

Nitrogen fluxes in a perennial energetic crop, Miscanthus x giganteus : experimental study and modelling elements

Strullu Loïc

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Flux d'azote dans une culture pérenne à vocation énergétique, *Miscanthus* x *giganteus* : étude expérimentale et éléments de modélisation

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Introduction

&

Problématique

L'atténuation des changements climatiques, et la sécurisation sur le long terme, des approvisionnements énergétiques, sont des défis majeurs du XXI^{ème} siècle. Le rapport du Groupement Intergouvernemental des Experts sur le Climat (GIEC) de 2007 met en avant l'implication de l'augmentation des gaz à effet de serres (GES) d'origine anthropique dans le réchauffement climatique observé depuis la moitié du XX^{ème} siècle. Au niveau mondial, les principales sources de GES d'origine anthropique (en particulier CO_2, N_2O et CH_4) des secteurs d'activité économique sont : la production d'électricité, l'industrie, l'agriculture et les transports (Stern, 2006). Dans ce contexte, et pour se conformer au protocole de Kyoto, l'Union Européenne a décidé de promouvoir l'utilisation d'énergies produites à partir de ressources renouvelables (énergie éolienne, solaire, biomasse, etc.). La Directive Européenne 2009/28/CE a fixé pour objectif aux états membres d'atteindre 20% la part d'énergie renouvelable dans la consommation finale brute d'énergie de la Communauté d'ici à 2020. Elle impose également une part minimale de 10% de biocarburants dans la consommation totale d'essence et de gazole destinés au transport, mais fixe des critères de durabilité à respecter. D'après la directive, les biocarburants doivent permettre de réduire d'au moins 35% les émissions de GES par rapport aux carburants d'origine fossile, avec un objectif de 60% à partir de 2018. De plus, la directive exclut certaines situations pour la production de cultures destinées à la fabrication de biocarburants, comme les prairies naturelles, les forêts primaires ou les zones humides.

En 2009, la part des énergies renouvelables représentait 8.1% de la consommation finale d'énergie en France (Service de l'observation et des statistiques, 2010). Les objectifs à 2020 pour la France, fixés par la directive européenne, sont de porter cette part à 23%. Pour diminuer ses émissions de GES, la France s'est notamment tournée vers les biocarburants car le secteur des transports est le premier secteur émetteur de GES en France du fait de son contexte énergétique (CITEPA, 2010). Dans son plan d'action en faveur du développement

des énergies renouvelables (2009-2020), la France prévoit que les biocarburants représenteront la majeure partie des énergies renouvelables dans la consommation d'énergie finale brute d'ici à 2020.

Les biocarburants actuels, produits à partir de parties de plantes utilisées initialement pour la production alimentaire (biocarburants de 1ère génération), soulèvent de nombreuses inquiétudes sur les bilans nets d'énergie et de gaz à effet de serre et sur la compétition potentielle avec les productions alimentaires (FAO, 2008). La possibilité d'utiliser la lignocellulose pour la production de biocarburants (biocarburants de 2ème génération) offre la perspective d'ouvrir le panel des ressources candidates (déchets urbains et industriels, coproduits agricoles et forestiers, cultures pérennes dédiées, etc.) et de trouver des cultures mieux adaptées pour faciliter la réponse au « trilemme alimentation, énergie, environnement » (Tilman *et al.*, 2009). L'utilisation de cultures dédiées pour la fabrication de biocarburants de 2ème génération ne sera acceptable qu'à condition de limiter les impacts des pratiques agricoles qui leur sont appliquées, au niveau global (émissions de GES), mais aussi local (lixiviation des nitrates ou de pesticides, consommation en eau, etc.). Les plantes candidates devront donc répondre à ces exigences tout en alliant un rendement élevé à l'hectare, afin de limiter la concurrence entre productions alimentaires et non alimentaires (Sims *et al.*, 2006). En effet, le développement des biocarburants conduira à l'utilisation de terres agricoles jusqu'ici dédiées à la production de nourriture. Plus une culture sera productive, moins la pression sur les terres arables sera forte.

