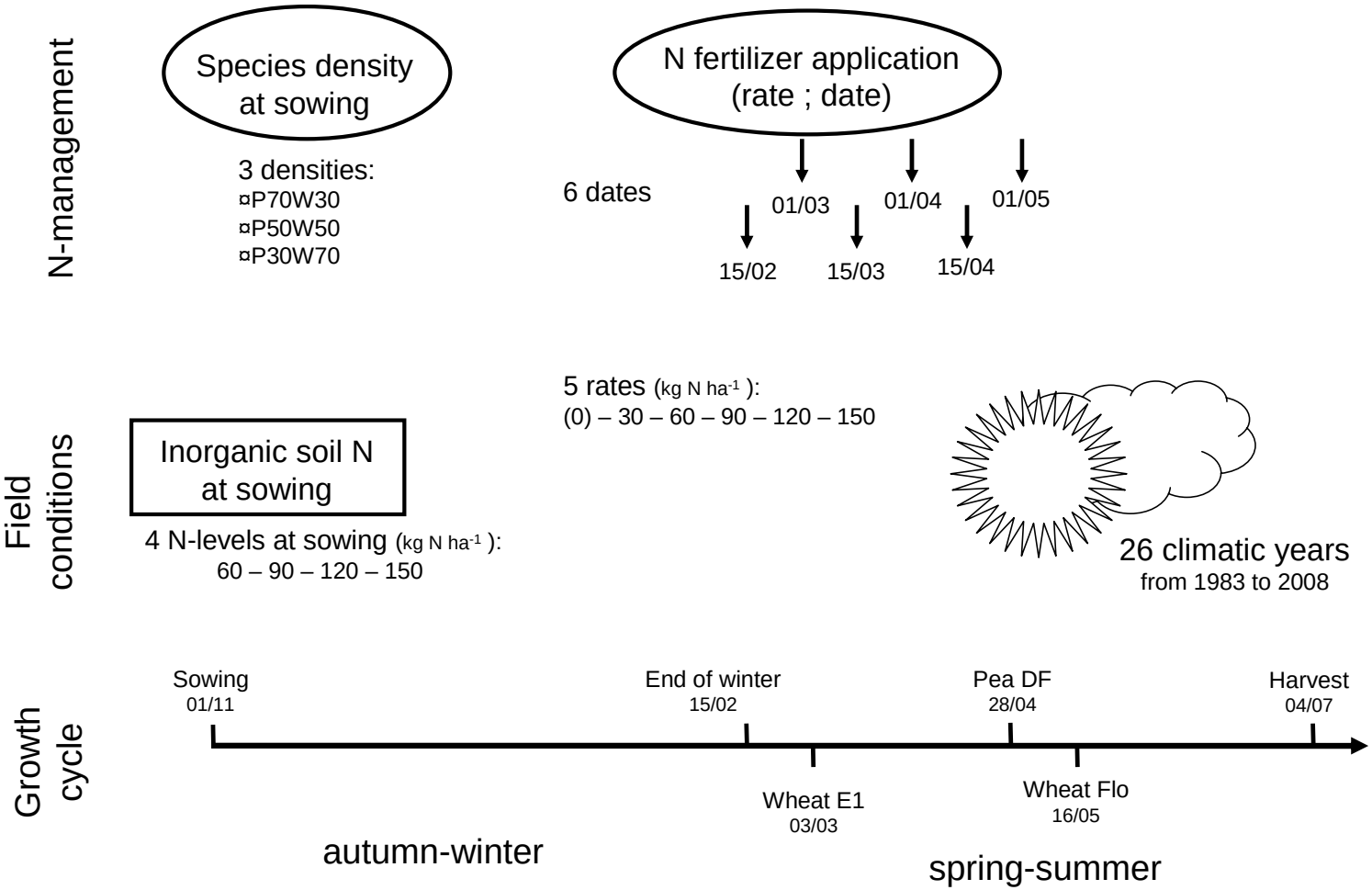

Figure 1: Detailed description of parameter value variation used to simulate a wide range of contrasted soil N availability.

Table 1: Simulated performances of pea wheat intercrop (proportion of intercropped wheat biomass after winter (Feb 15th), IC grain yield, IC wheat grain yield, IC pea grain yield, proportion of wheat grain yield, protein contents in wheat and pea grain, and simulated mineral soil N resources (kg ha^{-1}) from Feb 15th to harvest under low soil N availability conditions . Mineral soil nitrogen amount at sowing was 60 kg ha^{-1}. Mineral N resources result from summing mineral soil N on February 15th and the estimated N mineralization from then until harvest. Values are derived from simulations using a 26 climatic years dataset in La Jaillière (France). They are expressed as means ± s.e. CV: coefficient of variation.

a) P70W30	Mineral N resources from Feb 15th to harvest (kg ha^{-1})	% of wheat in IC biomass on Feb 15th	Total IC Grain Yield (q ha^{-1})	IC wheat Grain Yield (q ha^{-1})	IC pea Grain Yield (q ha^{-1})	% of wheat in IC grain yield	GPC of IC wheat (%)	GPC of IC pea (%)
Mean	125	35	61	21	40	35	8	23
SE	3.48	0.71	1.92	0.76	1.99	1.50	0.16	0.21
CV	14%	10%	16%	18%	25%	21%	10%	5%
2nd quintile	111	33	53	17	31	28	7	22
Median	125	35	63	21	40	35	8	23
4th quintile	139	38	68	23	47	41	8	24
b) P50W50	Mineral N resources from 15th february to harvest (kg ha^{-1})	Contribution of wheat in IC biomass on 15th february (%)	Total IC Grain Yield (q ha^{-1})	IC wheat Grain Yield (q ha^{-1})	IC pea Grain Yield (q ha^{-1})	Contribution of wheat in IC grain yield (%)	GPC of IC wheat (%)	GPC of IC pea (%)
Mean	125	55	59	24	35	41	8	23
SE	3.52	0.82	1.84	0.82	1.88	1.62	0.15	0.20
CV	14%	7%	15%	17%	27%	20%	9%	4%
2nd quintile	111	54	52	21	26	34	8	22
Median	125	55	61	24	34	42	8	23
4th quintile	140	59	66	27	43	48	9	24
c) P30W70	Mineral N resources from 15th february to harvest (kg ha^{-1})	Contribution of wheat in IC biomass on 15th february (%)	Total IC Grain Yield (q ha^{-1})	IC wheat Grain Yield (q ha^{-1})	IC pea Grain Yield (q ha^{-1})	Contribution of wheat in IC grain yield (%)	GPC of IC wheat (%)	GPC of IC pea (%)
Mean	126	74	56	27	29	49	9	23
SE	3.55	0.67	1.72	0.90	1.78	1.85	0.14	0.19
CV	14%	5%	15%	17%	30%	19%	8%	4%
2nd quintile	112	73	49	23	23	39	8	22
Median	126	74	57	27	27	49	8	23
4th quintile	141	77	63	31	38	55	9	24

2.2. Simulations

The model was run with a 26 year climatic dataset (temperatures, PAR, precipitations) (between 1983 and 2008) in La Jaillière (France; 47°26'N, 0°57'W) to assess intercrop performances by accounting for inter-annual climatic variations**.** The soil was a sand clayey loam (16.4% clay, 57.5% silt, 26.1% sand) with a high potential of N mineralization. For each simulation, the date of the end of winter (February 15th) was chosen when soil mineral nitrogen is commonly monitored under temperate lands. It will be referred as the "end of winter" further in the paper (Fig. 1).

It is now well established that soil mineral nitrogen plays a key role for driving intercrop management (Corre-Hellou et al., 2006; 2009; Hauggaard-Nielsen et al., 2001; Naudin et al., 2009a, b). Accordingly, intercrop performances (total intercrop yield, intercropped wheat and pea grain yields, wheat and pea grain protein contents), soil N (mineral soil N on Feb 15th, N mineralization after winter (from February 15th to harvest)) and proportion of intercropped wheat biomass after winter were simulated under either unfertilized (accounting for soil N mineralization from sowing to harvest) or fertilized (soil mineral N derived from mineralization + N fertilizer applications) plots varying soil N availability through (i) pea/wheat proportions at sowing, (ii) mineral soil nitrogen amount at sowing and (iii) rates and dates of N fertilizer applications. Range of value variation for each parameter is summarized in Fig. 1.

2.3. Statistics

A multiple linear regression procedure using R software (R Development Core Team, 2009) was used to describe intercrop performances as a linear combination of the various variables such as soil mineral N observed at the end of winter, proportion of wheat in intercrop biomass observed at the end of winter, mineralized soil N from end of winter to harvest, and rate of N inputs.

3. Results and discussion

3.1. Simulated intercrop performances under an unfertilized plot for 26 different climatic years with a low mineral N content at sowing (Table 1)

Total IC grain yields reach on average 61, 59 and 56 q ha^{-1} for P70W30, P50W50, P30W70 intercrops respectively. Species proportion at sowing has no effect on grain protein content (GPC), even though a trend to increased GPC in wheat is observed along with an increased wheat proportion (from 8 to 9%; Table 1). The percentage of wheat increased with the proportion of wheat at sowing. This is explained by both a decrease of intercropped pea (-11 q ha^{-1} between P70W30 and P30W70; Table 1) and, to a lesser extent, an increase of intercropped wheat grain yield at harvest (+6 q ha^{-1} between P70W30 and P30W70; Table 1). Pea biomass contributes to a larger extent to total biomass than wheat when no N fertilizer is applied, except in 50% of climatic situations in P30W70 intercrops.

For a given proportion at sowing, a high variability of the percentage of wheat at harvest was observed. Accordingly, simulations across the 26 climatic years suggest that proportions of wheat species at harvest can not be accurately predicted only from pea/wheat proportions at sowing under low soil N resources. Andersen et al. (2007) and Naudin et al. (2009a) have highlighted that proportions and competitive advantage of intercropped species highly vary along the crop cycle.

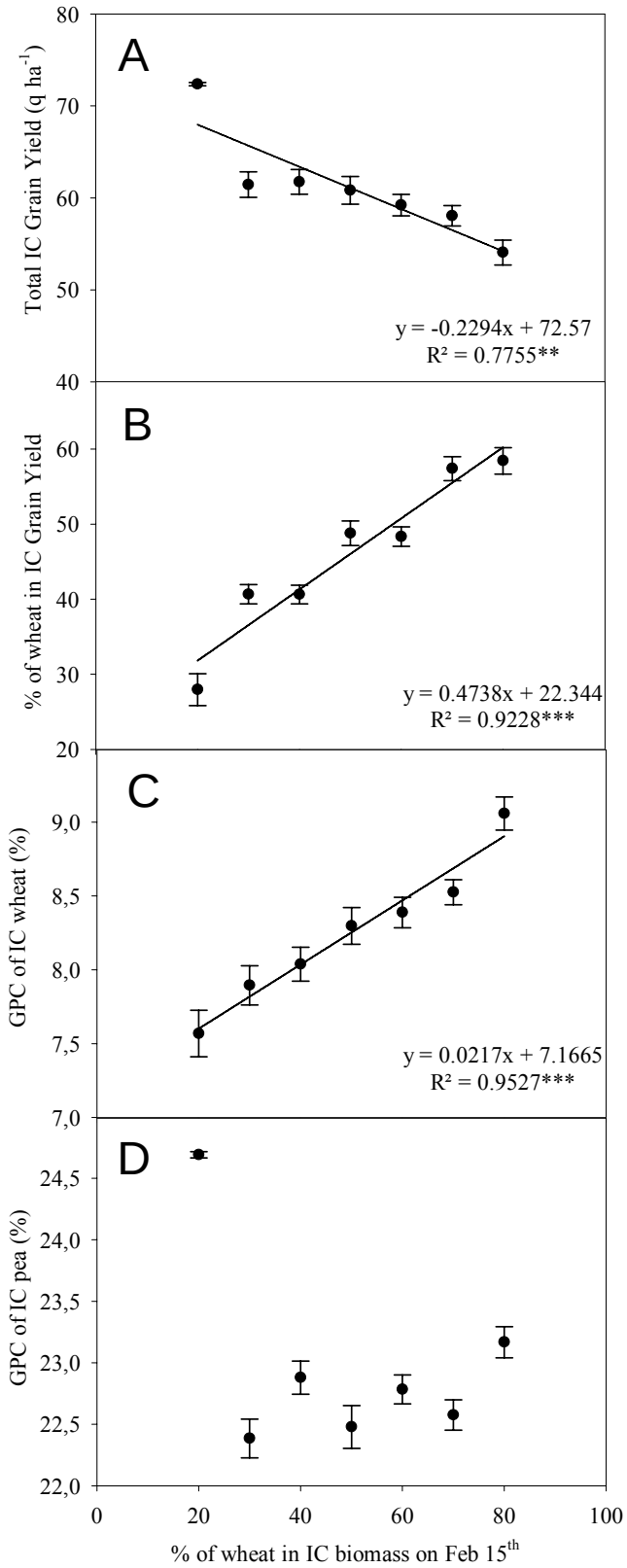

Figure 2: Effect of intercropped wheat biomass proportion after winter (on February 15th) on intercrop performances at harvest (total grain yield, proportion of wheat grain yield into intercrop, grain protein content in intercropped wheat and pea). Values are means ± SE when larger than the symbol.

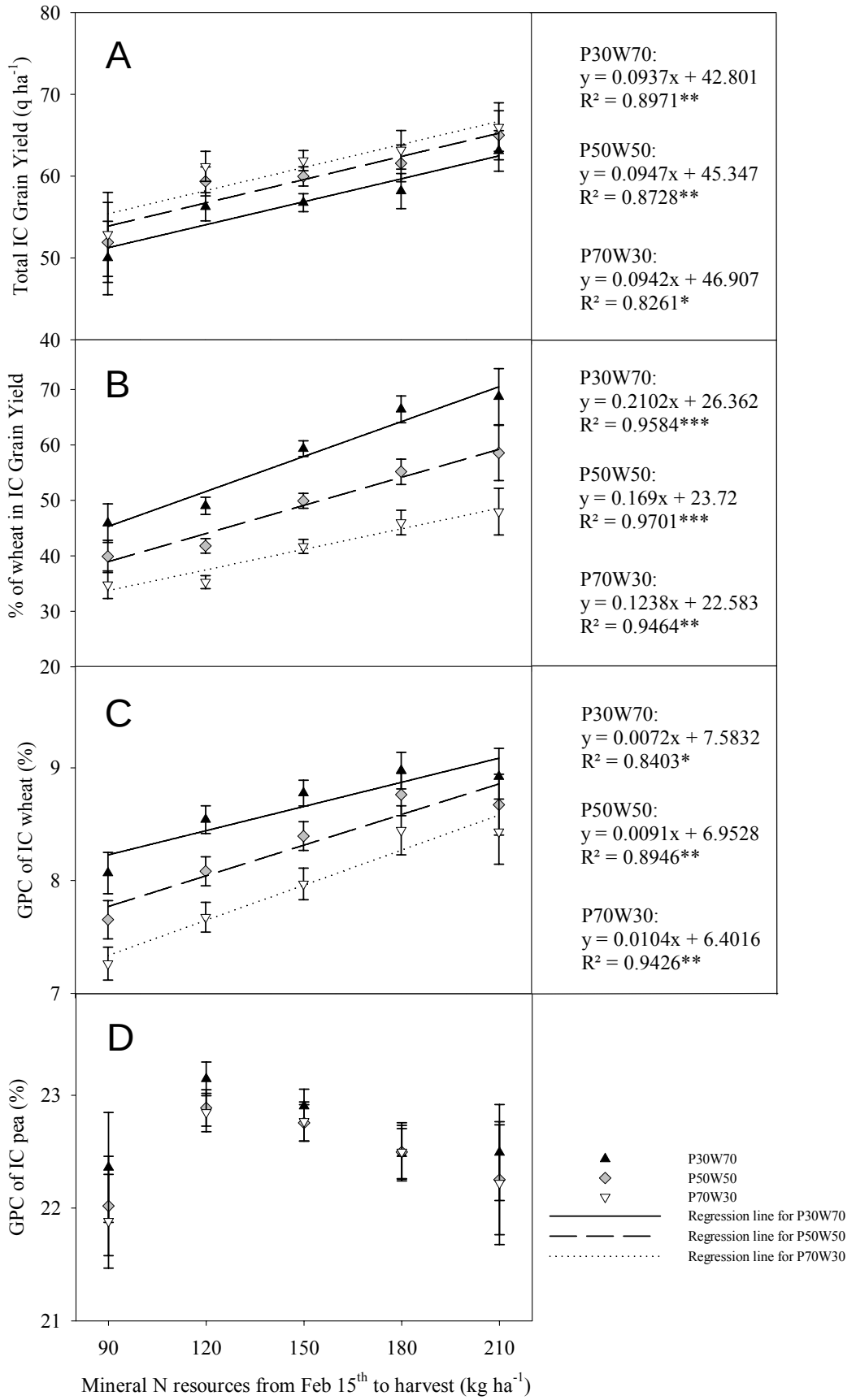

Figure 3: Effect of soil nitrogen amount mineralized from February 15th to harvest on intercrop performances at harvest (total grain yield, proportion of wheat grain yield into intercrop, grain protein content in intercropped wheat and pea) for three species proportions at sowing (P30W70: dashed line; P50W50: dashed bold line; P70W30; plain line). Values are means ± SE when larger than the symbol.

The three proportions of wheat at sowing resulted in three contrasting proportions of wheat at the end of the winter. However, wheat grain yield proportions at harvest are within a much narrower range than those ones at the end of the winter. Except for the simulation "P70W30", proportions of wheat in intercrop dramatically decrease from 55% and 74% of total IC biomass after winter to 41 and 49 % of intercrop grain yield at harvest, respectively. Such result based upon 26 year dataset simulations brings extra evidence about the very high variability of species proportions (coefficient of variation of IC wheat proportion = 20 %) at harvest for the tested range of soil mineral N based on soil mineral N content at sowing of 60 kg N ha^{-1}, which lead to soil mineral N content at the end of winter varying from 26 to 96 kg N ha^{-1}, and to N mineralization potential between the end of winter and the harvest varying from 50 to 91 kg N ha^{-1}).

3.2. Relationship between proportion of wheat on February 15th and soil mineral N availability from Feb 15th to harvest) into unfertilized intercrop on intercrop performances at harvest.

Intermediates variables (% of wheat biomass at the end of winter and soil mineral N amount available between February 15th and harvest) and final variables (total IC grain yield, % of wheat grain yield at harvest, grain protein content in wheat and pea) were simulated crossing a wide range of soil mineral nitrogen amount and species densities at sowing (Fig. 1 and 2) as described in the Material and Methods section. Such an approach allows stretching the range of wheat biomass proportion into intercrop observed at end of winter (varying from 23 to 79%; Fig. 2) as well as mineral N resources from Feb 15th to harvest (Figure 3). Mineral N resources of Figure 3 is defined as the sum of mineral soil N available at the end of winter (varying from 26 to 153 kg ha^{-1}) and the soil nitrogen mineralized from Feb 15th to harvest (varying from 50 to 91 kg ha^{-1}).