Au vu des exigences citées précédemment, *Miscanthus* x *giganteus* apparaît être l'une des cultures les plus prometteuses pour fournir la matière première pour la fabrication de biocarburants de 2ème génération (Somerville *et al.*, 2010). *M. giganteus*, originaire d'Asie du Sud-est, est un hybride interspécifique issu du croisement entre *M. sacchariflorus* et *M.*



Coupe précoce (mi-octobre)



Coupe tardive (fin février)

sinensis. Il est étudié depuis les années 80 en Europe pour son intérêt comme culture énergétique. C'est une plante pérenne en C4, appartenant à la famille des Poaceae, au potentiel de production élevé. La première année démarre par la plantation de morceaux d'un rhizome mère. Jusqu'aux premières gelées, la plante s'installe et développe principalement ses organes souterrains (Beale and Long, 1995). La culture est broyée en fin d'hiver de l'année d'implantation car la production de biomasse aérienne est très limitée. En Europe, le rendement de *M. giganteus* peut atteindre, à partir de la deuxième année de culture, 20 à 50 $tMS.ha^{-1}.an^{-1}$ quand la récolte a lieu en automne (récolte précoce) et 10 à 30 $tMS.ha^{-1}.an^{-1}$ quand la récolte a lieu fin d'hiver (récolte tardive) (Clifton-Brown *et al.*, 2000 et 2004 ; Tayot *et al.*, 1994). Si le rendement est supérieur lors d'une récolte précoce, les teneurs en éléments minéraux, notamment en azote, et en eau sont plus faibles lors de la récolte tardive (Lewandowski and Heinz, 2003). Pour la production de biocarburants de $2^{ème}$ génération, deux voies très différentes sont envisagées : la voie fermentaire ou voie humide, et la voie thermochimique ou voie sèche. L'efficacité des voies fermentaires dépend des composants de la paroi des cellules végétales (cellulose et hémicellulose) et de leur récalcitrance à la dégradation. L'efficacité des voies thermochimiques est liée, quant à elle, à des taux d'humidité et des contenus en cendres et éléments minéraux faibles (Karp and Shield, 2008). Selon la voie de transformation, et donc les critères de qualité de la biomasse qui sont attendus, l'une ou l'autre des stratégies de récolte du miscanthus pourrait être préférable.

Pour tout couvert végétal, la contribution de l'azote à l'élaboration de la biomasse est un élément majeur car i) l'azote est un facteur de croissance essentiel pour la plante (rôle clé sur le développement de l'indice foliaire et la production de biomasse), ii) l'utilisation d'engrais azotés a un impact fort sur le bilan énergétique, du fait du procédé de synthèse (Lewandowski and Schmidt 2006), et sur le bilan environnemental, du fait par exemple de pertes sous forme

de N_2O et/ou NH_3 (Crutzen *et al*., 2008) ou des pertes de NO_3 par lessivage (Beaudoin *et al.* 2005). Différentes études ont été menées en Europe afin de déterminer les réponses d'une culture de *M. giganteus* à un apport d'azote, mais leurs résultats sont contradictoires. Des expérimentations ont montré un effet positif de la fertilisation azotée sur la production de biomasse par une culture de *M. giganteus* récoltée précocement (Ercoli *et al.*, 1999 ; Acaroglu *et al.*, 2005), ou tardivement (Cosentino *et al.*, 2007 ; Boehmel *et al.*, 2008). Cependant, d'autres études concluent à l'absence d'effet de la fertilisation azotée sur la production de biomasse par une culture de *M. giganteus* récoltée tardivement (Beale *et al*., 1996 ; Himken *et al*., 1997 ; Clifton-Brown *et al*., 2007 ; Christian *et al*., 2008). Cette absence de réponse à la fertilisation azotée, ou les faibles besoins de cette culture en fertilisation azotée (Beale *et* Long, 1997 ; Himken *et al.*, 1997 ; Lewandowski *et al.*, 2000), peuvent s'expliquer par différentes caractéristiques, à commencer par une efficience d'utilisation de l'azote très élevée, qui varie entre 190 $gMSA.gN^{-1}$ et 350 $gMSA.gN^{-1}$ (Consentino *et al.*, 2007 ; Lewandowski and Schmidt, 2006). Ces valeurs sont supérieures aux valeurs habituellement observées pour les plantes annuelles en C3 (Lewandowski and Schmidt, 2006). D'autre part, à l'automne et pendant l'hiver, *M. giganteus* recycle une partie de l'azote accumulé pendant la croissance *via* la chute des feuilles et la mise en réserve de l'azote contenu dans les organes aériens vers les organes souterrains. L'azote issu de la chute des feuilles pourra être disponible pour la culture, les années suivantes, après minéralisation. Une partie de l'azote stocké dans les rhizomes est remobilisée au printemps, diminuant ainsi les besoins en azote externe (Beale and Long 1997 ; Himken *et al.*, 1997, Kahle *et al.*, 2001). Enfin, Eckert *et al.* (2001) ont isolé une bactérie du genre *Azospirillum* associée aux racines d'une culture de *M. giganteus* âgée de 5 ans en Allemagne, *Azospirillum* étant un genre constitué de bactéries endophytes facultatives, capables de fixer l'azote atmosphérique (Steenhoudt *et al.*, 2000). Ces différents phénomènes (efficience d'utilisation de l'azote élevée, recyclage de l'azote à