Regarding the effect of % of wheat biomass at the end of winter, simulations clearly show that the larger the wheat biomass proportion at end of the winter the lower the total IC grain yield at harvest (Fig. 2 A). Indeed a 60% increase of the % of wheat IC biomass on February 15th leads to a 15 q ha^{-1} IC yield increase. As shown in Fig. 2B, % of wheat IC yield concomitantly increases to the same proportion at the end of the winter. Hauggaard-Nielsen et al. (2006) have shown that proportion of species at harvest was in accordance from the expected proportions sown, except for high relative densities (additive intercrops). However, the slope value (0.47) lower than 1 indicates a lag between pea and wheat growth cycles so that proportion of wheat IC grain yield is decreased until harvest. Indeed, the proportion of species in intercrop biomass is known to be variable along the crop cycle (Andersen et al., 2007) and to be dependant on mineral N availability (Jensen, 1996; Hauggaard-Nielsen and Jensen, 2001; Ghaley et al., 2005; Corre-Hellou et al., 2006) and dynamics along the crop cycle. From a quality viewpoint, GPC values in wheat simulated by AZODYN-IC under such conditions are low (ranging from 7.5 to 9 %, Fig. 2C). Indeed, current observed GPC in intercrop wheat are currently higher than those observed in sole crops: in no fertilized conditions, intercropping is known to increase barley GPC by 13 % (Jensen, 1996), and mean GPC was about 9.1 % (from pooled data observed on unfertilized and fertilized intercrop) (Corre-Hellou, 2005). However, based on our simulations, a highly significant positive correlation (R^2=0.9527) was found between wheat GPC and the % of wheat IC biomass on February 15th. Indeed, a 1.5% variation in wheat GPC was simulated over the simulated range of the % of wheat IC biomass at the end-of-winter (Fig. 2C). Finally the % of wheat IC biomass on February 15th has no effect on pea GPC (Fig. 2D).

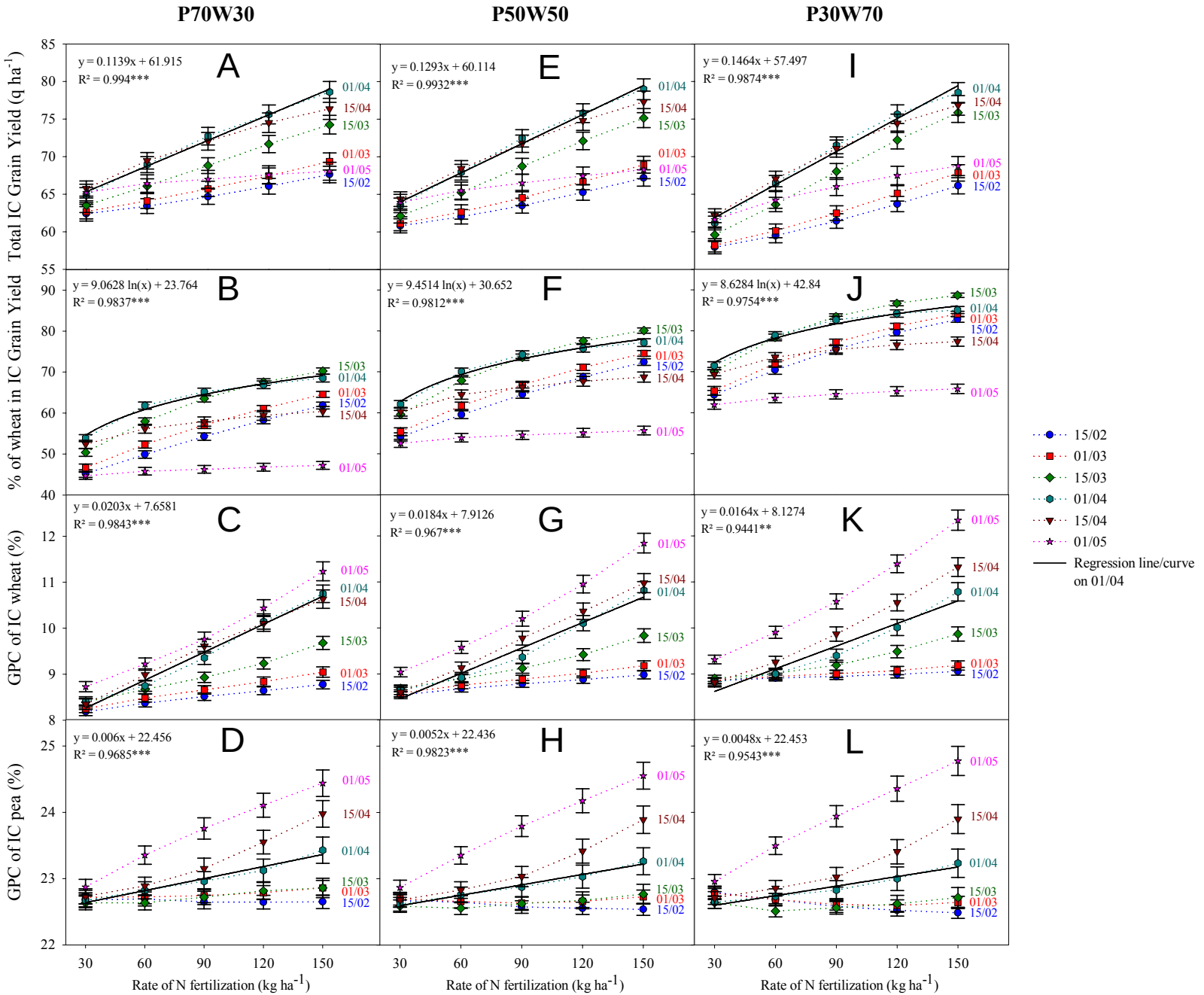

Figure 4: Effect of N fertilization applications (rates and dates detailed in Table 1) on intercrop performances at harvest (total grain yield, proportion of wheat grain yield into intercrop, grain protein content in intercropped wheat and pea) for three species proportions at sowing (P30/W70; P50/W50; P30/W70). Values are means ± SD when larger than the symbol.

When considering the mineral soil N amount available from the end-of-winter to harvest, simulations demonstrate that N resources during this period have a statistically significant effect on the total IC grain yield, but with a slow slope (about 0.09 q kg^{-1} N available; Fig. 3A), whatever the species density at sowing. For instance, a total IC grain yield gain of 11 q ha^{-1} is observed along with an increase of 120 kg N ha^{-1}.

Increasing mineral N resources from February 15th to harvest leads to a concomitant increase of both % of wheat in IC grain yield and GPC of IC wheat (Fig. 3 B and C). This increase is all the steeper that the pea/wheat proportion at sowing is high whereas the reverse is shown for the GPC of IC wheat, for which a plateau is suggested beyond 180 kg N ha^{-1}. Finally IC pea GPC hardly varies along with increasing mineral N resources. The absence of effect of growing conditions on GPC pea is in accordance with previous results (Jensen, 1996).

3.3. Combined effect of N fertilization (rate and date of applications) pea/wheat swing densities on wheat grain proportion and global protein content at harvest

As stated earlier, available soil mineral nitrogen is a key-element to drive intercrops. Results from Table 1 and Fig. 2 suggest that the proportion of intercropped wheat biomass varies from the end of winter until harvest. Thus, N fertilizer during spring may be usefully applied to increase either the wheat grain yield or the wheat grain quality or maximize intercrop biomass production along with minimal N input depending on production goals. To do so, increasing N fertilizer rate as well as various date (from early to late application; see Fig. 1 for detailed description) between February 15th and April 15th were crossed and added to "unfertilized" situation to investigate to what extent such tool can be optimally handled. Resulting simulations are presented in Fig. 4.

When compared to the unfertilized simulations, adding N fertilizer increased both the total IC grain yield and the proportion of IC wheat grain yield at harvest for all species proportions and all dates of N fertilizer application (Fig. 4 A, B, E, F, I, J). For instance, the total IC grain yield and the proportion of IC wheat grain yield increase from 60 to 80 q ha^{-1} (*i.e.* 0.13 q kg^{-1} N applied; Fig. 4E) and from 50 to 80% (Fig. 4 F) for the P50W50 treatment, respectively. Thus it shows that N fertilizer application effect is not as strong as it is on a sole cropped wheat (averaged gain of 0.33 q kg^{-1} N applied) (Meynard et al., 1981). More interestingly, simulations point out that the magnitude and the pattern of the response depend on the date of application. Indeed, the response of the total IC grain yield and the proportion of IC wheat grain to N input is maximal when the tested fertilizer derived-N amount is applied on March 15th (12 days after wheat ZGS30 stage) and on April 1st (wheat ZGS32 stage), respectively. Early (before March 1st) and late (from May 1st) applications of nitrogen fertilizer have little effect on intercropped wheat grain yield and proportion at harvest, whatever the N fertilizer rate applied. Finally, it has to be underlined that the response of total IC yield to date of N fertilizer application is weak for low N input (30 and 60 kg N ha^{-1}). Indeed, between February 15th and April 15th, wheat was at the beginning of growth acceleration and N application at this time is known to be more efficiently valorized by wheat crops (Limaux et al., 1999).

Table 2: Linear model describing total IC grain yield (q ha^{-1}), % of IC wheat yield at harvest and global protein content in IC wheat (%) as a function of % of IC wheat biomass on February 15th (Rdww), mineral soil nitrogen on February 15th (kg ha^{-1}; Nsoilw) and N mineralization from February 15th until harvest (kg ha^{-1}; Nmin). SE: standard error.

Total IC Grain Yield (q ha^{-1})

Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**45.2791**	±4.273	***
Rdww (%)	**-0.1263**	±0.031	***
Nsoilw (kg ha^{-1})	**0.0701**	±0.018	***
Nmin (kg ha^{-1})	**0.2335**	±0.050	***
	*R^2=0.126****		

% wheat in IC grain yield

Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**-16.6226**	±3.999	***
Rdww (%)	**0.4180**	±0.029	***
Nsoilw (kg ha^{-1})	**0.2080**	±0.017	***
Nmin (kg ha^{-1})	**0.3861**	±0.047	***
	*R^2=0.569****		

GPC of IC wheat (%)

Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**8.1070**	±0.279	***
Rdww (%)	**0.0231**	±0.002	***
Nsoilw (kg ha^{-1})	**0.0115**	±0.001	***
Nmin (kg ha^{-1})	**-0.0296**	±0.003	***
	*R^2=0.544****		

Table 3: General linear model describing total IC grain yield (q ha^{-1}), % of IC wheat yield at harvest and global protein content in IC wheat (%) as a function of rate of N fertilizer applied (Rate; kg ha^{-1}), % of IC wheat biomass on February 15th (Rdww), mineral soil nitrogen on February 15th (kg ha^{-1}; Nsoilw) and N mineralization from February 15th until harvest (kg ha^{-1}; Nmin). N fertilizer is applied on April 1st. SE: standard error.

Total IC Grain Yield (q ha^{-1}) (N applications on April 1st)			
Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**14,5166**	±2,141	***
Rate (kg ha^{-1})	**0,1332**	±0,006	***
Rdww (%)	**-0,0401**	±0,015	***
Nsoilw (kg ha^{-1})	**0,0711**	±0,009	***
Nmin (kg ha^{-1})	**0,6323**	±0,024	***
	*R^2=0.437****		

% of wheat in IC grain yield (N applications on April 1st)			
Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**-26,8060**	±2,259	***
ln Rate (ln kg ha^{-1})	**9,2071**	±0,349	***
Rdww (%)	**0,4346**	±0,012	***
Nsoilw (kg ha^{-1})	**0,1088**	±0,007	***
Nmin (kg ha^{-1})	**0,3952**	±0,019	***
	*R^2=0.629****		

GPC of IC wheat (%) (N applications on April 1st)			
Multiple Regression			
	Estimated coefficient	**±SE**	**Pr>|t|**
Intercept	**11,6956**	±0,238	***
Rate (kg ha^{-1})	**0,0180**	±0,001	***
Rdww (%)	**0,0067**	±0,002	***
Nsoilw (kg ha^{-1})	**0,0128**	±0,001	***
Nmin (kg ha^{-1})	**-0,0786**	±0,003	***
	*R^2=0.588****		

N fertilization leads to an increase of the GPC in wheat for the three tested species proportions at sowing (Fig. 4 C, G, K). For a late supply, this increase is all the larger that the P/W proportion at sowing decreases. Hence, this increase ranges from 8.5 % to 11, 11.5 and 12.5 % for P70/W30, P50/W50 and P30/W70 treatments, respectively. The highest GPC in wheat was predicted for the latest date of N fertilizer application (on May 1st), for all pea/wheat proportions at sowing. Such late date of N-applications is currently known to be efficient to increase GPC in sole cropped and intercropped wheat (Recous et al., 1988; Jeuffroy and Bouchard, 1999; Limaux et al., 1999; Bedoussac and Justes, 2009; Naudin et al. 2009b). Simulated GPC in pea do not significantly vary along with N fertilizer application as it ranges from 22.5 to 24. % (Fig. 3 D, H, L).
All these data taken together clearly put forward that N fertilizer application during spring combined with the proportion of each species at sowing is a powerful tool (i) to maximize total IC yield or (ii) to enhance proportion of IC wheat yield at harvest or (iii) to improve global protein content in wheat. It also highlights that the date of N application is of great importance confirming experimental results from Naudin et al. (2009a, b). It shows that the date of N application for reaching either a large wheat yield at harvest or a high GPC in wheat is similar to sole-crop wheat, yet applied N amounts are much lower. Indeed, a significant effect is observed beyond 60 kg N ha^{-1} applied for the soil of La Jaillière under the 26 climatic years dataset. Although this threshold can not be hold as an absolute value as the native available soil N resources are high in La Jaillière, multi-year simulations from AZODYN-IC strengthens the fact that IC yields are higher than SC yields along with lower N inputs.

3.4. Modeling intercrop yield at harvest based upon mineralized soil mineral nitrogen, N fertilizer applications and proportion of intercropped wheat biomass at the end of winter (Tables 2 and 3).

AZODYN-IC was used to simulate and assess the performances of winter-pea wheat intercrops for a wide range of soil N resources availability. From previous results, several identified key-variables can be proposed to predict intercrop performances either under low soil N resources or with the N fertilization tool. Two general linear models are then proposed to describe the total IC grain yield, % of IC wheat yield at harvest and global protein content in IC wheat (%) depending on (i) the proportion of IC wheat biomass on February 15th (Rdww), (ii) mineral soil nitrogen on February 15th (kg ha^{-1}; Nsoilw) and (iii) N mineralization from February 15th until harvest (kg ha^{-1}; Nmin) (Table 2) and (iv) rate of N fertilizer when applied (Table 3). These variables are easy to measure or to estimate in agricultural production context and most of them are already used for N-management decision in sole cropping systems (Machet et al., 1990). This is a key point for the choice of variables to integrate in such simplified model intended to contribute to build decision rules (Meynard et al., 2002).

When no fertilizer is applied (Table 2), the proposed model shows that % of IC wheat yield at harvest and global protein content in IC wheat (%) depending on Rdww, Nsoilw and Nmin can explain 56.9% and 54.4% of the variation of the proportion of wheat grain yield at harvest and GPC in IC wheat, respectively (Table 2). By contrast, the low R^2 value (0.126) for the total IC grain yield points out that those variables have little effect.
When the rate of N fertilizer is added to the model, the R^2 values increase for predicted outputs (0.437, 0.629 and 0.588 for total IC grain yield, proportion of IC wheat yield at harvest and global protein content in IC wheat, respectively) (Table 3). Thus it suggests that N fertilizer during spring can be used as a tool to improve the prediction and drive intercrop performances.

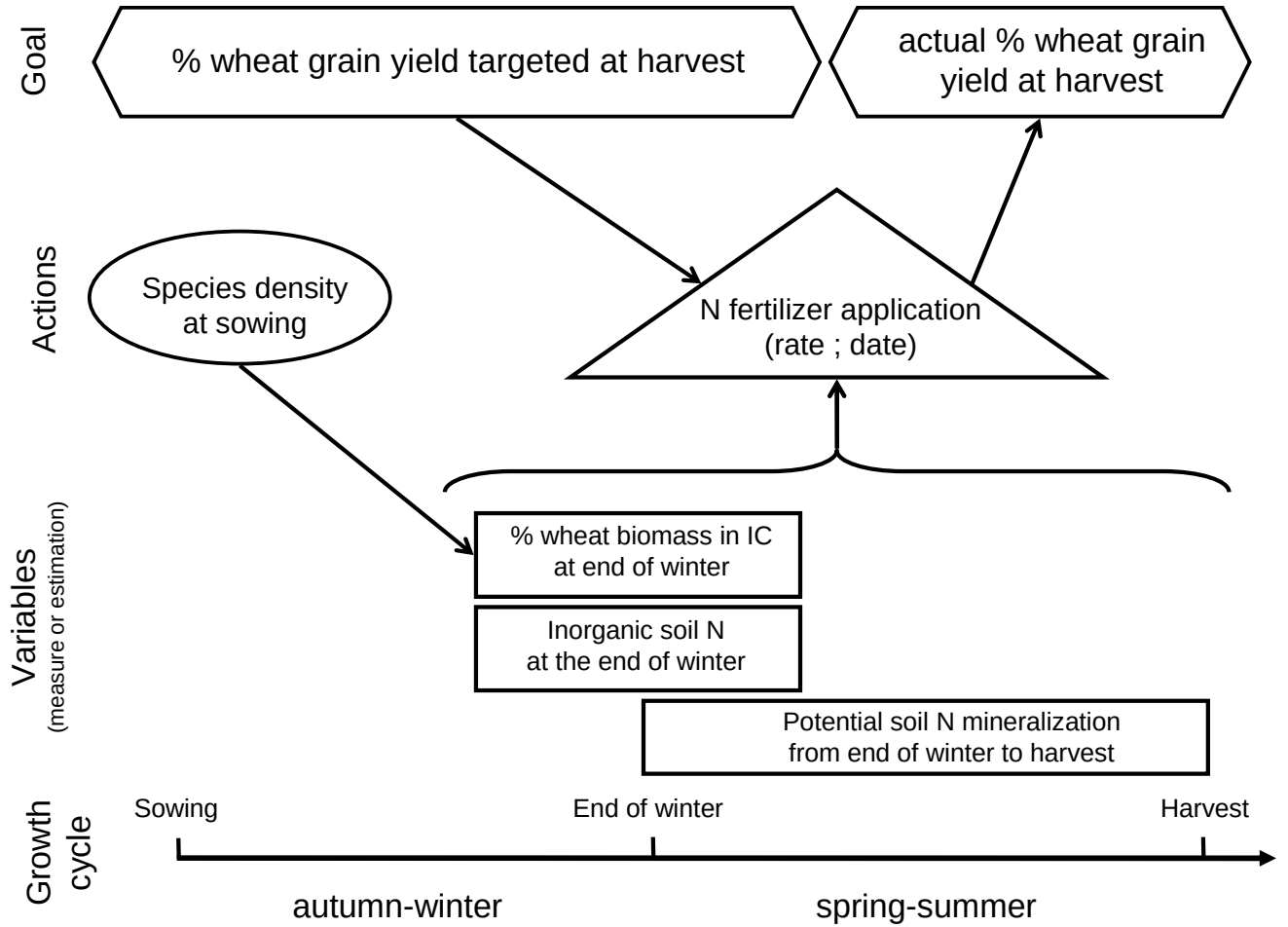

Figure 5: Overview summarizing steps requiring further investigation through experiment to propose decision rules guiding farming practices.