l'automne puis remobilisation au printemps et possible fixation d'azote atmosphérique), sont à l'origine de la faible réponse et/ou l'absence de réponse à la fertilisation azotée observée chez *M. giganteus* lors d'une récolte en coupe tardive (Cadoux *et al.* submitted).

L'effet de la date de coupe sur la production de biomasse et sur la nutrition azotée de la plante par une culture de *Miscanthus* x *giganteus* n'a, à notre connaissance, jamais été étudié. Or une coupe précoce en automne, lorsque la production de biomasse par la culture est maximale empêche la chute des feuilles et pourrait limiter tout ou partie de la mise en réserve des nutriments, notamment l'azote. Différentes études sur les plantes pérennes comme les arbres (Millard and Grelet, 2010), la luzerne (Avice *et al*, 1996 ; Teixeira *et al*, 2007 ; Ourry *et al*., 1994) ou les graminées fourragères (Thornton and Millard, 1997; Louahlia *et al.*, 1999), montrent que les réserves azotées ont un rôle primordial sur la nutrition azotée et la croissance de la plante lors du redémarrage de la culture au printemps et donc sur la production de biomasse. En effet, les réserves en azote influent sur la mise en place de l'indice foliaire des plantes (Avice *et al.*, 1996 ; Thornton and Millard, 1997 ; Teixeira *et al.*, 2007) ainsi que sur leur efficience de conversion des rayonnements (Avice *et al.*, 1996). Il semble donc important de comprendre les implications d'une coupe précoce, potentiellement intéressante pour des voies de valorisations fermentaires, et plus largement de comprendre les déterminants des flux de remobilisation ainsi que leur implication dans le fonctionnement de la plante.

Différents auteurs ont montré que l'efficience d'utilisation des rayonnements et la vitesse de mise en place de l'indice foliaire dans une culture de *M. giganteus* augmentaient avec l'âge de la culture pendant la phase d'installation, notamment entre la 1ère et la 3ème année de culture (Cosentino *et al.*, 2007 ; Jørgensen *et al.*, 2003). Cependant, aucune étude à ce jour n'a déterminé le rôle des réserves azotées des rhizomes dans ces phénomènes. L'absence de

compréhension des déterminants des flux d'azote au sein de la plante, notamment les flux de remobilisation de l'azote, rend difficile la compréhension et la prévision des besoins en azote externe à apporter à la culture.

La modélisation fournit un outil puissant pour étudier la production de biomasse actuelle et potentielle d'une culture dans différentes conditions, et peut également être utilisée pour tester différentes hypothèses sur l'effet de l'altération de caractéristiques physiologiques et/ou phénotypiques ainsi que les effets du changement climatique. Les modèles sol-plante-atmosphère permettent également la simulation des impacts environnementaux liés à l'eau, l'azote et l'énergie (Brisson *et al.*, 1998) et peuvent donc être utilisés pour aider au choix des plantes les mieux adaptées au débouché bioénergie en prenant en compte les différents critères recherchés. Les modèles empiriques sont utiles pour aider à prédire le rendement d'une culture dans différents contextes climatiques, cependant, les modèles mécanistes sont plus informatifs quant aux processus impliqués dans la croissance de la plante. A ce jour, il n'y a pas de modèle mécaniste prenant en compte la dynamique de l'azote entre la partie aérienne de la plante et sa partie souterraine pour les plantes dédiées à la production de biomasse. Toutefois, différents modèles permettant de simuler la production de biomasse par une culture de *M. giganteus* ont été paramétrés et publiés. Le premier modèle publié est un modèle empirique simulant la production potentielle : MISCANMOD (Clifton-Brown *et al.*, 2000). Ce modèle n'intègre ni les effets des stress hydriques et/ou azotés, ni les réserves contenues dans les rhizomes. Ce modèle a ensuite été amélioré en intégrant de nouvelles connaissances sur la physiologie de la plante et en prenant en compte l'effet d'un stress hydrique sur la production de biomasse ainsi que l'effet de la température: MISCANFOR (Hastings *et al.*, 2009). MISCANFOR présente donc une avancée importante, mais il ne considère pas les fonctions sources et puits du rhizome et ne prend en compte le stress azoté

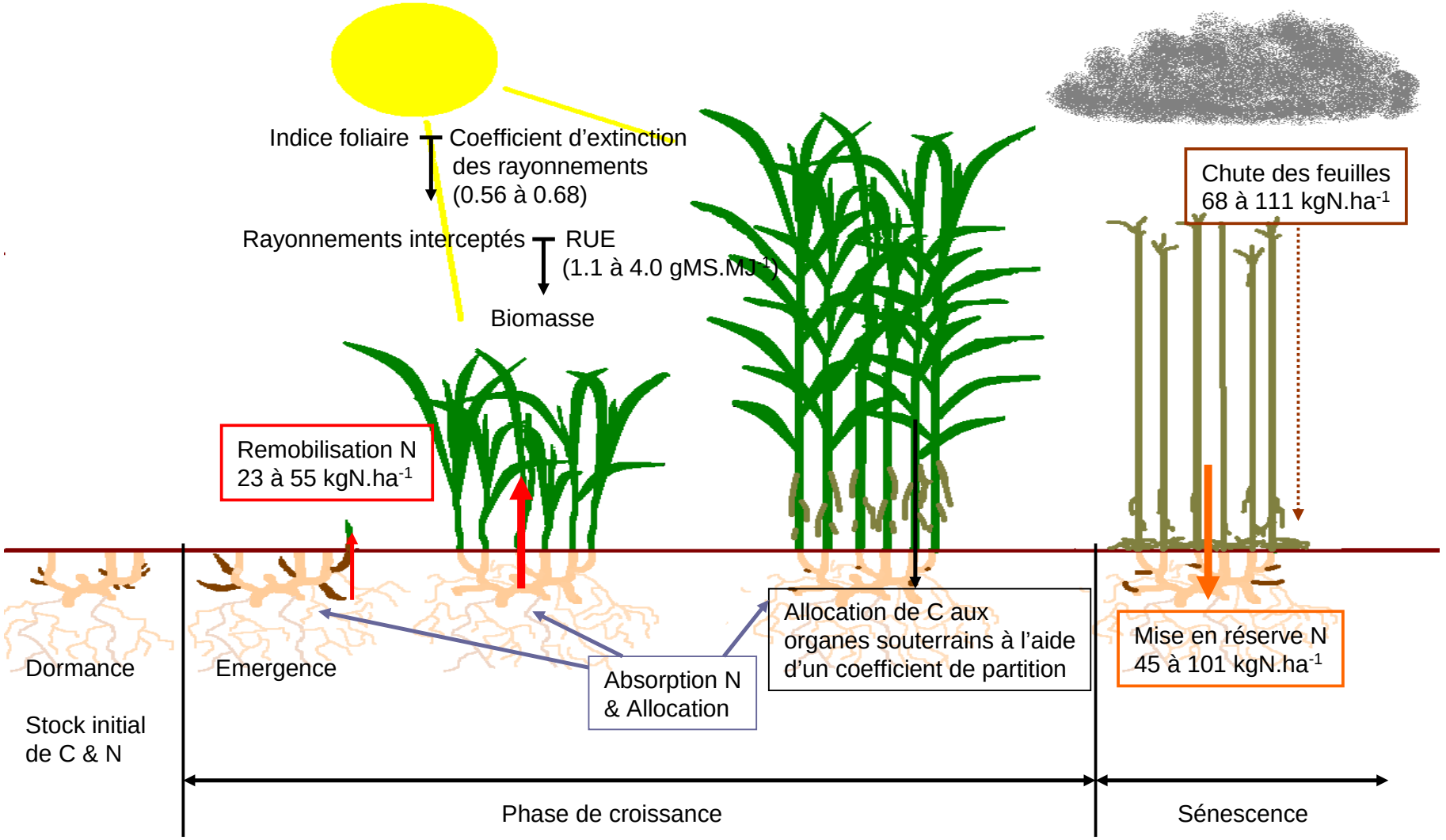


Figure 1: Schéma du fonctionnement d'une culture de *M. giganteus* à partir de la bibliographie de l'introduction.