Linear models proposed here aim at contributing to the definition of decision rules for N-management of cereal-legume intercrops and to bring guidelines for future experimentations (Bergez et al., 2009). These simplified models are proposed to supply straightforward relationships between targeted harvested variables values and easy-assessing real-time variables along the growth cycle. Indeed, it is out of purpose to run AZODYN-IC to help farmers drive intercrop management as too many parameter values and numerous expensive measurements from sowing to harvest are required to run AZODYN-IC.

Accordingly, these models clearly demonstrate that key-variables at harvest (such as total IC yield, proportion of wheat yield, GPC in wheat) can be convincingly predicted through % of wheat biomass at the end of the winter and variables describing soil mineral N availability between the end of the winter and the harvest (Nsoilw, Nmin, Rate). Despite a high R^2 value averaging 0.5, using such simplified models point out that there is a lack of information explaining the full variation of simulated final variables. Thus, additional factors jeopardizing intercrop performances may later be included into those models such as water deficit, weeds competition, pest attack, for instance. Such improvements would allow a more accurate prediction a wider range of limiting factors occurring under field conditions with low N inputs.

4. Conclusions

This work was able to give propositions to test decision rules for N-management of intercrop according to soil N mineral content at the end of winter, contribution of wheat in intercrop biomass at the end of winter, estimation of N-mineralization form end of winter to harvest, and rate and date of N-application (Figure 5). This could be use to design future experiments to test our propositions and define strong decision rules. Moreover, to date, our work only focus on agronomic performances through biomass, yield or wheat GPC gain. However, a further development may also deals with the N balance at the field levels to address the environmental benefit, and with economic benefits. As AZODYN-IC is a soil-plant process based model, some pertinent variables such as N leaching or soil N at harvest should be considered. It would also provide some helpful elements to rank intercrop into the crop system. Another way of improving intercrop performances may also go through the definition of adapted cultivars, better fit or architectured to optimize their growths under intercrop design. As a consequence, a potential use of AZODYN-IC would stress on cultivar parameters such as the effect of precocity or leaf properties on their competitiveness.

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References

Andersen, M.K., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2007. Competition for and utilisation of sulfur in sole and intercrops of pea and barley. Nutr. Cycl. Agroecosys. 77, 143-153.

Bédoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil. in press (doi: 10.1007/s11104-009-0082-2)

Bellostas, N., Hauggaard-Nielsen, H., Andersen, M.K., Jensen, E.S., 2003. Early interference dynamics in intercrops of pea, barley and oilseed rape. Biol. agric. hortic. 21, 337-348.

Bergez, J.E., Colbach, N., Crespo, O., Garcia, F., Jeuffroy, M.H., Justes, E., Loyce, C., Munier-Jolain, N., Sadok, W., 2009. Designing crop management systems by simulation. European Journal of Agronomy (doi:10.1016/j.eja.2009.06.001)

Biarnès, V., Lecoeur, J., Jeuffroy, M.-H., Vocanson, A., Ney, B., 2009. A model which integrates knowledge on pea crop physiology and agronomic diagnosis. In: Physiology of the pea crop. Science Publishers, Enfield, USA, pp 197-208. in press.

Bulson, H.A.J., Snaydon, R.W., Stopes, C.E., 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. Journal of Agricultural Science 128, 59-71.

Corre-Hellou, G., 2005. Acquisition de l'azote dans des asociations pois-orge (*Pisum sativum* L.-*Hordeum vulgare* L.) en relation avec le fonctionnement du peuplement. PhD Thesis, Université d'Angers, Angers, France.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific Competition for soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208.

Corre-Hellou, G., Faure, M., Launay, M., Brisson, N., Crozat, Y., 2009. Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea-barley intercrops. Field Crops Res. 113, 72-81.

Cruz P., Soussana, J.-F. Mixed crops. 1997. In: Lemaire, G. (ed.), Diagnosis of the Nitrogen Status in Crops. Springer Verlag, Berlin, Heidelberg, pp. 131-144.

Ghaley, B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E. 2005. Intercropping of Wheat and Pea as Influenced by Nitrogen Fertilization. Nutr. Cycl. Agroecosyst. 73, 201-212.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E. S., 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res. 70, 101-109.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.

Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. Field Crop Res. 95, 256-267.

Jensen, E.S., 1996. Grain yield, symbiotic N_2 fixation and interspecific competition for mineral N in pea-barley intercrops. Plant Soil. 182, 25-38.

Jeuffroy, M.-H., Bouchard, C., 1999. Intensity and duration of nitrogen deficiency on wheat grain number. Crop Sci. 39, 1385-1393.

Jeuffroy, M.-H., Recous, S., 1999. AZODYN: a simple model simulating the date of nitrogen deficiency for decision support in wheat fertilization. Eur. J. Agron. 10,129-144.

Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huché, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74, 397-407.

Limaux, F., Recous, S., Meynard, J.-M., Guckert, A., 1999. Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat. Plant and Soil 214, 49-59.

Machet, J.M., Dubrulle, P., Louis, P., 1990. AZOBIL: a computer program for fertilizer N recommandations based on a predictive balance sheet method. In: Scaife, A. (Ed.), Proc. 1st Congress of the European Society for Agronomy, Paris pp. 21–22.

Malagoli P, Naudin C, Goulevant G, Sester M, Corre-Hellou G and Jeuffroy M-H. 2009. Simulation of the response of a winter pea-wheat intercrop to contrasted N-fertilization applications: design and assessment of AZODYN-IC model. Eur. J. Agron. Under review. EURAGR1748.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S., Valantin-Morison, M. 2008. Mixing plant species in cropping systems: concepts, tools and models. A review. Agron. Sustain. Dev. 28, 1-20.

Meynard, J.-M., Boiffin, J., Caneill, J., Sébillotte, M., 1981. Elaboration du rendement et fertilisation azotée du blé d'hiver en Champagne crayeuse. II. Types de réponse à la fumure azotée et application de la méthode du bilan prévisionnel. Agronomie 1, 795-806.

Meynard, J.-M., Cerf, M., Guichard, L., Jeuffroy, M.-H., Makowski, D., 2002. Which decision support tools for the environmental management of nitrogen? Agronomie 22, 817-829.

Monteith, J.-L., 1977. Climate and efficiency of crop production in Britain. Phil. Trans. R. Soc. Lond. 281, 277-294.

Naudin C, Corre-Hellou G, Pineau S, Crozat Y and Jeuffroy M-H, 2009a. Timing effect of nitrogen fertilization on winter pea-wheat intercrops. I- Growth dynamics and grain yield. Field Crops Res. Under review. FIELD-D-09-00419.

Naudin C, Corre-Hellou G, Pineau S, Crozat Y and Jeuffroy M-H. 2009b – Timing effect of nitrogen fertilization on winter pea-wheat intercrops. II- N-partitioning and Symbiotic Nitrogen Fixation variations. Field Crops Res. Under review. FIELD-D-09-00420.

Neumann, A., Schmidtke, K., Rauber, R. 2007. Effects of crop density and tillage system on grain yield and N uptake from soil and atmosphere of sole and intercropped pea and oat. Field Crop Res., 100, 285-293.

Ney, B., Doré, T., Sagan, M. 1997. Grain legumes. In: Lemaire, G., (Ed.), Diagnosis of the nitrogen status in crops. Springer, pp. 107-118.

R Development Core Team , 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988. The fate of labelled15N urea and ammonium nitrate applied to a winter wheat crop. I. Nitrogen transformations in the soil. Plant and Soil 112, 205-214.Tsubo, M., Walker, S., 2002. A model of radiation interception and use by a maize-bean intercrop canopy. Agric. Meteorol. 110, 203-215.

Tsubo, M., Walker, S., 2002. A model of radiation interception and use by a maize-bean intercrop canopy. Agric. Meteorol. 110, 203-215.

Vocanson, V., 2006. Evaluation ex ante d'innovations variétales en pois d'hiver (*Pisum sativum* L.) : approche par modélisation au niveau de la parcelle et de l'exploitation agricole. Ph. D. manuscript, Institut National Agronomique Paris-Grignon, 293 p.

Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil. 243, 31-42.

Willey, R.W., 1979. Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. Field Crop Abstr. 32, 1-10.

Synthèse partielle

Notre démarche de modélisation a permis d'aboutir à l'obtention, à partir de deux modèles de culture pures (AZODYN pour le blé et AFISOL pour le pois), d'un modèle dynamique du fonctionnement de ces associations (AZODYN-IC) dont l'intérêt et l'originalité se situent dans :

- sa capacité à bien simuler la réponse à des disponibilités en azote variées permettant ainsi d'être directement opérationnel et utilisé comme outil d'aide à la gestion de la fertilisation ;
- ses formalismes relativement simples de partage des ressources (lumière, eau, azote) et comprenant un lien très étroit entre le partage de la lumière et l'acquisition de l'azote déterminant dans le fonctionnement du peuplement ;
- l'absence de paramétrage spécifique en association. Le modèle a permis de prolonger les expérimentations en simulant des stratégies de fertilisation plus larges (combinaisons de proportions de semis x doses x dates de fertilisation) et ceci pour une gamme importante d'années climatiques.

Ce travail a également permis de dégager des pistes de règles de décision pour adapter la fertilisation en fonction :

- des reliquats d'azote observés sortie hiver
- de la proportion de blé dans la biomasse de l'association observée sortie hiver
- et de l'estimation de la minéralisation depuis sortie hiver jusqu'à la récolte
- de la dose et de la date d'apport

Ces résultats devraient permettre d'orienter les expérimentations futures finalisant la définition de règles de décision pour la conduite azotée des associations pois-blé.

Discussion générale et perspectives

1. Effet de la fertilisation azotée sur les dynamiques de croissance, de partage des ressources azotées, et sur les performances finales à la récolte

1.1. La fertilisation azotée comme levier de pilotage de la proportion des espèces

Nos travaux ont montré que la fertilisation azotée était un levier efficace pour modifier l'évolution, au cours du cycle, de la proportion de chaque espèce dans le mélange (hypothèse 1), critère aujourd'hui peu maîtrisé.
Ils confirment, pour des associations pois-blé conduites en semis d'hiver, les connaissances acquises précédemment sur le fonctionnement d'une association céréale-légumineuse (pois-orge semée au printemps, essentiellement) en réponse à la disponibilité en azote : un apport d'azote favorise la croissance de la céréale et pénalise celle de la légumineuse (Jensen, 1996 ; Ghaley et al., 2005, Corre-Hellou et al., 2006). La céréale apparaît plus compétitive que la légumineuse pour l'azote minéral. Un apport d'azote est principalement valorisé par la céréale qui voit son statut azoté amélioré et par conséquent sa compétitivité aérienne pour la lumière accrue. En situation de fertilisation azotée, le pois subit donc une compétition accrue pour les ressources en présence d'une forte disponibilité en azote. Un apport d'azote accroît les écarts de dynamique de croissance entre espèces et ralentit la diminution au cours du cycle du pourcentage de blé dans la biomasse.

1.2. Les conditions initiales du couvert au moment de l'apport conditionnent la réponse à la fertilisation azotée

Ces travaux ont permis aussi d'élargir la compréhension du fonctionnement d'une association dans le cas de disponibilités en azote minéral variables dans le temps.

Nous avons mis en évidence que la céréale est plus compétitive que le pois pour l'azote minéral (somme de l'azote du sol et de l'engrais) jusqu'au stade début du remplissage des grains. Avant DRG, le blé acquiert environ 80% de l'azote minéral accumulé par le couvert. Un apport au stade début remplissage est au contraire principalement valorisé par le pois.

Nous avions formulé l'hypothèse 2 selon laquelle une fertilisation azotée intervenant avant le démarrage de l'accélération de la croissance du blé devrait favoriser la croissance de la céréale au détriment de celle de la légumineuse et ainsi modifier la part des espèces au profit de la céréale. Une fertilisation azotée réalisée plus tard dans le cycle (en fin de montaison du blé) pénaliserait moins la croissance du pois, en lui permettant d'installer ses capteurs racinaires et aériens ainsi que son appareil fixateur sans trop souffrir de la compétition exercée par la céréale.

Nos travaux montrent, toutefois, que l'effet de la date de l'apport ne s'exprime pas de façon aussi chronologique que ce qui était envisagé dans l'hypothèse initiale. L'intensité de la réponse à la date de fertilisation varie en fonction des écarts de dynamique de croissance et de phénologie de chaque espèce avant l'apport, qui se mettent en place notamment au moment de l'installation du couvert. L'ampleur des écarts de besoins entre espèces varie au cours du cycle et conditionne le partage des ressources. Il est ainsi montré que la proportion de blé dans la biomasse totale de l'association au moment de l'apport est déterminante de la compétition pour la lumière observé un mois après l'apport et du partage des ressources en azote minéral entre les deux espèces pendant cette même période. Ainsi, c'est la demande relative des espèces au moment de l'apport qui est déterminante de la réponse du couvert plurispécifique à l'apport azoté.

Par ailleurs, un apport précoce favorise effectivement la croissance de la céréale mais cet effet reste ponctuel. Le statut azoté du blé, amélioré juste après l'apport, décroît ensuite rapidement et pénalise la croissance ultérieure du blé. Le maintien de la satisfaction des besoins du blé est d'autant plus difficile que l'apport d'azote a accentué la croissance foliaire et donc les besoins. Avec un apport plus tardif, le statut azoté est légèrement amélioré par rapport à une association non fertilisée aux alentours de la floraison, période importante pour la formation du rendement et de la qualité, avec des conséquences favorables principalement sur le taux de protéines.

Par ailleurs, éviter un apport en début de cycle pour laisser la croissance du pois s'installer, n'est pas si efficace que ce qui était envisagé puisque le pois a intrinsèquement une faible vitesse de croissance en début de cycle et n'est donc pas capable de profiter d'une faible compétitivité du blé.

Par conséquent, dans les conditions testées, bien que la croissance de chaque espèce soit affectée au cours du cycle par la fertilisation azotée, le levier de la date de l'apport n'entraîne pas des écarts très importants et systématiques sur la part de chaque espèce à la récolte.

Afin de prédire l'effet de la date de l'apport, il apparaît donc essentiel de prendre en compte non seulement le niveau de fourniture en azote mais aussi les écarts de croissance entre espèces avant l'apport. Le modèle AZODYN-IC, développé dans le cadre de cette thèse, permet de rendre compte, avec une précision satisfaisante, de l'effet de différentes dates de fertilisation azotée. Le travail réalisé à partir des simulations a permis d'élargir la gamme de stratégies de fertilisation testées et proposer des outils pour définir des règles de décision pour agir de façon plus prononcée sur les performances finales non seulement par la date de l'apport mais par des combinaisons dose x date x proportions des espèces au semis (cf ci-dessous point 3).

2. Dynamique de la fixation symbiotique du pois en réponse à la date de fertilisation (effet inhibiteur et capacité de reprise)

Les expérimentations de plein champ ont démontré que le statut azoté du pois n'était jamais modifié et toujours à l'optimum, et ce, quel que soit le régime de fertilisation appliqué à l'association. En effet, si l'appareil fixateur n'est pas pénalisé par la structure du sol, le statut hydrique du sol, ou la présence de larves de sitones, le pois se montre capable de « switcher » rapidement entre les deux voies d'alimentation azotée en fonction du niveau de ressources en azote minéral du sol. La céréale est plus compétitive que le pois pour l'azote du sol entraînant systématiquement une augmentation de la contribution de la fixation symbiotique par rapport au pois pur même dans les associations fertilisées, comme précédemment montré dans plusieurs travaux (Jensen, 1996 ; Corre-Hellou et al., 2006 ; Hauggaard-Nielsen et al., 2009b). Dans nos expérimentations, tous essais et sites confondus, la contribution de la fixation dans l'azote total accumulé dans les parties aériennes du pois atteignait, à maturité, 86 et 92 % pour les pois associés (respectivement associations non fertilisées et fertilisées), ce qui est significativement supérieur aux 77 % observés en moyenne sur pois pur.

Le pic d'accumulation d'azote du sol par la céréale est plus précoce que celui d'accumulation d'azote par la fixation symbiotique du pois. Par conséquent, la complémentarité entre les deux espèces pour l'utilisation des deux sources d'azote n'est pas totale sur l'ensemble du cycle. Par ailleurs, un apport d'azote modifie la période de réalisation de ces pics en favorisant l'accumulation de l'azote de la céréale dans le mois suivant l'apport. Ainsi, la fertilisation azotée réduit la complémentarité entre espèces pour l'azote.

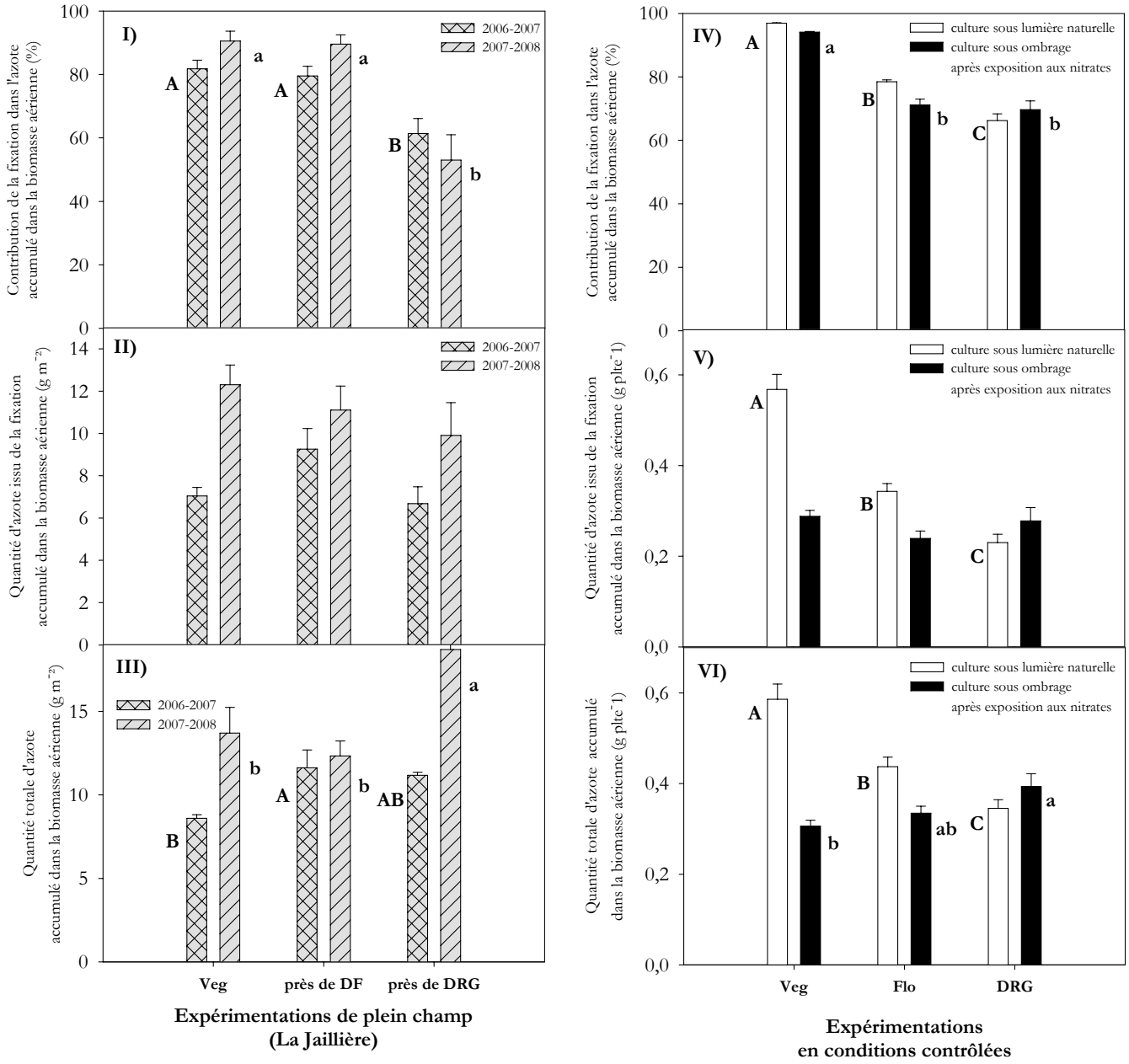

Figure 1 : Contribution de la fixation dans l'azote total accumulé dans la biomasse aérienne du pois, quantité d'azote fixée accumulé dans les parties aériennes, et quantité totale d'azote accumulé dans les parties aériennes : Résultats cumulés observés en fin de cycle du pois.

Période d'exposition aux nitrates : Veg (stades végétatifs), Flo (floraison du pois), DF (début de floraison), DRG (début de remplissage des grains)

Une analyse de variance (somme des carrés de type III, α=5 %) a été réalisée afin de tester un effet date d'exposition aux nitrates. Les traitements avec les mêmes lettres de même casse, ou sans lettres, ne sont pas significativement différents (Test HSD de Tukey, α=5 %).

2.1. Contribution de la fixation symbiotique et quantité d'azote fixé par le pois associé

Les **expérimentations de plein champ** mettent en évidence un effet de la date de l'apport azoté sur le fonctionnement de l'appareil fixateur :

- dans le cas d'un apport très précoce (dès la fin de l'hiver – modalité B-IC3 du Chapitre 2) et en comparaison d'une association non fertilisée, le blé, plus compétitif pour les ressources d'azote minéral du sol, voit sa biomasse augmentée et exerce ainsi une plus forte compétition aérienne sur le pois. Il en résulte une diminution de la biomasse de pois et donc une diminution de la quantité d'azote total accumulée par le pois, sans pour autant diminuer la contribution de l'azote issu de la fixation dans l'azote total accumulé.

- dans le cas d'un apport tardif (vers début de remplissage des grains du pois), la croissance du pois est moins affectée que dans le cas d'un apport précoce intervenant durant les phases végétatives du pois. L'apport azoté intervient à un stade où le pois a encore une forte demande en azote alors que le blé est moins compétitif. Le pic de nitrate dans le sol inhérent à la fertilisation azotée inhibe durablement l'activité de la fixation du pois. Le pois satisfait alors sa demande en azote principalement à partir de l'absorption racinaire. On observe donc une forte diminution de la contribution de l'azote fixé à l'azote total accumulé par le pois par rapport à un apport précoce (graphe I de la Figure 1). Lors d'un apport tardif, la quantité d'azote total accumulé par le pois tend à être supérieure en comparaison d'un apport précoce (graphes III de la Figure 1). La quantité d'azote fixé, résultante du produit entre quantité totale d'azote et taux de fixation, est similaire quelle que soit la date de l'apport (graphes II de la Figure 1).

Ces apports d'azote (45 kg N ha^{-1}) réalisés en plein champ sur associations pois-blé à différentes dates conduisent à exposer les pois associés à une courte exposition aux nitrates. En effet, la quantité d'azote minéral dans le sol est rapidement absorbée par le couvert associé et sa quantité décroît rapidement après apport sous le seuil d'inhibition mis en évidence par Voisin et al. (2002b). Dans les 30 premiers centimètres de sol, après apport d'azote, la quantité d'azote minéral passe sous le seuil d'inhibition de 40 kg N ha^{-1}, généralement sous environ une quinzaine de jours après l'apport. Ainsi, les régimes de fertilisation testés sur nos expérimentations reviennent à induire une courte exposition aux nitrates. Les expérimentations en conditions contrôlées ont été mises en place afin d'approfondir, à l'échelle de la plante de pois, les mécanismes en jeu dans une telle situation.

Les **expérimentations en conditions contrôlées** ont mis en évidence que plus la date d'exposition aux nitrates était tardive, et plus la contribution de l'azote fixé dans l'azote total accumulé dans les parties aériennes était diminuée, et ce, quelle que soit la disponibilité en photosynthétats après retrait des nitrates (graphe IV de la figure 1).
Pour les plantes cultivées sous lumière naturelle, la quantité d'azote fixé accumulée dans les parties aériennes (graphe V de la figure 1), tout comme la quantité d'azote total accumulée dans les parties aériennes (graphe VI de la figure 1), décroît significativement au fur et à mesure que la date d'exposition aux nitrates est tardive.
Dans le cas d'une culture sous ombrage après retrait des nitrates, la quantité d'azote fixé n'est pas modifiée par la date d'exposition aux nitrates, alors que la quantité totale d'azote accumulée dans les parties aériennes augmente avec des dates d'exposition plus tardives.

Les très fortes différences de conditions de cultures en serre en comparaison des conditions de plein champ (hydroponie vs substrat de terre, climat contrôlé vs climat incontrôlé, différences de variétés, *etc*) ne permettent pas une transposition directe des résultats mais **des tendances communes dans les résultats peuvent être mises en évidence**. En effet, dans les expérimentations en serre, on retrouve le même effet dépressif sur le taux de fixation d'un apport à DRG que celui observé en champ (graphes I et IV de la figure 1).

2.2. Inhibition et capacités de réversibilité en fonction du stade d'exposition

Les expérimentations de plein champ comme les expérimentations en conditions contrôlées ont démontré que la fixation symbiotique du pois était réversible après une courte phase d'inhibition liée à la présence de nitrate mais que cette réversibilité n'était possible que si l'exposition au nitrate intervenait avant le stade de début du remplissage des grains.

Les expérimentations en serre ont permis de dissocier l'effet inhibiteur des nitrates pour différentes dates d'exposition sur la structure et la fonction des nodosités. Nous avons mis en évidence que l'impact inhibiteur des nitrates sur la structure de l'appareil fixateur était différent selon le stade du pois pendant la période d'exposition (Hypothèse 3). Une exposition en tout début de cycle pénalise la croissance des nodosités, mais ralentit aussi leur vitesse d'apparition. Une exposition aux nitrates pendant les stades reproducteurs (floraison et remplissage du grain) ne pénalise que la croissance des nodosités car les nodosités d'une première vague de nodulation sont toutes en place. La fonction de fixation, caractérisée par l'activité spécifique de fixation des nodosités, est diminuée quel que soit le stade d'exposition.

Une exposition aux nitrates en tout début de cycle favorise la croissance de la plante, des nodosités, et une plus forte acquisition en azote après la période d'exposition au nitrate. De plus, dans le cas d'une exposition aux nitrates en tout début de cycle ou pendant la floraison du pois, on observe l'apparition d'une deuxième vague de nodosités. Cette deuxième vague n'est pas observée sur les pois témoins (c'est-à-dire ceux qui n'ont jamais été exposés aux nitrates), ni sur les pois exposés pendant le remplissage du grain, ni sur les pois cultivés sous ombrage après exposition aux nitrates. En conséquence, et contrairement à des observations précédentes de plein champ (Tricot, 1993 ; Voisin et al., 2002a), le pois est capable de fabriquer de nouvelles nodosités après le stade début floraison. Cependant, cette deuxième vague ne dispose que de peu de carbone pour assurer la croissance des nodosités. De plus, le délai entre l'apparition de nodosités et la mise en place de leur activité fixatrice peut être trop long pour être véritablement efficace avant maturité physiologique. Le niveau d'accumulation total en azote des plantes exposée aux nitrates pendant floraison et cultivés sous lumière naturelle, parvient au niveau de celui des plantes témoins sans toutefois le dépasser.

Nous avons par ailleurs démontré que la réversibilité de la fixation symbiotique après une courte exposition aux nitrates est fonction du niveau de nutrition carbonée des nodosités (Hypothèse 3). En effet, l'allocation carbonée aux nodosités décroît tout au long du cycle du pois et les parties aériennes deviennent prioritaires pendant la phase de remplissage des grains (Voisin et al., 2003). Ainsi, la réversibilité de l'appareil fixateur ne s'est observée que dans les cas où le ratio nodules / racines nodulées augmentait à nouveau après l'exposition aux nitrates.

2.3. Effet tampon de la fixation symbiotique par rapport aux ressources azotées

Cette capacité du pois à exploiter les deux voies de nutrition azotée en fonction des disponibilités en azote minéral dans le milieu permet d'obtenir une bonne efficience d'utilisation de l'apport azoté notamment à des dates tardives (diminution des risques de lixiviation liées à une faible valorisation) et confirme de précédentes observations (Jensen, 1986 ; Bédoussac et Justes, 2009). Ainsi, dans nos expérimentations, les reliquats d'azote minéral du sol observés à la récolte ne variaient pas significativement, quelle que soit la conduite azotée appliquée aux associations. Cependant ceci reste à confirmer pour des niveaux de doses plus élevés.

3. Démarche de modélisation : intérêts multiples

3.1. Construction du modèle

Le modèle proposé ici, AZODYN-IC, a démontré qu'il était capable de rendre compte de situations de fertilisation contrastées en termes de doses et de dates. En effet, les simulations d'acquisition d'azote total, de partage des ressources entre espèces et de croissance des espèces associées, sont satisfaisantes pour les différents régimes de fertilisation testés.

AZODYN-IC présente la particularité d'être construit autour d'un lien étroit entre surface foliaire et satisfaction de la demande azotée via la courbe de dilution maximale de l'azote dans la biomasse. Il a également été construit de manière à partager les ressources en azote minéral en fonction des besoins de chaque espèce et en fonction des écarts de profondeur d'enracinement quand l'offre s'avère limitante. Ainsi, les formalismes intégrés au modèle permettent une forte interaction entre partage du rayonnement et partage de l'azote.

De plus, le partage du rayonnement, dans le modèle Azodyn-IC, est calculé au prorata des kLAI des deux espèces. Un tel formalisme est très simple en comparaison de ceux intégrés aux autres modèles existants (STICS, FASSET) mais n'en est pas moins efficace à rendre compte du partage des ressources, d'autant plus que les deux espèces et variétés utilisées dans ce travail ne présentent pas de différences de hauteur. Cette simplicité, ajoutée au peu de paramètres exigés en entrée par le modèle, en fait un outil adapté à l'aide à la décision stratégique.

Plusieurs points restent cependant à améliorer :

- pour des raisons de temps, les conclusions de nos observations sur la réversibilité de la fixation symbiotique n'ont pas été intégrées au modèle. Or AZODYN-IC sous-estime les taux de fixation observés sur pois associés dans les expérimentations au champ, notamment sous conduite avec fertilisation. Il serait donc judicieux de remédier à ce problème, par exemple en testant un formalisme permettant une reprise plus rapide de la fixation ;
- nous avons démontré à partir des observations de plein champ, que l'impact d'une fertilisation azotée sur les performances des associations pois-blé et sur le partage des ressources entre les espèces était fonction des dynamiques de croissance et des stades phénologiques des deux espèces au moment de l'apport. Nous avons également observé sur nos deux années d'expérimentations de plein champ des décalages différents entre les stades de développement des deux espèces associées. Or, l'incapacité d'AZODYN-IC à rendre compte de la date de début floraison du pois en fonction du climat nous a conduit à considérer le stade « début floraison » du pois comme une entrée fixe du modèle. Nos simulations considèrent donc toujours la même synchronisation de stades entre les deux espèces, quelque soit la variabilité climatique. Des travaux en cours à l'INRA de Dijon permettront sans doute d'inclure un module fiable de prévision du stade DF du pois et de pallier ce problème ;

- le modèle a été évalué sur des expérimentations réalisées sur deux sites (La Jaillière et Grignon) et sur 2 à 3 années. Il pourrait être opportun de l'évaluer encore plus largement, notamment en valorisant les résultats du programme CASDAR n° 8058 actuellement en cours et piloté par le LEVA.

3.2. Outils pour identifier de nouvelles stratégies et faire émerger des règles de décision

Le modèle AZODYN-IC a été utilisé pour décrire les performances moyennes des associations et leur variabilité à partir de simulations basées sur 26 années climatiques. En situation sans fertilisation azotée, la proportion des espèces varie en fonction des dynamiques de minéralisation fortement influencées par le climat, et est donc difficilement prévisible. Ainsi, une fertilisation azotée adaptée permet d'orienter le couvert en diminuant le poids de la cinétique de minéralisation dans l'évolution de la proportion d'espèces. En ce sens, la fertilisation azotée est bel et bien un outil de pilotage de ces cultures.

La réponse des associations pois-blé à de larges gammes de dose et de date d'apport a également été étudiée par modélisation.
Les simulations mettent en évidence que l'intensité de réponse à la fertilisation est très variable selon la date d'apport. La réponse du rendement total de l'association est la plus forte au 01/04 (*ie* vers le stade 1 à 2 nœuds du blé). La réponse de la proportion de blé dans le rendement total est la plus forte au 15/03 (*ie* 10 jours après stade épi 1 cm). De plus, plus la date de fertilisation s'éloigne de la plage 15/03 – 01/04 (avant ou après cette plage), et moins elle a d'impact sur le rendement et les proportions d'espèces. Ainsi, le rendement total et la proportion de blé sont peu modifiés par un apport au 01/05 (*ie* vers le stade gonflement du blé). Ces conclusions issues des simulations peuvent être expliquées à deux niveaux. Premièrement, le CAU (Cœfficient Apparent d'Utilisation) de la dose apportée est calculée dans AZODYN-IC en fonction des vitesses de croissance des cultures. Or, c'est pendant la montaison du blé que cette culture voit une accélération de sa croissance. Un apport avant épi 1 cm ou près de floraison est donc mal valorisé car le CAU simulé est faible. Par ailleurs, plus l'apport est tardif (et s'approche de la floraison du blé), et plus les gains de biomasse possibles sont limitées.
La plage optimale de fertilisation pour agir sur le rendement total de l'association et sur la proportion des espèces se situe entre 10 jours après le stade épi 1 cm et le stade 1 à 2 nœuds. Il faut remarquer que c'est une plage de temps très courte d'environ 15 jours.

De plus, à partir de simulations réalisées, il ressort que le rendement total des associations est beaucoup moins réactif à une dose d'azote que ne l'est celui d'une culture pure telle que le blé, ce qui a déjà été observé expérimentalement par différents auteurs (Jensen, 1996 ; Hauggaard-Nielsen et Jensen, 2001 ; Ghaley et al., 2005 ; Corre-Hellou et al., 2006). En effet, dans le cas d'un apport en début de montaison du blé (stade auquel la réponse du rendement de l'association est la plus forte dans nos simulations) et pour des associations substitutives et quelle que soit la proportion de blé au semis (30 %, 50 % ou 70 % de la densité semée en culture pure), on observe un gain de 0.11 à 0.15 q ha^{-1} de grains par unité d'azote apportée (contre 0.33 q ha^{-1} par unité d'azote pour un blé pur ; Meynard et al., 1981). En tenant compte du coût élevé de l'engrais azoté actuel (et probablement encore pour une longue période, en lien avec la réduction de la disponibilité des ressources en énergie fossile), la pratique de la fertilisation azotée à des doses élevées sur ce type d'associations n'apparaît donc pas recommandable.

Dans les conditions pédoclimatiques testées, la proportion simulée de céréales dans le rendement de l'association n'augmente plus au-delà d'une fertilisation de 90 kg N ha^{-1}. Pour une fertilisation réalisée en début de montaison du blé, et en comparaison d'un apport de 30 kg N ha^{-1}, un apport de 90 kg N ha^{-1} permet d'augmenter la proportion de céréales dans le rendement de 60 % à environ 75 %.

Ces résultats sont en accord avec les résultats expérimentaux. Il apparaît clairement, par exemple en 2008 sur le site de la Jaillière, qu'il est possible de combiner rendement et réduction d'intrants en cultures associées par rapport à la culture pure. Avec un faible apport de 45 kg N ha^{-1} au stade épi 1 cm, le rendement total de l'association est supérieur à la moyenne des cultures pures cultivées avec une conduite optimale de fertilisation azotée. Cette association produit davantage de pois et autant de blé avec deux fois moins d'azote que si on cultivait les deux espèces séparément. Avec un apport de 90 kg N ha-^{1}, le rendement total n'est pas modifié mais la part de blé augmente.