que de manière très simplifiée, au travers d'un indicateur basé sur le niveau de fertilisation azotée. Un autre modèle a été récemment paramétré pour *M. giganteus* : WIMOVAC (Miguez *et al.*, 2009). Ce modèle fonctionne à partir de la simulation de la photosynthèse au niveau du peuplement végétal et les assimilats carbonés sont ensuite distribués dans les différents organes de la plante en fonction du stade de développement de la culture. Ce modèle prend en compte les rhizomes en intégrant les flux de carbone entre partie aérienne et souterraine à l'aide d'un coefficient de partition du carbone assimilé, mais il n'intègre pas les flux d'azote entre parties aériennes et souterraines.

L'ensemble des connaissances issues de la bibliographie nous permet de proposer un schéma conceptuel du fonctionnement de *M. giganteus* présentant les principaux processus ou paramètres connus et ceux à déterminer pour la modélisation de la plante (Figure 1). L'approche de la production de biomasse basée sur l'efficience d'interception et de conversion du rayonnement lumineux (Monteith *et al.*, 1977) semble *a priori* adaptée au miscanthus. Ainsi, pour modéliser la production de biomasse par la culture, il est nécessaire de simuler le développement de l'indice foliaire et de déterminer le coefficient d'extinction de la culture afin de pouvoir simuler l'interception des rayonnements par le peuplement végétal. Les rayonnements interceptés seront convertis en biomasse en fonction d'une efficience de conversion. Il faut ensuite déterminer la partition des assimilats carbonés dans la plante entre partie aérienne et partie souterraine, comme suggéré par Miguez *et al.* (2009), en prenant en compte la phase d'entrée en pleine production, qui peut prendre de 3 à 5 ans (Miguez *et al.*, 2008). Tous ces paramètres vont varier en fonction des stress biotiques ou abiotiques. L'effet des stress hydriques et azotés devra en particulier être renseigné. Finalement, la simulation des flux d'azote au sein de la plante est nécessaire pour prévoir la contribution des organes de réserve souterrains à la nutrition azotée de la plante et pouvoir simuler l'évolution des

réserves azotées sur le long terme. Cette modélisation de l'évolution des réserves est très importante car elle pourrait influencer directement la production de biomasse par la culture comme discuté précédemment, mais surtout avoir une influence très importante sur le bilan d'azote à long terme et donc sur les impacts environnementaux potentiels.

Nous avons donc orienté la thèse vers l'analyse et la quantification du rôle des organes de réserve dans la nutrition azotée de la plante et dans la gestion à long terme de l'azote par la culture, en vue d'une modélisation de ces processus.

Objectifs

&

Plan de la thèse

Les objectifs de la thèse sont donc les suivants :

1) Quantifier les impacts de la date de coupe et de la fertilisation azotée sur le rendement d'une culture de *M. giganteus* déjà installée (en 3ème et 4ème années de production) ainsi que sur les paramètres écophysiologiques (développement de l'indice foliaire, efficience d'utilisation des rayonnements, stock d'azote et de carbone dans les rhizomes).

2) Quantifier les flux de remobilisation au printemps (des rhizomes vers la partie aérienne) et de mise en réserve à l'automne (de la partie aérienne vers les rhizomes) de l'azote, au cours d'un cycle de culture et étudier les déterminants de ces flux.

L'enjeu de la thèse étant de proposer les éléments d'un modèle déterministe fonctionnel permettant de modéliser la production de biomasse par une culture de *M. giganteus* ainsi que l'évolution des réserves azotées des organes souterrains, en prenant en compte sur plusieurs années l'effet de la nutrition azotée de la plante (effet cumulatif), notamment sur les mises en réserve dans le rhizome. Le cahier des charges de ce modèle est présenté en annexe. Les objectifs étant de déterminer les bases écophysiologiques d'un module plante qui prenne en compte les réserves azotées.