Concernant la teneur en protéines des grains de blé, la date optimale d'apport est sans conteste la plus tardive (gonflement du blé), ce qui est cohérent avec le fonctionnement des espèces pures (Recous et al., 1988; Jeuffroy and Bouchard, 1999; Limaux et al., 1999). Pour des associations substitutives et quelle que soit la proportion de blé au semis (30 %, 50 % ou 70 % de la densité semée en culture pure), on observe un gain de 0.016 à 0. 2 % de protéines par unité d'azote apportée en début de montaison du blé, et un gain de 0.021 à 0.025 % de protéines pour un apport à gonflement. Toutefois, les valeurs absolues de taux de protéines issues des simulations sont très en deçà des valeurs couramment observées sur des blés associés. Ce problème avait déjà été relevé avec des simulations réalisées sur blé pur avec AZODYN (Barbottin et al., 2006).
Nos résultats expérimentaux indiquent qu'en association non fertilisée, ou fertilisée avec un apport de faible quantité positionné tardivement, on peut obtenir des teneurs en protéines au moins équivalentes à celles obtenues en culture de blé pur conduit avec une fertilisation à l'optimum.

L'analyse des simulations a permis de proposer des équations simplifiées de prédiction du rendement total de l'association, de la proportion de céréales dans le rendement et de la teneur en protéines des grains de blé à partir de quelques variables explicatives clés et faciles d'accès en situation de production agricole : reliquat azoté observé sortie hiver, potentiel de minéralisation depuis sortie hiver à la récolte, proportion des espèces observée sortie hiver, et dose d'azote apportée. Ces modèles peuvent servir à orienter les essais de test de stratégies de fertilisation azotée à réaliser pour confirmer les stratégies *a priori* recommandées à l'issue des simulations.
Cependant, il faut garder à l'esprit le contexte pédologique choisi pour ces simulations (notamment sol à fort potentiel de minéralisation), les gammes de variations des variables explicatives et l'absence de prise en compte des facteurs climatiques dans les équations simplifiées de prédiction. Des facteurs climatiques tels qu'un stress hydrique ou des fortes températures à des périodes importantes pour l'élaboration du rendement pourraient être insérés pour tenir compte de leurs effets sur le rendement et la proportion des espèces. Le test de l'impact de ces stress et leurs interactions nécessitera au préalable une validation du modèle sur des conditions pédoclimatiques plus larges que dans le cadre de cette thèse où le principal facteur limitant étudié était l'azote.

Enfin, dans la partie II du Chapitre 4, nous avons testé des stratégies basées sur un seul apport d'azote. Il serait intéressant d'utiliser le modèle pour tester l'effet de différents fractionnements de la fertilisation sur les performances des associations.

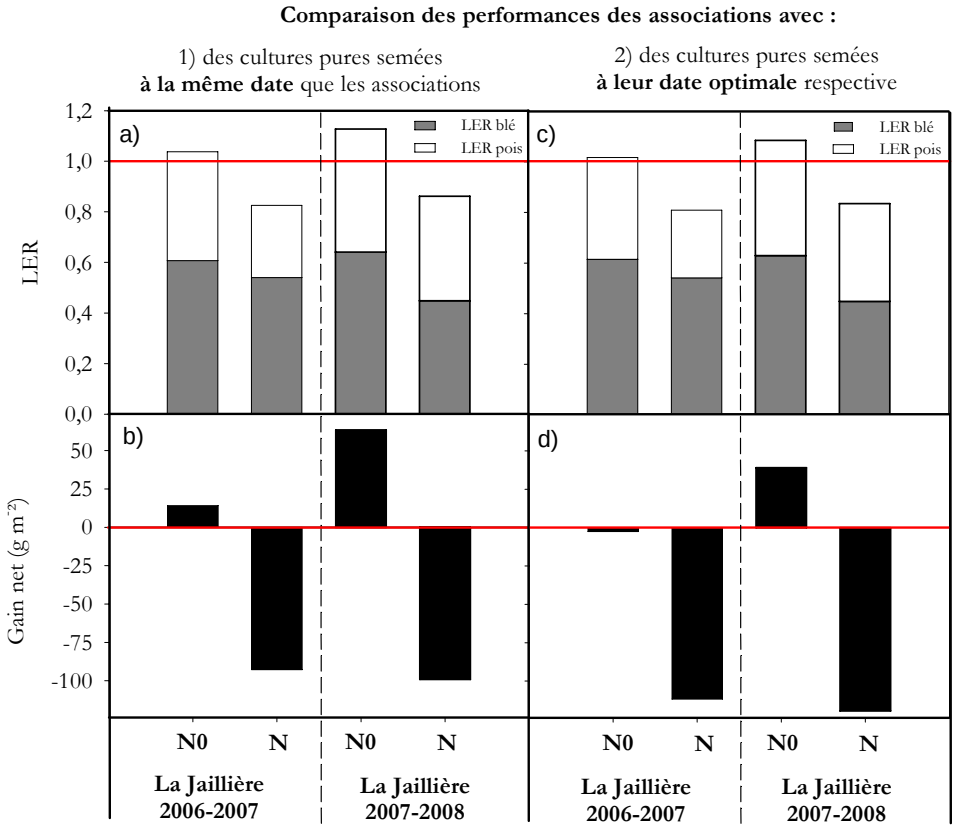

Figure 2 : Évaluation des associations pois-blé en fonction de cultures pures semées à différentes dates.
Cette évaluation est basée sur des simulations réalisées avec Azodyn-IC à partir du contexte pédoclimatique des expérimentations de La Jaillière (saisons 2006-2007 et 2007-2008) et des stratégies de conduites azotées testées dans ces expérimentations. Ces simulations ont été complétées par les performances simulées de cultures pures semées à date optimale, et non simultanément aux associations. Les dates de semis et les stratégies de fertilisations sont les suivantes :

- les associations ont été semées le 26/10
- les dates optimales de semis pour les cultures pures : 07/10 pour le blé et 10/11 pour le pois.
- Les associations fertilisées ont reçues 45 kg N ha^{-1} au stade épi 1 cm du blé
- Les blés purs fertilisées ont reçues 188 kg N ha^{-1} (saison 2006-2007) et 185 kg N ha^{-1} (saison 2007-2008) en deux apports (au stade épi 1 cm et gonflement du blé)
- Les pois purs n'ont jamais été fertilisés

Note méthodologique :
Le LER (Land Equivalent Ratio) (De Wit et Van den Bergh, 1965) permet d'évaluer la productivité des associations en comparaison des cultures pures. Un LER supérieur à 1 indique qu'il faut plus d'un hectare de cultures pures pour produire le même rendement que celui obtenu sur un hectare d'associations. Le LER est calculé comme suit :

$$LER = LER\ blé + LER\ pois$$

$$LER = \frac{Rdt\ blé_{CA}}{Rdt\ blé_{CP}} + \frac{Rdt\ pois_{CA}}{Rdt\ pois_{CP}}$$

Avec : $Rdt\ blé_{CA}$ et $Rdt\ pois_{CA}$, le rendement en grains du blé et du pois dans un hectare d'associations
Et $Rdt\ blé_{CP}$ et $Rdt\ pois_{CP}$, le rendement en grains d'un hectare de blé pur et d'un hectare de pois pur.

Le gain net (Δ) de production des associations Δ (Loreau et Hector, 2001) est calculé comme la différence entre le rendement total d'un hectare d'association avec le rendement de la moyenne des rendements des cultures pures, moyenne pondérée au pro rata des densités respectives. Dans notre cas, les associations étant des associations substitutives (P50W50) (*ie* dont les densités relatives de chaque espèce au semis sont respectivement de moitié celles semées en cultures pures), la moyenne des rendements des cultures pures revient à sommer le rendement du blé pur sur 0.5 ha avec le rendement du pois pur sur 0.5 ha.
Ainsi, le LER est un critère d'évaluation de la productivité des associations qui indique les surfaces nécessaires en cultures pures pour obtenir précisément la même quantité de blé que celle obtenue sur un hectare d'association, et précisément la même quantité de pois que celle obtenue sur un hectare d'association : la surface nécessaire en culture pure de blé est le « LER blé » et la surface nécessaire en culture pure de pois est le « LER pois ».
Le gain net (Δ) est un indicateur d'évaluation du niveau de production d'un hectare d'association en comparaison, dans notre cas, de 0.5 ha de blé pur et de 0.5 ha de pois pur : le gain net (Δ) indique un gain net de production entre deux stratégies d'assolement, en comparant des niveaux de production totaux qui ne sont pas forcément composés de la même proportion de rendement de blé et de rendement de pois.

3.3. Le modèle comme outil d'évaluation des associations en comparaison de cultures pures conduites à l'optimum

Notre approche délibérée tout au long de cette thèse a davantage consisté à analyser l'impact d'une fertilisation azotée sur le fonctionnement des associations, qu'à chercher à les évaluer en comparaison des cultures pures.
La plupart des évaluations agronomiques et environnementales des associations ont été jusqu'ici souvent réalisées en comparaison de cultures pures conduites à l'identique des associations en termes de dates de semis, de fertilisation et de protection phytosanitaire. Si cette approche a pour intérêt de comparer l'efficience d'utilisation des ressources des associations et des cultures pures, elle ne permet pas de fournir aux agriculteurs des éléments de comparaison, en terme de rendement total produit, avec les performances des cultures pures qu'ils connaissent. Il serait donc important de comparer les associations à des cultures pures conduites à l'optimum tel qu'elles sont couramment conduites dans les agrosystèmes.

Là encore, le recours aux simulations issues d'un modèle de culture permet d'éviter des dispositifs complexes et coûteux de mise en œuvre.

La figure 2 présente les performances simulées des associations en comparaison des cultures pures semées à la même date que les associations, ou semées à leur date optimale respective (07/10 pour le blé et 10/11 pour le pois). Cette comparaison a été menée à partir des conditions pédoclimatiques de La Jaillière (saisons 2006-2007 et 2007-2008). Elle présente une situation sans aucune fertilisation (ni sur associations, ni sur cultures pures), et une situation avec stratégies de fertilisation (à l'optimum sur les cultures pures : 185 kg N ha^{-1} sur le blé et 0 kg N ha^{-1} sur le pois ; et de 45 kg N ha^{-1} pour les associations). Si on fait une comparaison avec des cultures pures semées simultanément aux associations, les associations semblent moins performantes par rapport aux cultures pures en situation fertilisée qu'en situation non fertilisée : les LER (Land Equivalent Ratio) sont de 1.04 à 1.13 en situation non fertilisées et de 0.83 à 0.86 en situation fertilisée (Graphe a et b de la Figure 2), ce qui est en accord avec les observations précédentes (Jensen, 1996 ; Ghaley et al., 2005 ; Corre-Hellou et al., 2006). De même, cultiver des associations non fertilisées représente un gain net positif de 14 à 64. g m^{-2} en comparaison de cultures pures non fertilisées, mais une perte (gain net négatif) de 93 à 99 g m^{-2} en situation fertilisée (associations fertilisées comparées aux cultures pures fertilisées à l'optimum, *ie* blé pur fertilisé et pois pur non fertilisé).
Si on fait une comparaison avec des cultures pures semées à dates optimales, les performances des associations sont encore davantage diminuées. Par rapport aux résultats obtenus en comparant associations et cultures pures semées à la même date, les résultats d'une comparaison entre cultures semées à dates optimales sont diminués d'environ 0.03 pour les LER, et d'environ 20 g m^{-2} pour les gains nets, quelques soient les conduites azotées.

En conclusion, la fertilisation pénalise bien les performances des associations en comparaison des cultures pures. Cependant, si on considère que la fertilisation permet également d'augmenter le rendement total de l'association (telle que nous le suggère les simulations d'AZODYN-IC), alors il doit exister une dose de fertilisation qui permette aux associations d'être aussi performantes que les cultures pures (en terme de LER et de gain net). Cette dose ne doit cependant pas être trop élevée pour que les associations restent pertinentes en terme de réduction d'intrant. Ainsi, ces résultats demandent à être approfondis afin d'étudier l'effet de la dose d'azote sur les performances des associations en comparaison des cultures pures, et d'étendre l'analyse sur de plus nombreuses années climatiques.

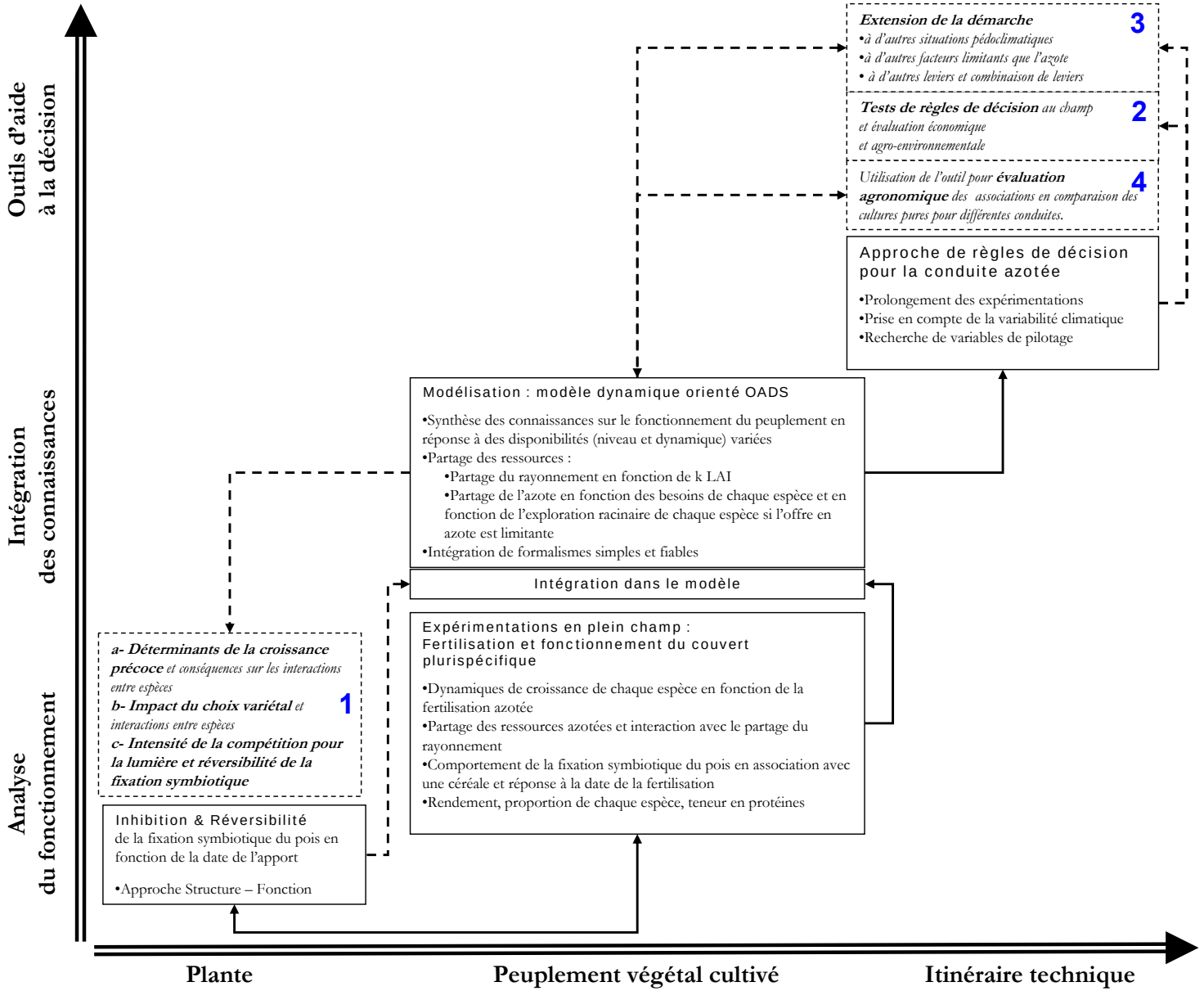

Figure 3 : Perspectives intégrées à la démarche générale de la thèse
OADS : Outil d'Aide à la Décision Stratégique ; kLAI : coefficient d'extinction * Leaf Area Index

4. Perspectives

La figure 3 reprend les axes étudiés dans la thèse et indique les perspectives directement liées aux résultats des travaux menés.

4.1. Nouvelles questions de recherche

Phase d'installation du couvert : déterminants de la croissance précoce de chaque espèce et conséquences sur les interactions entre espèces (perspective n°1a - Figure 3)

Les travaux expérimentaux ont montré l'importance des rapports de dominance établis en tout début de cycle sur le fonctionnement du couvert plurispécifique durant le reste du cycle. Au début du cycle, dans une association de cultures, une espèce qui a une croissance plus rapide va progressivement devenir dominante et cette dominance va s'accentuer dans la suite du cycle en raison de son avantage acquis pour capter les ressources (lumière, eau, azote). Les rapports de dominance entre espèces peuvent donc se déterminer très tôt dans le cycle. Les observations d'interactions dynamiques entre espèces dans les phases précoces du cycle sont rares. Dans des associations de pois et d'orge, un effet dépressif de l'orge sur la croissance du pois est toutefois observé dès 15 jours après la levée (Bellostas et al., 2003) indiquant que la croissance rapide de l'orge à des stades précoces est déterminante dans la dominance exercée par cette espèce quand elle est cultivée dans une association. La phase d'implantation (levée-6 feuilles du pois) s'avère cruciale. Les facteurs de variation de la levée sont bien connus. Le pourcentage et la vitesse de levée sont dépendants des caractéristiques des semences et des états physiques du milieu. Ils sont bien décrits par le modèle SIMPLE (SIMulation of Plant Emergence) pour de nombreuses espèces (Dürr et al., 2001; Moreau-Valancogne et al., 2008). En revanche, les déterminants de la croissance précoce ont été peu étudiés. L'initialisation du modèle AZODYN-IC se fait par la biomasse de chaque espèce à la levée qui est arbitrairement fixée en fonction de la biomasse de graines semées. Plusieurs facteurs peuvent influencer cette croissance précoce mais leur importance n'est pas connue pour une plus grande gamme d'espèces et, dans tous les cas, la hiérarchisation et la quantification sont insuffisantes pour alimenter des modèles.
Des expérimentations au champ et en conditions contrôlées seront nécessaires pour préciser quels sont les principaux déterminants de la croissance précoce de chaque espèce et l'impact d'écarts de croissance précoce sur les interactions de compétition et facilitation dans la suite du cycle. Une large gamme d'espèces et de variétés au sein d'une même espèce pourrait être utilisée pour créer une gamme d'intensité pour certains facteurs à priori déterminants pour la croissance précoce : taille de semences, localisation des réserves, levée épigée ou hypogée, absorption plus ou moins précoce d'azote (Dürr et Mary, 1998; Dürr et Boiffin, 1995; Tamet et al., 1996).

Impact du choix variétal sur les interactions entre espèces (perspective n°1b – Figure 3)

Un gain de rendement en cultures associées peut se produire si les espèces utilisent les ressources à différents moments, dans différentes parties du sol ou du couvert ou encore sous différentes formes. Il est souvent suggéré que la complémentarité temporelle donne de plus forts gains de rendements que la complémentarité spatiale. Dans nos expérimentations, des écarts de pics de croissance entre le blé et le pois ont été observés mais restent modérés. Le choix variétal pourrait accentuer ces écarts de dynamiques entre espèces. Quelques travaux ont étudié l'impact du choix variétal sur les performances des associations céréale-pois (Hauggaard-Nielsen et Jensen, 2001) mais peu de travaux ont cherché à déterminer et hiérarchiser les caractéristiques variétales qui minimisent la compétition entre espèces et maximisent la complémentarité. Il est souvent difficile au champ de séparer les gains de rendement dus aux différentes formes de complémentarité et

d'identifier le rôle de différentes caractéristiques variétales sur chacune d'elle. L'utilisation de la modélisation par la création de scénarios et d'analyse de sensibilité à différents paramètres variétaux devrait être une approche complémentaire intéressante aux expérimentations de plein champ.
De plus, les stratégies d'innovation variétale ont jusqu'alors été raisonnées pour des systèmes de cultures conventionnels, où les associations sont quasiment absentes. L'optimisation de la complémentarité interspécifique dans l'exploitation des ressources introduit des contraintes et des objectifs différents de ceux retenus pour une culture pure : par exemple gestion de la compétition aérienne (forme des feuilles, répartition verticale de la surface foliaire, hauteur de la tige), gestion de la compétition racinaire (profondeur d'enracinement, densité de répartition des racines). Il faut donc prolonger notre travail pour aboutir à des critères de choix variétaux adaptés aux différentes finalités de production.
Le test d'une large gamme de combinaisons variétales peut conduire à l'étude d'associations de blés et de pois présentant des hauteurs significativement différentes, ce qui n'était pas le cas dans notre étude. Ainsi, l'élargissement de notre modèle à d'autres couples variétaux impliquera sans doute de simuler la hauteur et d'adapter le module de partage du rayonnement en conséquence.

Inhibition et réversibilité de la fixation symbiotique (perspective n°1c – Figure 3)

Nous avons démontré que la réversibilité de la fixation symbiotique après une courte exposition aux nitrates est fonction de la disponibilité en carbone. Cet effet de la disponibilité en carbone a été étudié en conditions contrôlées, sur du pois pur, par la mise en place de situations ombrées comparativement à des situations éclairées après une période d'exposition aux nitrates. Au champ, la capacité de reprise de la fixation après un apport d'azote a été étudiée en culture associée où le pois subit aussi une réduction de sa croissance en réponse à la compétition pour la lumière exercée par le blé. On dispose de peu d'éléments sur la comparaison de l'intensité de la compétition pour la lumière et ses conséquences sur la croissance du pois en culture de pois pur et en culture associée. La compétition interspécifique du blé sur le pois pour la lumière n'est peut-être pas plus forte que la compétition intraspécifique entre plantes de pois. Il serait intéressant d'analyser la capacité de reprise de la fixation après un apport d'azote pour un peuplement de pois pur comparativement à du pois associé et approfondir l'impact de la compétition pour la lumière entre plantes. Une gamme d'intensité de compétition pourrait être créée par la variation de la densité de peuplement et/ou du choix variétal.

4.2. Applications et autres actions tournées vers le test, l'évaluation et l'opérationnalité

Continuer la construction de l'itinéraire technique adapté à différents objectifs de production (perspective n°2 et 3 – Figure 3)

Notre travail de modélisation aboutissant à des propositions pour la définition de règles de décision s'inscrit dans la méthodologie proposée par Bergez et al. (2009). Les équations de prédiction mises au point dans cette thèse et qui pourront être adaptés à des conditions pédoclimatiques plus variés, constituent des outils d'aide à la définition de règles de décision pour la fertilisation azotée qui seront ensuite testées au champ (perspective n°2 – Figure 3). Cette démarche peut être élargie à l'étude d'autres leviers techniques.

Certains facteurs de l'itinéraire technique (fertilisation azotée, choix variétal, *etc.*) doivent être étudiés séparément pour comprendre leur impact sur le fonctionnement (perspective n°3 – Figure 3). Mais c'est surtout la combinaison de plusieurs facteurs qui permettront d'atteindre des objectifs de production contrastés. Dans un objectif de transfert et de démonstration de ces

innovations auprès des agriculteurs, les expérimentations en plein champ doivent être construites à partir des objectifs de production visés et par la proposition d'une conduite combinant choix variétal, proportions des espèces au semis, fertilisation azotée (dose, date), ceci en fonction des connaissances de l'effet de chacun des facteurs en interaction avec les conditions du milieu (fournitures en azote du sol notamment).

Quelques stratégies peuvent être dors et déjà être envisagées :

- production de **blé riche en protéines avec moins d'intrants azotés** : une association P30W70 (Pois 30 Blé 70, en pourcentage des densités semées en cultures pures) pourrait être testée avec un premier apport au stade épi 1 cm pour accroître la proportion de blé dans le mélange dont la dose serait déterminée en fonction du reliquat sortie hiver, des fournitures et de l'état de croissance de chaque culture à la sortie de l'hiver (cf modèles linéaires proposés dans la partie II du Chapitre 4). Un deuxième apport serait réalisé au stade gonflement du blé pour améliorer la teneur en protéines si besoin. La variété de blé testée serait un blé ayant une forte capacité de tallage et une bonne efficience d'utilisation de l'azote en situations de faibles fournitures.
- production d'un **mélange équilibré de blé riche en protéines et de pois,** avec peu d'intrants azotés : une association P50W50 serait choisie et seul un dernier apport pour favoriser la teneur en protéines serait réalisé si besoin.
- production de **pois avec moins de facteurs limitants** que ceux rencontrés en culture pure de pois : une association de type P70W30, voire P100W25, pourrait être pertinente. Aucun apport d'azote ne serait appliqué pour ne pas défavoriser le pois. Une variété de blé n'exerçant pas trop de compétition pour la lumière (variété courte) pendant la période de forte croissance du pois serait alors préconisée.

De nombreux travaux ont par le passé étudié l'intérêt des associations par rapport à la gestion des adventices (Baumann et al., 2000; Hauggaard-Nielsen et al., 2001; Poggio, 2005), et par rapport à la gestion des maladies et des ravageurs (Litsinger and Moody, 1976; Trenbath, 1993; Finch and Collier, 2000; Kinane and Lyngkjaer, 2002). Les conclusions de ces études permettent d'envisager les associations céréale-légumineuse comme une culture plus économe en intrants phytosanitaires. Il serait toutefois important dans la construction des itinéraires techniques d'étudier l'impact de différents facteurs (variété, densité, arrangement spatial) pour accroître la performance de l'association pois-blé sur la maîtrise des adventices, des maladies et ravageurs.

Étudier l'introduction des associations dans les systèmes de culture et dans le bassin de production

Une introduction plus large des associations dans les systèmes de cultures implique d'en étudier l'impact sur la culture suivante en termes de rendement mais aussi d'effet sur la structure du sol, la richesse en nutriments du sol et le niveau d'infestation potentielle en maladies et ravageurs. Une étude récente (Hauggaard-Nielsen et al., 2009a) a initié cette approche mais doit être complétée par des expérimentations et des observations pluriannuelles afin de définir l'effet précédent des associations et leur délai de retour minimum.

Enfin, la culture d'associations dans un objectif de production de grains destinés à la vente peut impliquer une organisation particulière des organismes collecteurs stockeurs. En effet, la collecte de ce type de récolte imposera le plus souvent une procédure de tri (notamment si la céréale associée est destinée à la meunerie). Il sera alors indispensable d'analyser le coût supplémentaire imputable au tri, coût à prendre en compte dans la viabilité économique des cultures associées, au même titre que l'intérêt de la réduction des intrants qu'elles rendent possibles.

Évaluater plus largement les associations adaptées à l'agriculture conventionnelle (Perspective 4 – Figure 3) :

Comme nous l'avons abordé précédemment, il serait intéressant de prolonger la comparaison des performances agronomiques des associations avec des cultures pures semées à leurs dates optimales espectives, en se basant sur un plus large jeu de données.
De plus, cette étude pourrait être associée à une analyse économique permettant de chiffrer l'intérêt des associations pour la pérennité financière des exploitations agricoles.
Enfin, il est d'ores et déjà prévu dans le prolongement de cette thèse de réaliser une analyse environnementale des associations céréale-légumineuse et des conduites de fertilisation par une méthodologie d'Analyse de Cycle de Vie (ACV). Nous avons démontré que les associations pois-blé sont d'intérêt pour la réduction des intrants azotés. Il est désormais important de caractériser plus largement leurs impacts environnementaux (consommation d'énergie fossile, pouvoir de réchauffement global, potentiel d'eutrophisation, utilisation de surface arable, *etc.*).

La méthodologie ACV présente plusieurs avantages (Jolliet et al., 2005) :

- la prise en compte de toute la chaîne de fabrication du produit avec l'intégration de la fabrication des intrants (méthodologie dite « du berceau à la tombe ») ;
- le consensus international sur la méthodologie et les paramètres utilisés par défaut ;
- la transparence de la méthodologie (normalisation ISO 14040)

Ce travail serait alors l'occasion d'une comparaison des performances environnementales des associations fertilisées ou non avec les cultures pures, mais aussi une comparaison de différentes conduites de fertilisation adaptées aux associations pour un même objectif de production. Enfin, ce sera également l'occasion d'une réflexion sur l'adaptation méthodologique de l'ACV, notamment en termes de choix d'allocations des impacts environnementaux sur les espèces en associations.

Références bibliographiques

Barbottin, A., Le Bail, M., Jeuffroy, M.-H., 2006. The AZODYN crop model as a decision support tool for choosing cultivars. Agron. Sustain. Dev. 26, 107-115.

Baumann, D.T., Kropff, M.J., Bastiaans, L., 2000. Intercropping lekks to suppress weeds. Weed Research 40, 359-374.

Bedoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil, in press, doi: 10.1007/s11104-009-0082-2.

Bellostas, N., Hauggaard-Nielsen, H., Andersen, M.K., Jensen, E.S., 2003. Early interference dynamics in intercrops of pea, barley and oilseed rape. Biol. agric. hortic. 21, 337-348.

Bergez, J.E., Colbach, N., Crespo, O., Garcia, F., Jeuffroy, M.H., Justes, E., Loyce, C., Munier-Jolain, N., Sadok, W., 2009. Designing crop management systems by simulation. European Journal of Agronomy, in press, doi:10.1016/j.eja.2009.06.001.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for Soil N and its interaction with N2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant and Soil 282, 195-208.

De Wit, C.T., Van den Bergh, J.P., 1965. Competition between herbage plants. Neth. J. Agric. Sci. 13, 212-221.

Dürr C, Boiffin J.,1995. Sugarbeet seedling growth from germination to first leaf stage. J. Agric. Sci., Camb. 124, 427-435

Dürr C., Mary, B., 1998. Effects of nutrient supply on preemergence growth and nutrient absorption in wheat (*Triticum aestivum* L.) and sugarbeet (*Beta vulgaris* L.). Ana. Bot.-London, 81: 665-672.

Dürr, C., Aubertot, J.N., Richard, G., Dubrulle, P., Duval, Y., Boiffin, J., 2001. Simple: A model for simulation of plant emergence predicting the effects of soil tillage and sowing operations. Soil Sci Soc Am J 65, 414-423.

Finch, S., Collier, R.H., 2000. Host-plant selection by insects – a theory based on `appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata 96, 91-102.

Ghaley, B.B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E.S., 2005. Intercropping of wheat and pea as influenced by nitrogen fertilization. Nutr. Cycl. Agroecosys. 73, 201-212.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res. 70, 101-109.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E., 2009a. Pea and barley intercropping and short-term subsequent crop effects across European organic cropping conditions. Nutri. Cycl. Agroecosys. 85, 141-155.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009b. Pea-barley intercropping for efficient symbiotic N_2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crop Res. 113, 64-71.

Jensen, E.S., 1986. The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (Pisum sativum L.). Fert. Res. 10, 193-202.

Jensen, E.S., 1996. Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant and Soil 182, 25-38.

Jeuffroy, M.-H., Bouchard, C., 1999. Intensity and duration of nitrogen deficiency on wheat grain number. Crop Sci. 39, 1385-1393.

Jolliet, O., Saadé, M., Crettaz, P., 2005. Analyse du cycle de vie. Comprendre et réaliser un écobilan. Presse Polytechniques et Universitaires Romandes (Ed.). Collection Gérer l'environnement. Lausanne, Switzerland.

Kinane, J., Lyngkjaer, M.F., 2002. Effect of barley-legume intercrop on disease frequency in an organic farming system. In: Efpp, t.C. (Ed.), Plant Prot. Sci. Plant Prot. Sci., Prague, Czech Republic, pp. 227-231.

Limaux, F., Recous, S., Meynard, J.-M., Guckert, A., 1999. Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat. Plant and Soil 214, 49-59.

Litsinger, J.A., Moody, K., 1976. Integrated pest management in multiple Cropping Systems. In: Asa, C.S. (Ed.), Multiple Cropping. ASA, pp. 293-316.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72-76.

Meynard, J.-M., Boiffin, J., Caneill, J., Sébillotte, M., 1981. Elaboration du rendement et fertilisation azotée du blé d'hiver en Champagne crayeuse. II. Types de réponse à la fumure azotée et application de la méthode du bilan prévisionnel. Agronomie 1, 795-806.

Moreau-Valancogne, P., Coste, F., Crozat, Y., Dürr C., 2008. Assessing emergence of bean (*Phaseolus vulgaris* L.) seed lots in France: Field observations and simulations. Eur. J. Agron. 28, 309-320.

Poggio, S.L., 2005. Structure of weed communities occuring in monoculture and intercropping of field pea and barley. Agr. Ecosyst. Environ. 109, 48-58.

Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988. The fate of labelled15N urea and ammonium nitrate applied to a winter wheat crop. I. Nitrogen transformations in the soil. Plant and Soil 112, 205-214.

Tamet, V., Boiffin, J., Dürr, C., Souty, N., 1996. Emergence and early growth of an epigeal seedling (*Daucus carota* L.): influence of soil temperature, sowing depth, soil crusting and seed weight. Soil and Tillage Research, 40, 25-38.

Trenbath, B.R., 1993. Intercroppping for the management of pests and diseases. Field Crops Research 34, 381-405.

Tricot F. 1993. Mise en place des nodosités du pois protéagineux de printemps (Pisum sativum L.). Influence de la nutrition carbonée. PhD thesis, Université Paris-Sud Orsay, France.

Voisin, A.-S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002a. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (Pisum sativum L.). Plant and Soil 242, 251-262.

Voisin, A.-S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002b. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (Pisum sativum L.). Plant and Soil 243, 31-42.

Voisin, A.S., Salon, C., Jeudy, C., Warembourg, F.R., 2003. Seasonal patterns of 13C partitioning between shoots and nodulated roots of N2- or nitrate-fed Pisum sativum L. Ana. Bot.-London 91, 539-546.

Liste récapitulative des références bibliographiques

Adu-Gyamfi, J., Ito, O., Yoneyama, T., Devi, G., Katayama, K., 1997. Timing of N fertilization on N2 fixation, N recovery and soil profile nitrate dynamics on sorghum/pigeonpea intercrops on Alfisols on the semi-arid tropics. Nutrient Cycling in Agroecosystems 48, 197-208.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agr. Ecosyst. Environ. 74, 19-31.

Altieri, M.A., 1993. Ethnoscience and biodiversity: key elements in the design of sustainable pest management systems for small farmers in developing countries. Agr. Ecosyst. Environ. 46, 257-272.

Altieri, M.A., 1989. Agroecology: a new research and development paradigm for world agriculture. Agr. Ecosyst. Environ. 27, 37-46.

Amarger, N., Mariotti, A., Mariotti, F., Dürr, J.C., Bourguignon, C., Lagacherie, A., 1979. Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in 15N natural abundance. Plant soil. 52, 269-280.

Andersen, M.K., Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266, 273-287.

Andersen, M.K., Hauggaard-Nielsen, H., Høgh-Jensen, H., Jensen, E.S., 2007. Competition for and utilisation of sulfur in sole and intercrops of pea and barley. Nutr. Cycl. Agroecosys. 77, 143-153.

Andrews, D.J., Kassams, A.H., 1976. The importance of multiple cropping in increasing world food supplies. In: Papendick, R.I. et al. (Eds.), American Society of Agronomy, Madison, Wisconsin, pp. 1-11.

Anil, L., Park, J., Phipps, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53, 301-317.

Bacanamwo M, Harper JE. 1997. The feedback mechanism of nitrate inhibition of nitrogenase activity in soybean may involve asparagines and/or products of its metabolism. Physiologia Plantarum 100, 371-377.

Baker CM, Blamey FPC. 1985. Nitrogen fertilizer effects on yield and nitrogen uptake of sorghum and soybean, grown in sole cropping and intercropping systems. Field Crops Rese.12, 233-240.

Ball, D.A., Shaffer, M.J., 1993. Simulating resource competition in multispecies agricultural plant communities. Weed Res. 33, 299-310.

Ballot, R., 2009. Prise en compte dans les indicateurs de rentabilité de facteurs agronomiques intervenant entre cultures se succédant. Master Thesis, Groupe ESA, Angers France, 166pp.

Bandyopadhyay AK, Veena J, Nainawatee HS, 1996. Nitrate alters the flavonoid profile and nodulation in pea (*Pisum sativum* L.). Biol. Ferti. Soils, 21, 189-192.

Barbottin, A., Le Bail, M., Jeuffroy, M.-H., 2006. The AZODYN crop model as a decision support tool for choosing cultivars. Agron. Sustain. Dev. 26, 107-115.

Baumann, D.T., Kropff, M.J., Bastiaans, L., 2000. Intercropping lekks to suppress weeds. Weed Research 40, 359-374.

Bedoussac, L., Justes, E., 2009. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil, in press, doi: 10.1007/s11104-009-0082-2.

Bellostas, N., Hauggaard-Nielsen, H., Andersen, M.K., Jensen, E.S., 2003. Early interference dynamics in intercrops of pea, barley and oilseed rape. Biol. agric. hortic. 21, 337-348.

Bellostas, N., Jensen, E.S., 2004. Intercropping of cereals and grain legumes in European organic farming systems.-INTERCROP. Grain Legumes 39, 14-15.

Bergez, J.E., Colbach, N., Crespo, O., Garcia, F., Jeuffroy, M.H., Justes, E., Loyce, C., Munier-Jolain, N., Sadok, W., 2009. Designing crop management systems by simulation. European Journal of Agronomy, in press, doi:10.1016/j.eja.2009.06.001.

Berntsen, J., Haugaard-Neilsen, H., Olesen, J.E., Petersen, B.M., Jensen, E.S., Thomsen, A., 2004. Modelling dry matter production and resource use in intercrops of pea and barley. Field Crop Res. 88, 69-83.

Biarnès, V., Lecoeur, J., Jeuffroy, M.-H., Vocanson, A., Ney, B., 2009. A model which 14 integrates knowledge on pea crop physiology and agronomic diagnosis. In: Physiology of the pea crop. Science Publishers, Enfield, USA, pp 197-208. in press.