Ces objectifs ont été développés en quatre chapitres :

1. Le 1er chapitre, présenté sous forme d'article (Soumis à « Field Crops Research », accepté avec révisions), vise à répondre à deux questions scientifiques importantes pour la compréhension et la modélisation d'une culture de *M. giganteus* :

   - Quels sont les déterminants de l'accumulation d'azote dans la plante ?

- Quels sont les déterminants des flux d'azote dans une culture de *M. giganteus* ?

Pour répondre à ces questions, un suivi dynamique de la production biomasse aérienne et souterraine, ainsi que de la composition de cette biomasse en carbone et en azote, a été effectué, en fonction de la date de coupe et du niveau de fertilisation azotée.

Hypothèses de travail :

- Les réserves en azote des organes souterrains (rhizome et racines) couvrent une grande partie des besoins en azote du peuplement.
- Une coupe précoce empêche tout ou partie de la mise en réserve de l'azote depuis la partie aérienne de la plante vers les organes souterrains (rhizome et racines). Il en résulterait une interaction entre la date de coupe et la fertilisation azotée sur la production de biomasse et l'accumulation d'azote par la plante l'année suivante.

2. Le 2ème chapitre, présenté sous forme d'article (Soumis à « Biomass and Bioenergy »), aborde la question scientifique suivante :

   - Quel est l'effet du niveau de nutrition azotée de *M. giganteus* sur le développement de l'indice foliaire et l'efficience d'utilisation des rayonnements et quelle est l'implication des réserves azotées des organes souterrains ?

Pour répondre à cette question, nous avons régulièrement mesuré l'indice foliaire au cours du cycle de croissance et effectué en parallèle un bilan radiatif de la culture afin de déterminer le coefficient d'extinction des rayonnements ainsi que l'efficience d'utilisation des rayonnements par une culture de *M. giganteus* en fonction de la date de coupe et du niveau de fertilisation azotée.

Hypothèses de travail :

- Le niveau de nutrition azotée permet d'expliquer les variations de production de biomasse observées
- Le niveau des réserves azotées du rhizome en fin d'hiver influence le développement de l'indice foliaire et/ou l'efficience d'utilisation des rayonnements par *M. giganteus*.

3. Le 3ème chapitre, présenté sous forme d'article (à soumettre), traite des questions scientifiques suivantes :

   - Quelles sont l'origine et la partition de l'azote dans la culture ?

   - Quel est le devenir de l'azote apporté par la fertilisation à une culture de *M. giganteus* ?

Pour répondre à ces questions, nous avons apporté de l'azote marqué (^{15}N) en 4ème année de culture dans les traitements fertilisés. Nous avons pu déterminer l'allocation de l'azote dans la plante entière et calculer des flux bruts d'azote dans la plante au cours d'un cycle de culture. Cette expérience a permis une meilleure compréhension des flux d'azote dans la plante.

4. Le Chapitre 4 permet une discussion des résultats et des éléments de modélisation du fonctionnement pluriannuel d'une culture de *Miscanthus* x *giganteus*.

Chapitre 1

Biomass production, nitrogen accumulation and remobilisation by *Miscanthus* x *giganteus* as influenced by nitrogen stocks in belowground organs

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Abstract

The nitrogen (N) requirement of dedicated crops for bioenergy production is a particularly significant issue, since N fertilisers are energy-intensive to make and have environmental impacts on the local level (NO_3 leaching) and global level (N_2O gas emissions). Nitrogen nutrition of *Miscanthus* x *giganteus* aboveground organs is assumed to be dependent on N stocks in belowground organs, but the precise quantities involved are unknown. A kinetic study was carried out on the effect of harvest date (early harvest in October or late harvest in February) and nitrogen fertilisation (0 or 120 $kgN.ha^{-1}$) on aboveground and belowground biomass production and N accumulation in established crops. Apparent N fluxes within the crop and their variability were also studied.

Aboveground biomass varied between 24 and 28 $tDM.ha^{-1}$ in early harvest treatments, and between 19 and 21 $tDM.ha^{-1}$ in late harvest treatments. Nitrogen fertilisation had no effect on crop yield in late harvest treatments, but enhanced crop yield in early harvest treatments due to lower belowground biomass nitrogen content. Spring remobilisation, *i.e.* nitrogen flux from belowground to aboveground biomass, varied between 36 and 175 $kgN.ha^{-1}$, due to the variability of initial belowground nitrogen stocks in the different treatments. Autumn remobilisation, *i.e.* nitrogen flux from aboveground to belowground organs, varied between 107 and 145 $kgN.ha^{-1}$ in late harvest treatments, and between 39 and 93 $kgN.ha^{-1}$ in early harvest treatments. Autumn remobilisation for a given harvest date was linked to aboveground nitrogen accumulation in the different treatments. Nitrogen accumulation in aboveground biomass was shown to be dependent firstly on initial belowground biomass nitrogen stocks and secondly on nitrogen uptake by the whole crop.

The study demonstrated the key role of belowground nitrogen stocks on aboveground biomass nitrogen requirements. Early harvest depletes belowground nitrogen stocks and thus increases the need for nitrogen fertiliser.

Keywords

Harvest date; apparent nitrogen fluxes; rhizome; perennial reserves; nitrogen fertilisation; biomass crop.

1. Introduction

The use of dedicated crops for production of biofuels to replace fossil fuels is one way to reduce anthropogenic greenhouse gas emissions (Smith *et al.*, 2000). *Miscanthus* x *giganteus* is a perennial rhizomatous grass employing the C4 photosynthetic pathway, which originates from Asia and was introduced into Europe in the 1930s. It has been described as having high potential biomass production with a low nitrogen requirement (Lewandowski *et al.*, 2000). These traits are likely to lead to significant energy production per hectare and high reductions in greenhouse gas emissions when used for fossil fuel substitution (Clifton-Brown *et al.*, 2007; Heaton *et al.*, 2008). The nitrogen (N) requirement is a particularly significant issue, because N fertilisers are energy-intensive to manufacture and so greatly affect the energetic balance of crops (Boehmel *et al.*, 2006). Moreover, losses following N fertilisation have environmental impacts on the local level (*e.g.* NO_3 leaching) and the global level (*e.g.* N_2O gaseous emissions). Biomass production by *M. giganteus* has been described as being dependent on soil water availability, air temperature and precipitation (Richter *et al.*, 2008), but there is no consensus yet in terms of this crop's nitrogen fertilisation requirement. Indeed, many authors suggest that N fertilisation has no effect on biomass production (Christian *et al.*, 2008; Clifton-Brown *et al.*, 2007; Danalatos *et al.*, 2007; Himken *et al.*, 1997) whereas others report that nitrogen fertilisation is needed to achieve maximum biomass production (Boehmel *et al.*, 2006; Cosentino *et al.*, 2007; Ercoli *et al.*, 1999). However, a consensus view is that the nitrogen requirement of *M. giganteus* to achieve maximum biomass yields is low compared with that of other crops (Lewandowski and Schmidt, 2006). This is mainly due to N cycling within the crop. In spring, part of the rhizome nitrogen stocks are remobilised from belowground to aboveground organs (hereafter referred to as spring remobilisation). Part of the nitrogen accumulated in aboveground parts is subsequently remobilised from aboveground to belowground organs (hereafter referred to as autumn remobilisation) during

autumn and winter (Beale and Long, 1997; Christian *et al.*, 2006; Himken *et al.*, 1997). However, the exact crop requirements in terms of N fertilisation have not been defined. Few studies have taken into account the contribution of the rhizome in the nitrogen nutrition of the crop, and the factors that affect spring and autumn remobilisation are not known. In fact, the amounts of remobilised nitrogen in spring and autumn reported by Beale and Long (1997) and Himken *et al.* (1997) differ, possibly owing to differences in belowground biomass nitrogen stocks, aboveground biomass nitrogen accumulation and climate conditions.

M. giganteus is currently used in combustion to produce heat and electricity, and is thus harvested in late winter to benefit from improved quality with regard to combustion processes, i.e. low mineral and moisture content (Lewandowski *et al.*, 2003). The development of an industrial process for converting cellulose to ethanol is likely to make early harvest of green material interesting, since the quality criteria for this type of conversion relate to lignocellulose content and recalcitrance (Karp and Shield, 2008). In a recent study, Le Ngoc Huyen *et al.* (2010) showed that saccharification yields of early harvested biomass were higher than those of late harvested plants. However, early harvest could increase the crop nitrogen requirement due to preventing or limiting leaf losses and autumn remobilisation, which in turn could prevent or limit nitrogen recycling in the soil-crop system.