Bilalis DJ, Sidiras N, Kakampouki I, Efthimiadou A, Papatheohari Y, Thomopoulos P. 2005. Effects of organic fertilization on maize-legume intercrop in a clay loam soil and Mediterranean climate - Can the Land Equivalent Ratio (LER) index be used for root development? J. Food Agric. Environ. 3, 117-123.

Brisson, N., Bussière, F., Ozier-Lafontaine, H., Tournebize, R., Sinoquet, H., 2004. Adaptation of the crop model STICS to intercropping. Theoretical basis and parameterization Agrononomie 24, 409-421.

Brisson, N., Mary, B., Ropoche, D., Jeuffroy, M.-H., Ruget, F., Nicoullaud, B., Gate, P., Devienne-Barret, F., Antonioletti, R., Durr, C., 1998. STICS: a generic model for the simulation of crops and their water and nitrogen balances: I. Theory and parameterization applied to wheat and corn. Agronomie 18, 311–346.

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth. J. Agric. Sci. 10, 399-408.

Brun, D., 2002. Développement du pois d'hiver en zone Nord. Master report, ENSAIA, Nancy, France.

Bulson, H.A.J., Snaydon, R.W., Stopes, C.E., 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. J. Agric. Sci. 128, 59-71.

Burns, I.G., 1974. A model for predicting the redistribution of salts applied to fallow soils after excess rainfall or evaporation. J. Soil Sci. 25, 165-178.

Caldwell R M 1995 Simulation models for intercropping systems. In Ecophysiology of tropical intercropping. Eds. H Sinoquet and P Cruz. pp. 353-368. INRA, Paris.

Chauvel, B., Guillemin, J.P., Colbach, N., Gasquez, J., 2001. Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). Crop Prot. 20, 127-137.

Colbach, N., Maurin, N., Huet, P., 1996. Influence of cropping system on foot rot of winter wheat in France. Crop Prot. 15, 295-305.

Corre-Hellou, G., 2005. Acquisition de l'azote dans des asociations pois-orge (*Pisum sativum* L.-*Hordeum vulgare* L.) en relation avec le fonctionnement du peuplement. PhD Thesis, Université d'Angers, Angers, France.

Corre-Hellou, G., Crozat, Y., 2005. Assessment of Root System Dynamics of Species Grown in Mixtures under Field Conditions using Herbicide Injection and 15N Natural Abundance Methods: A Case Study with Pea, Barley and Mustard. Plant Soil 276, 177-192.

Corre-Hellou, G., Crozat, Y., 2005. N_2 fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems as affected by weeds and peaweevil (*Sitona lineatus* L.). Eur. J. Agron. 22, 449-458.

Corre-Hellou, G., Crozat, Y., 2004. Interspecific competition for soil N in pea-barley mixtures during the vegetative phase and consequences of N_2 fixation. 5th Conference on Grain Legumes, Dijon, France, pp. 65-66.

Corre-Hellou, G., Faure, M., Launay, M., Brisson, N., Crozat, Y., 2009. Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea-barley intercrops. Field Crops Res. 113, 72-81.

Corre-Hellou, G., 2007. Using a modelling approach to study interactions between species in intercropping systems. 6th European Conference on Grain Legumes, Lisbon, Portugal.

Corre-Hellou, G., Brisson ,N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crop Res. 103, 76-85.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for Soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282, 195-208;

Cruz P., Soussana, J.-F. Mixed crops. 1997. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 131–144.

Daimon H, Hori K, Shimizu A, Nakagawa M. 1999. Nitrate-induced inhibition of root nodule formation and nitrogenase activity in the peanut (*Arachis hypogaeg* L.). Plant Proc. Sci. 2, 81-86.

Darwin, C., 1859.On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life . Eds John Murray, London, UK.

David, C., Jeuffroy, M.-H., 2009. A sequential approach for improving Azodyn crop model under conventional and low-input conditions. Eur. J. Agron. in press. doi:10.1016/j.eja.2009.05.007

David, C., Jeuffroy, M.-H., Laurent, M., Mangin, M., Meynard, J.M. 2005. The assessment of AZODYN-ORG model for managing nitrogen fertilization of organic winter wheat. Eur. J. Agron. 23, 225-242.

David, C., Jeuffroy, M.-H., Recous, S., Dorsainvil, F., 2004. Adaptation and assessment of the AZODYN model for managing the nitrogen fertilization of organic winter wheat. Eur. J. Agron. 21, 249-266.

Davidson I, Robson M. 1986. Interactions between nitrate uptake and N_2 fixation in white clover. Plant and Soil 91, 401-404.

Davidson IA, Robson MJ. 1986. Effect of contrasting patterns of nitrate application on the nitrate uptake, N2 fixation, nodulation and growth of white clover. Annals of Botany 57, 331-338.

Dazzo FB, Brill WJ, 1978. Regulation by fixed nitrogen of host-symbiont recognition in the Rhizobium-clover symbiosis. Plant Physiol., 62, 18-21.

De Wit, C.T., 1960. On competition. Versl Landbouwk Onderzoek 66, 1-81.

De Wit, C.T., Van den Bergh, J.P., 1965. Competition between herbage plants. Neth. J. Agric. Sci. 13, 212-221.

Doré, T., Le Bail, M., Roger-Estrade, J., Martin, P., Jeuffroy, M.H., 2006. L'agronomie aujourd'hui. Quae Editions, Versailles, France

Dürr C, Boiffin J.,1995. Sugarbeet seedling growth from germination to first leaf stage. J. Agric. Sci., Camb. 124, 427-435

Dürr C., Mary, B., 1998. Effects of nutrient supply on preemergence growth and nutrient absorption in wheat (*Triticum aestivum* L.) and sugarbeet (*Beta vulgaris* L.). Ana. Bot.-London, 81, 665-672.

Dürr, C., Aubertot, J.N., Richard, G., Dubrulle, P., Duval, Y., Boiffin, J., 2001. Simple: A model for simulation of plant emergence predicting the effects of soil tillage and sowing operations. Soil Sci Soc Am J 65, 414-423.

Fan, F., Zhang, F., Song, Y., Sun, J., Bao, X., Guo, T., Li, L., 2006. Nitrogen Fixation of Faba Bean (*Vicia faba* L.) Interacting with a Non-legume in Two Contrasting Intercropping Systems. Plant and Soil 283, 275-286.

Faurie, O., Soussana, j.-F., Sinoquet, H. 1996. Radiation interception, partitioning and use in Grass-Clover mixtures. Ann. Bot. 77, 35-45.
Finch, S., Collier, R.H., 2000. Host-plant selection by insects – a theory based on "appropriate/inappropriate landings" by pest insects of cruciferous plants. Entomol. Exp. Appli. 96, 91-102.
Fisher, R.A., Yates, F., 1938. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Edinburg.
Francisco PB, Akao S, 1996. Autoregulation and Nitrate Inhibition of Nodule Formation in Soybean cv. Enrei and its Nodulation Mutants. J. Exp. Bot. 44, 547-553.
Fujikake H, Yamazaki A, Ohtake N, Sueyoshi K, Matsuhashi S, Ito T, Mizuniwa C, Kume T, Hashimoto S, Ishioka N-S, Watanabe S, Osa A, Sekine T, Uchida H, Tsuji A, Ohyama T. 2003. Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. J. Exp. Bot. 54, 1379-1388.
Fujikake H, Yashima H, Sato T, Ohtake N, Sueyoshi K. 2002. Rapid and reversible nitrate inhibition of nodule growth and N2 fixation activity in soybean (*Glycine max* (L.) Merr.). Soil Sci. Plant Nutr. 48, 211-217.
Fujita, K., Ofosu-Budu K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant Soil 141, 155-175.
Gate, P., 1995. Ecophysiologie du blé: de la plante à la culture. Lavoisier Tec&Doc.
Ghaley, B., Hauggaard-Nielsen, H., Hogh-Jensen, H., Jensen, E. 2005. Intercropping of Wheat and Pea as Influenced by Nitrogen Fertilization. Nutr. Cycl. Agroecosyst. 73, 201-212.
Gordon AJ, Skot L, James CL, Minchin FR. 2002. Short-term metabolic responses of soybean root nodules to nitrate. J. Exp. Bot. 53, 423-428.
Gosse, G., Varlet-Grancher, C., Bonhomme, R., Chartier, M., Allirand, J.M., Lemaire, G., 1986. Production maximale de matière sèche et rayonnement solaire intercepté par un couvert végétal. Agron. 6, 47–56.
Grant RF, 1992 Simulation of competition among plant populations under different managements and climates. Agron. Abstr., American Society of Agronomy, Madison, Wisconsin, USA.
Greenwood, D., Gerwitz, A., Stone, D., Barnes, A., 1982. Root development of vegetable crops. Plant Soil 68, 75-96.
Gregory P J, McGowan M and Biscoe P V 1978 Water relations of winter wheat. 2. Soil water relations. J. Agr. Sci. 91, 103-116.
Griffon, M., 1999. Développement durable et agriculture: la révolution doublement verte. Cah. Agric. 8, 259-267.
Griffon, M., 2006. Nourrir la planète. (Eds) Odile Jacob, Paris, France.
Hamblin, A.P., Hamblin, J., 1985. Root characteristics of some temperate legume species and varieties on deep, free-draining entisols. Aust. J. Agr. Res. 36, 63-72.
Hansen, B., 1989. Determination of nitrogen as elementary N, an alternative to Kjeldhal. Acta Agr. Scan. 39:113-118.
Hansen, S., Jensen, H.E., Nielsen, N.E., Svenden, H., 1991. Simulation of nitrogen dynamics and biomass production in winter using the Danish simulation model DAISY. Fert. Res. 27, 245-259.
Hauggaard-Nielsen, H., 2001. Competitive interactions, resource use and nitrogen dynamics in annual intercrops in low-input cropping systems. PhD thesis, Risø National Laboratory, Roskilde, pp110.
Hauggaard-Nielsen, H., Andersen, M.K., 2000. Intercropping grain legumes and cereals in organic cropping systems. Grain Legumes 30, 18-19.
Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N avaibility. Field Crop Res. 72, 185-196.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res. 70, 101-109.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Reintroducing grain legume-cereal intercropping for increased protein production in European cropping systems. *In* 4th European conference on grain legumes, Cracow, Poland (Eds) AEP, pp. 52-53.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutr. Cycl. Agroecosys. 65, 289-300.

Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. Field Crop Res. 95, 256-267.

Hauggaard-Nielsen, H., Jornsgaard, B., Kinane, J., Jensen, E., 2008. Grain-legume-cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agr. Food Syst. 23, 3-12.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N_2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crop Res. 113, 64-71.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E., 2009. Pea and barley intercropping and short-term subsequent crop effects across European organic cropping conditions. Nutri. Cycl. Agroecosys. 85, 141-155.

Izaurralde RC, Juma NG, McGill WB. 1990. Plant and Nitrogen Yield of Barley-Field Pea Intercrop in Cryoboreal-Subhumid Central Alberta. Agron. J. 82, 295-301.

Jacobsen, B.H., Petersen, B.M., Berntsen, J., Boye, C, Sorensen, C.G., Sogaard, H.T., Hansen, J.P., 1998. An integrated economic and environmental farm simulation model (FASSET). Report No. 102. Danish Institute of Agricultural and Fisheries Economics, Copenhagen, Denmark.

Jamieson, P.D., Semenov, M.A., 2000. Modelling nitrogen uptake and redistribution in wheat. Field Crops Res. 68, 21-29.

Jensen, E.S., 1986. The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (*Pisum sativum* L.). Fert. Res. 10, 193-202.

Jensen, E.S., 1987. Seasonal patterns of growth and nitrogen fixation in field-grown pea. Plant Soil 101, 29-37.

Jensen, E.S., 1996. Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil 182, 25-38.

Jensen, E.S., 1996. Barley uptake of N deposited in the rhizosphere of associated field pea. Soil Biol. Biochem. 28, 159-168.

Jensen ES, Hauggaard-Nielsen H. 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? Plant and Soil 252, 177-186.

Jeuffroy, M.H., Bergez, J.-E., David, C., Flénet, F., Gate, P., Loyce, C., Maupas, F., Meynard, J.-M., Reau, R., Surleau-Chambenoit, C., 2008. Utilisation des modèles pour l'aide à la conception at à l'évaluation d'innovations techniques en production végétales: bilan et perspectives. In: Reau, R., Doré, T. (Eds.), Des systèmes de culture innovants et durables: quelles méthodes pour les mettre au point et les évaluer? Educagri, Dijon, pp. 11-27.

Jeuffroy, M.-H., Bouchard, C., 1999. Intensity and duration of nitrogen deficiency on wheat grain number. Crop Sci. 39, 1385-1393.

Jeuffroy, M.-H., Recous, S., 1999. AZODYN: a simple model simulating the date of nitrogen deficiency for decision support in wheat fertilization. Eur. J. Agron. 10,129-144.

Jeuffroy, M.-H., Warembourg, F.R., 1991. Carbon transfer and partitioning between vegetative and reproductive organs in Pisum sativum L. Plant Physiol. 97, 440-448.

Jolliet, O., Saadé, M., Crettaz, P., 2005. Analyse du cycle de vie. Comprendre et réaliser un écobilan. Presse Polytechniques et Universitaires Romandes (Ed.). Collection Gérer l'environnement. Lausanne, Switzerland.

Justes, E., Jeuffroy, M.H.,Mary, B., 1997. Wheat, barley, and durum wheat. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 73–91.

Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huché, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74, 397-407.

Karimi, M.M., Siddique, K.H.M., 1991. Crop growth and relative growth rates of old and modern wheat cultivars. Aust. J. Agr. Res. 42, 13-20.

Keating, B. A., Carberry, P. S., 1993. Resource capture and use in intercropping: solar radiation. Field Crops Res. 34, 273-301.

Keeney, D.R., Wilson, D.W., 1989. Nitrogen-inorganics forms. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis. Part II: Chemical and Microbiological properties, 2nd edn. American Society of Agronomy, Madison, Wisconsin.

Kinane, J., Lyngkjaer, M.F., 2002. Effect of barley-legume intercrop on disease frequency in an organic farming system. In: Efpp, t.C. (Ed.), Plant Prot. Sci. , Prague, Czech Republic, pp. 227-231.

Kouchi H, Akao S and Yoneyama T 1986 Respiratory utilization of 13C-labelled photosynthate in nodulated root systems of soybean plants. J. Exp. Bot. 37, 985–993.

Kropff, M. J. and H. H. van Laar. 1993. Modelling crop-weed interactions. Wallingford, Great Britain: CAB International and International Rice Research Institute. 274 p

Lecoeur, J., Sinclair, T.R., 1996. Field pea (*Pisum sativum* L.) transpiration and leaf growth in response to soil water deficits. Crop Sci. 36, 331-335.

Leitch, M., Musa, M., 1998. Agronomic characteristics and nitrogen balance of mixtures of barley and peas grown in different proportions at two levels of applied nitrogen. In: 3rd European Conference on Grain Legumes, Valladolid, pp 425.

Lemaire, G., van Oosterom, E., Jeuffroy, M.-H., Gastal, F., Massignam, A., 2008. Crop species present different qualitative types of response to N deficiency during their vegetative growth. Field Crops Res. 105, 253-265.

Lemaire, G., Jeuffroy, M.H., Gastal, F., 2008. Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. Eur. J. Agron., 28, 61-624.

Lemaire, G., Oosterom, E.v., Sheery, J., Jeuffroy, M.-H., Massignam, A., Rossato, L., 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? Field Crop Res. 100, 91-106.

Lemaire, G., Meynard, J.M., 1997. Use of the Nitrogen Nutrition Index for the analysis of agronomical data.In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 45–55.

Li, Y.-Y., Yu, C.-B., Cheng, X., Li, C.-J., Sun, J.-H., Zhang, F.-S., Lambers, H., Li, L., 2009. Intercropping alleviates the inhibitory effect of N fertilization on nodulation and symbiotic N_2 fixation of faba bean. Plant and Soil 323, 295-308.

Limaux, F., Recous, S., Meynard, J.-M., Guckert, A., 1999. Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat. Plant and Soil 214, 49-59.

Litsinger, J.A., Moody, K., 1976. Integrated pest management in multiple Cropping Systems. In: Asa, C.S. (Ed.), Multiple Cropping. ASA, pp. 293-316.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72-76.

Machet, J.M., Dubrulle, P., Louis, P., 1990. AZOBIL: a computer program for fertilizer N recommandations based on a predictive balance sheet method. In: Scaife, A. (Ed.), Proc. 1st Congress of the European Society for Agronomy, Paris pp. 21–22.

Malagoli P, Naudin C, Goulevant G, Sester M, Corre-Hellou G and Jeuffroy M-H. 2009. Simulation of the response of a winter pea-wheat intercrop to contrasted N-fertilization applications: design and assessment of AZODYN-IC model. Eur. J. Agron. Under review. EURAGR1748.

Malagoli, P., Naudin, C., Goulevant, G., Sester, M., Corre-Hellou, G., Jeuffroy M.H., 2009. Modeling competition for below-ground resources and light within a winter pea (*Pisum sativum* L.) – wheat (*Triticum aestivum* L.) intercrop (Azodyn-InterCrop): towards a decision making oriented-tool. 7th European Conference on Precision Agriculture, Joint International Agricultural Conference (JIAC), Wageningen (Pays-Bas). In: van Henten, E.J., Goense, D. Lokhorst C. (Eds), Precision Agriculture,Wageningen Academic publishers. , pp. 659-666.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., Tourdonnet, S.d., Valantin-Morison, M., 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. Agron. Sustain. Dev. 29, 43-62.

Mariotti, A., Mariotti, F., Amarger, N., 1980. Fractionnements isotopiques de l'azote lors de processus d'absorption des nitrates et de fixation de l'azote atmosphérique par les plantes. Physiol . Veg. 18, 163-181.

Marshall, B., Willey, R. W., 1983. Radiation interception and growth in an intercrop of pearl millet/groundnut. Field Crop Res. 7, 141-160.

Meynard, J.M., 2008. Produire autrement: réinventer les systèmes de culture. In: Reau, R., Doré, T. (Eds.), Des systèmes de culture innovants et durables: quelles méthodes pour les mettre au point et les évaluer? Educagri, Dijon, pp. 11-27.

Meynard, J.-M., Cerf, M., Guichard, L., Jeuffroy, M.-H.l.n., Makowski, D., 2002. Which decision support tools for the environmental management of nitrogen? Agronomie 22, 817-829.

Meynard, J.-M., Boiffin, J., Caneill, J., Sébillotte, M., 1981. Elaboration du rendement et fertilisation azotée du blé d'hiver en Champagne crayeuse. II. Types de réponse à la fumure azotée et application de la méthode du bilan prévisionnel. Agronomie 1, 795-806.

Minchin, F.R., Becana, M., Sprent, J.I., 1989. Short-term inhibition of legume N_2 fixation by nitrate. II Nitrate effects on nodule oxygen diffusion. Planta 180, 46-52.

Monteith, J.-L., 1977. Climate and efficiency of crop production in Britain. Phil. Trans. R. Soc. Lond. 281, 277-294.

Moreau-Valancogne, P., Coste, F., Crozat, Y., Dürr C., 2008. Assessing emergence of bean (*Phaseolus vulgaris* L.) seed lots in France: Field observations and simulations. Eur. J. Agron. 28, 309-320.

Morgado LB, Willey RW. 2003. Effects of plant population and nitrogen fertilizer on yield and efficiency of maize-bean intercropping. Pesqui. Agropecu. Bras. 39, 1257-1264.

Munier-Jolain, N., Biarnès, V., Chaillet, I., Lecoeur, J., Jeuffroy, M.-H., Carrouée, B., Crozat, Y., Guilioni, L., Lejeune, I., Tivoli, B., 2005. Agrophysiologie du pois protéagineux. Lavoisier, Paris.

Naudin C, Corre-Hellou G, Pineau S, Crozat Y and Jeuffroy M-H, 2009. Timing effect of nitrogen fertilization on winter pea-wheat intercrops. I- Growth dynamics and grain yield. Field Crops Res. Under review. FIELD-D-09-00419.

Naudin C, Corre-Hellou G, Pineau S, Crozat Y and Jeuffroy M-H. 2009 – Timing effect of nitrogen fertilization on winter pea-wheat intercrops. II- N-partitioning and Symbiotic Nitrogen Fixation variations. Field Crops Res. Under review. FIELD-D-09-00420.

Nemecek, T., von Richthofen, J.-S., Dubois, G., Casta, P., Charles, R., Pahl, H., 2008. Environmental impacts of introducing grain legumes into European crop rotations. Eur. J. Agron. 28, 380-393.

Neo HH, Layzell DB. 1997. Phloem glutamine and the regulation of O_2 diffusion in legume nodules. Physiologia Plantarum 113, 259-267.

Neumann, A., Schmidtke, K., Rauber, R. 2007. Effects of crop density and tillage system on grain yield and N uptake from soil and atmosphere of sole and intercropped pea and oat. Field Crop Res., 100, 285-293.
Ney, B., Doré, T., Sagan, M., 1997. The nitrogen requirement of major agricultural crops: grain legumes. In: Lemaire G., (Eds), Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, pp. 107–117.
Ney, B., 1994. Modélisation de la croissance aérienne d'un peuplement de pois, *In* UNIP-ITCF-INRA, ed. Agrophysiologie du pois protéagineux.
Ofori, F., Stern, W.R., 1987. Cereal-legume intercropping systems. Adv. Agron. 41, 41-90.
Ofori F, Stern WR. 1986. Maize/cowpea intercrop system: Effect of nitrogen fertilizer on productivity and efficiency. Field Crops Res. 14, 247-261.
Olesen, J.E., Petersen, B.M., Bernsten, J., Hansen, S., Jamieson, P.D., Thomsen, A.G., 2002. Comparison of methods for simulating effects of nitrogen on green leaf area index and dry matter growth in winter wheat. Field Crop Res. 74, 131-149.
Ozier-Lafontaine, H., Vercambre, G., Tournebize, R., 1997. Radiation and transpiration partitioning in a maize-sorghum intercrop: Test and evaluation of two models. Field Crop Res. 49, 127-145.
Paillard, C., 1991. Incidence de l'état structural du sol sur le développement et la croissance racinaire du blé tendre d'hiver. Répercussion sur l'alimentation hydrique et minérale. ENSAIA, Nancy, p. 32.
Parmentier, B., 2007. Nourrir l'humanité, les grands problèmes de l'agriculture mondiale. (Eds) Éditions La Découverte, Paris, France.
Pate JS, Dart PJ. 1961. Nodulation studies in legumes. Plant and Soil 15, 329-346.
Peoples MB, Herridge DF, Ladha JK. 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant and Soil 174, 3-28.
Pline l'Ancien (23-79). Historiae Naturalis (Traduction E. Littré). Eds Dubochet et al., Paris, 1850.
Poggio, S.L., 2005. Structure of weed communities occuring in monoculture and intercropping of field pea and barley. Agr. Ecosyst. Environ. 109, 48-58.
R Development Core Team , 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
Raggio M, Raggio N, Torrey JG. 1965. The Interaction of Nitrate and Carbohydrates in Rhizobial Root Nodule Formation. Plant Physiology 40, 601-606.
Rao MR, Rego TJ, Willey RW. 1987. Response of cereals to nitrogen in sole cropping and intercropping with different legumes. Plant Soil 101, 167-177.
Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988. The fate of labelled ^{15}N urea and ammonium nitrate applied to a winter wheat crop. I. Nitrogen transformations in the soil. Plant Soil 112, 205-214.
Rennie, R.J., Rennie, D.A., 1983. Techniques for quantifying N_2 fixation in association with non-legumes under field and greenhouse conditions. Can. J. Microbiol. 29, 1022–1035.
Rerkasem K, Rerkasem B. 1988. Yields and nitrogen nutrition of intercropped maize and ricebean (*Vigna umbellata* [Thumb.] Ohwi and Ohashi). Plant Soil 108, 151-162.
Sampaio EVSB, Tiessen H, Antonino ACD, Salcedo IH. 2004. Residual N and P fertilizer effect and fertilizer recovery on intercropped and sole-cropped corn and bean in semiarid northeast Brazil. Nutr. Cycl. Agroecosys.70, 1-11.
Schoonhoven, L.M., van Loon, J.J.A., ,Marcel Dicke, M., 2005. Insect-Plant Biology, Eds Oxford Biology, Oxford United kingdom, 421pp.
Siame J, Willey RW, Morse S. 1998. The response of maize/Phaseolus intercropping to applied nitrogen on Oxisols in northern Zambia. Field Crops Res. 55, 73-81.

Silsbury, J.H., 1990. Grain yield of wheat in rotation with pea, vetch or medic grown with three systems of management. Aust. J. Exp. Agr. 30, 645-649.
Sosulski, F.W., Imafidon, G.I., 1990.Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38, 1351-1356.
Stevenson, F.C., van Kessel, C., 1996. A Landscape-Scale Assessment of the Nitrogen and Non-Nitrogen Rotation Benefits of Pea. Soil Sci Soc Am J 60, 1797-1805.
Streeter JG. 1988. Inhibition of legume nodule formation and N_2-fixation by nitrate. CRC Critical Reviews in Plant Science 7, 1-23.
Streeter JG. 1985. Nitrate inhibition of legume nodule growth and activity. I. Long term studies with a continuous supply of nitrate. Plant Physiol. 77, 321-324.
Streeter JG. 1985. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Plant Physiol. 77, 325-328.
Tamet, V., Boiffin, J., Dürr, C., Souty, N., 1996. Emergence and early growth of an epigeal seedling (*Daucus carota* L.): influence of soil temperature, sowing depth, soil crusting and seed weight. Soil and Tillage Research, 40, 25-38.
Teller, G.L., 1932. Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for protein in cereals and bread. Cereal Chem. 9, 261-274.
Thorup-Kristensen, K., 1998. Root Growth of Green Pea (*Pisum sativum* L.) Genotypes. Crop Sci 38, 1445-1451.
Tilman, D., Cassman, K.G., Matsons, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671-677.
Tobita S, Ito O, Matsunaga R, Rao TP, Rego TJ, Johansen C, Yoneyama T. 1994. Field evaluation of nitrogen fixation and use of nitrogen fertilizer by sorghum/pigeonpea intercropping on an Alfisol in the Indian semi-arid tropics. Biol. Fert. Soils 17, 241-248.
Trenbath, B.R., 1993. Intercroppping for the management of pests and diseases. Field Crops Research 34, 381-405.
Trenbath, B.R., 1976. Plant interactions in mixed crop communities. In: Pappendick et al. (Eds) Multiple cropping, American Society of Agronomy, Madison, Wisconsin, pp. 129-169.
Tricot F. 1993. Mise en place des nodosités du pois protéagineux de printemps (Pisum sativum L.). Influence de la nutrition carbonée. PhD thesis, Université Paris-Sud Orsay, France.
Tsubo, M., Walker, S., 2002. A model of radiation interception and use by a maize-bean intercrop canopy. Agric. Meteorol. 110, 203-215.
Tsubo, M., Walker, S., Mukhala, E., 2001. Comparisons of radiation use efficiency of mono/inter-cropping systems with different row orientations. Field Crop Res. 71, 17-29.
UNIP, 2008. Cultiver des associations céréales-protéagineux : des intérêts agronomiques, économiques et environnementaux à découvrir. Rapport technique final. Appel à Projets Casdar 2005, n°431. 112 pages.
Vandermeer, J., 1989. The ecology of intercropping. Cambridge University Press, Cambridge, UK.
Vocanson A., 2006. Evaluation ex ante d'innovations variétales en pois d'hiver (*Pisum sativum* L.) : approche par modélisation au niveau de la parcelle et de l'exploitation agricole. PhD Thsesis INAPG, Paris, 250p. +annexes.
Vocanson, A., Estrade, J.-R., Boizard, H., Jeuffroy, M.-H., 2006. Effects of soil structure on pea (*Pisum sativum* L.) root development according to sowing date and cultivar. Plant Soil. 281, 121-135.
Vocanson, A., Jeuffroy, M.-H., Estrade, J.-R., 2006. Effect of sowing date and cultivar on root system development in Pea (*Pisum sativum* L.). Plant Soil. 283, 339-352.
Voisin A-S, Bourion V, Duc G, Salon C. 2007. Using an Ecophysiological Analysis to Dissect Genetic Variability and to Propose an Ideotype for Nitrogen Nutrition in Pea. Ann Bot, 1-12.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003. Seasonal patterns of ^{13}C partitioning between shoots and nodulated roots of N_2- or nitrate-fed *Pisum sativum* L. Ana. Bot.-London 91, 539-546.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003. Symbiotic N_2 fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. J. Exp. Bot. 54, 2733-2744.

Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003. Root and Nodule Growth in *Pisum sativum* L. in Relation to Photosynthesis: Analysis Using ^{13}C-labelling. Ann Bot 92, 557-563.

Voisin A-S, Salon C, Munier-Jolain NG, Ney B. 2002. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). Plant and Soil 242, 251-262.

Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil 243, 31-42.

Wallace, J.S., Batchelor, C.H., Dabeesing, D.N., Soopramanien, G.C., 1990. The partitioning of light and water in drip irrigated plant cane with a maize intercrop. Agr. Water Manage. 17, 235-256.

Waterer, J.G., Vessey, J.K., Stobbe, E.H., Soper, R.J., 1994. Yield and symbiotic nitrogen fixation in a pea-mustard intercrop as influenced by N fertilizer addition. Soil Biol. Biochem. 26, 447-453.

Willey, R.W., 1979. Intercropping - Its importance and research needs. Part 1. Competition and Yield advantages. Field Crops Abstr. 32, 1-10.

NITROGEN NUTRITION OF WINTER PEA-WHEAT INTERCROPS : (*Pisum sativum* L. – *Triticum aestivum* L.) : ANALYSIS, MODELLING, AND PROPOSITIONS FOR N-MANAGEMENT STRATEGIES

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Abstract

Cereal-legume intercrops are gaining increasing interest in Europe because of low-input preoccupations, preservation of environment and biodiversity. Previous studies had shown that intercropping performances are highly dependant on soil N availability. This practice could provide interesting applications to develop crops for multi-service outcomes along with lower inputs (particularly N). However, very few references are nowadays available to allow N management of intercrops for contrasted production targets.
The objectives of this work was (i) to use current knowledge on winter pea-wheat intercrop in order to study whether N fertilization could be a helpful tool to manage such intercrop towards production targets under a conventional agriculture; (ii) to improve knowledge on the response of intercrop to different N-availability dynamics (N resources sharing; inhibition and reversibility of Symbiotic Nitrogen Fixation (SNF)); (iii) to give propositions towards decision rules about N-management of pea-wheat intercrops for different production goals.
Our field experiments demonstrated that N-fertilization is an efficient tool to manage pea-wheat intercrop, notably contribution of cereal in total intercrop yield, which is nowadays a badly controlled criterion. N-supply favoured cereal growth to the detriment to the legume. Cereal is more competitive than the legume for mineral N resources if N-application occurs before pea beginning of seed filling. However, the intensity of the response to date of N-fertilizer application is variable according to gap between intercrops species in growth dynamics and stage of development. This is determinant for N-sharing and SNF response.
Experiments under greenhouse conditions investigated inhibition effect of nitrate and ability of pea SNF to recover through a structure –function analysis according to different stages of nitrate exposure. Nitrate reduced rate of nodule establishment when nodulated root were exposed to nitrate during vegetative phases while it entailed damage on existing nodules when applied during flowering and seed filling. Nitrate exposure always decreased specific activity of nodules. Moreover, the ability to recover of SNF after nitrate removal is partly dependant on level of C availability to nodules. Thus, pea SNF can recover when a short-term inhibition by nitrate occurs before seed filling stages, which is in agreement with our own field observations.
Crop model AZODYN-IC was built to simulate pea-wheat intercrop. It is based on two sole crop models (AZODYN and AFISOL for wheat and pea, respectively). Its main interests are (i) to be able to satisfactorily simulate response of such intercrops to various soil mineral N availability dynamics in order to be used as decision support tool; (ii) to use simple formalisms to simulate resources sharing (light, water, and nitrogen), tightly linking light sharing and N-acquisition, (iii) to run with the sole-crop parameters. This model was used to extend field experimentations by simulating wider range of N-management strategies (sowing densities x N-supply rates x N-supply dates) using numerous climatic datasets. This work was able to give propositions towards decision rules for N-management of intercrop according to soil N mineral content at the end of winter, contribution of wheat in intercrop biomass at the end of winter, estimation of N-mineralization from end of winter to harvest, and rate and date of N-application.

Keywords: Intercrop; N nutrition; Symbiotic Nitrogen Fixation, Competition, Modelling, Pea, Wheat

NUTRITION AZOTEE DES ASSOCIATIONS POIS-BLE D'HIVER
(*Pisum sativum* L. – *Triticum aestivum* L.) **:**
ANALYSE, MODELISATION ET PROPOSITIONS DE STRATEGIES DE GESTION

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Résumé

Avec l'émergence des préoccupations d'économie d'intrants, de préservation de l'environnement et de la biodiversité, les associations, qui consistent en la culture d'au moins deux espèces différentes sur la même surface pendant une période significative de leur développement, présentent un regain d'intérêt en Europe. Ces associations pourraient avoir des applications intéressantes pour le développement de cultures « multiservices » à moindre niveau d'intrants (azote particulièrement). Des travaux antérieurs ont montré que les performances des associations céréale-légumineuse dépendent fortement du niveau d'azote minéral du sol Cependant on ne dispose pas à l'heure actuelle de références suffisantes pour piloter la fertilisation azotée de l'association en fonction de différents objectifs de production.

Les objectifs de la thèse étaient (i) d'utiliser les connaissances précédemment acquises sur le fonctionnement dynamique d'une association pois-blé d'hiver pour étudier la pertinence de la fertilisation azotée comme levier pour orienter les performances des associations pois-blé vers différents objectifs de production en agriculture conventionnelle ; (ii) d'approfondir les connaissances sur le fonctionnement de l'association en réponse à différentes dynamiques de disponibilité en azote (partage des ressources azotées ; inhibition et réversibilité de la fixation symbiotique) ; (iii) d'apporter des pistes vers des règles de décision pour la gestion la fertilisation azotée de ces associations pour différents objectifs de production.

Nos expérimentations de plein champ démontrent que la fertilisation azotée est un levier efficace pour orienter les performances finales notamment la proportion de chaque espèce dans le mélange, critère aujourd'hui mal maîtrisé. Un apport d'azote favorise la croissance de la céréale et pénalise celle de la légumineuse. La céréale apparaît plus compétitive que la légumineuse pour les ressources d'azote minéral pour une date d'apport intervenant avant début du remplissage des grains du pois. Cependant, l'intensité de la réponse à la date de fertilisation varie en fonction des écarts de dynamiques de croissance et de phénologie de chaque espèce avant l'apport, facteurs qui apparaissent déterminants dans le partage de l'N minéral et le comportement de la fixation symbiotique.

Des expérimentations en conditions contrôlées ont permis d'approfondir l'effet inhibiteur des nitrates et la réversibilité de la fixation chez le pois en analysant séparément l'impact sur la structure et sur l'activité de l'appareil fixateur en fonction du stade phénologique et de la disponibilité en carbone. Une exposition aux nitrates pendant la phase végétative réduit la vitesse d'apparition des nodosités alors qu'une exposition durant la floraison et le remplissage des grains réduit la croissance des nodosités existantes. Les nitrates réduisent fortement l'activité fixatrice quelle que soit la date d'exposition aux nitrates. De plus, il a été démontré que la réversibilité de la fixation symbiotique après courte exposition aux nitrates était fonction de l'allocation carbonée aux nodosités. Ainsi, la réversibilité de la fixation symbiotique est possible chez le pois si une courte inhibition due nitrates survient avant les stades de remplissage du grain, ce qui est confirmé en situations de plein champ.

Notre démarche de modélisation a par ailleurs aboutit au développement, à partir de deux modèles de culture pures (AZODYN pour le blé et AFISOL pour le pois), d'un modèle dynamique du fonctionnement de ces associations (AZODYN-IC) dont l'intérêt et l'originalité se situent dans (i) sa capacité à bien simuler la réponse à des disponibilités en azote variées permettant ainsi d'être directement opérationnel et utilisé comme outil d'aide à la gestion de la fertilisation azotée (ii) des formalismes relativement simples de partage des ressources (lumière, eau, azote) et comprenant un lien très étroit entre le partage de la lumière et l'acquisition de l'azote, déterminant dans le fonctionnement du peuplement, (iii) l'absence de paramétrage spécifique pour simuler le fonctionnement de l'association. Le modèle a permis de prolonger les expérimentations en simulant une gamme de stratégies de fertilisation plus large (combinaisons de proportions de semis x doses x dates de fertilisation) et ceci pour une gamme importante d'années climatiques. Ce travail a également permis de proposer des pistes vers des règles de décision de conduites azotées en fonction des reliquats d'azote observés sortie hiver, de la proportion de blé dans la biomasse de l'association observée sortie hiver, et de l'estimation de la minéralisation depuis la sortie hiver jusqu'à la récolte.

Mots-clés : Association ; Nutrition Azotée ; Fixation Symbiotique ; Compétition ; Modélisation ; Pois ; Blé