The aims of this study were to determine: i) the impact of harvest date and nitrogen fertilisation on aboveground and belowground biomass production, ii) the impact of harvest date and nitrogen fertilisation on aboveground and belowground nitrogen accumulation, and iii) the contribution and determinants of nitrogen cycling on nitrogen accumulation in *M. giganteus*.

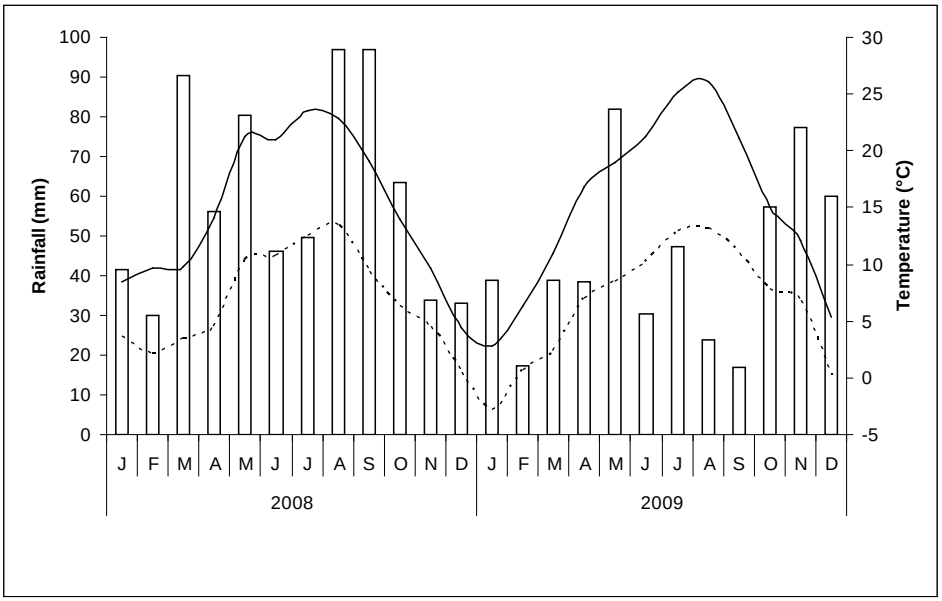


Figure 1: Monthly rainfall and temperature during the two years of *Miscanthus* x *giganteus* growth studied (2008 and 2009). Bars represent mean rainfall (mm), the broken line mean minimum temperature (°C) and the continuous line mean maximum temperature (°C) per month.

2. Materials and methods

2.1 Experimental site and trial setup

The experimental site is located in the Picardie region of Northern France (49°52'N, 3°00'E). The soil is a deep silt loam (Ortic luvisol) and is characterised by pH 7.6, 19% clay, 74% silt and 5% sand. The climate is oceanic, with mean rainfall of 625 mm per year and mean temperature of 10.7°C for the past 10 years. *Miscanthus* x *giganteus* was planted in May 2006 at a density of 15,625 plants ha^{-1} in a randomised block design. The previous crop was wheat, harvested in July 2005. After planting in 2006, two applications of herbicide were necessary to control weeds but no fertiliser was applied. The density after the first season of growth was 14,941 plants ha^{-1}. During the second year (2007), four different treatments with three replicates were established. Treatments varied in terms of nitrogen (N) fertiliser rate: 0 $kgN.ha^{-1}$ (N0) or 120 $kgN.ha^{-1}$ (N1), and harvest date: early harvest (E) or late harvest (L). The whole plots were harvested in October for early harvest and in February for late harvest. Plot size was 360 m^2 (12 m x 30 m), with 540 plants per plot. Each year, from 2007, nitrogen was applied as ammonium nitrate in late April. The soil mineral nitrogen (SMN) content was determined in March, before N fertilisation. In each plot, six soil cores were divided into five layers of 30 cm thickness. The six soil cores for each layer were pooled before N analysis. The temperature measured during the two years of growth was comparable to the 10-year average. The weather was drier in 2009 than in 2008. It was wetter than average in 2008 and drier in 2009 (Figure 1).

2.2 Biomass sampling

In each of the four treatments, aboveground biomass production was estimated on six occasions in 2008 (third year of growth) and 2009 (fourth year of growth) and belowground biomass production on five and six occasions in the third and fourth years of growth,

respectively. Six adjacent plants were harvested to measure the aboveground biomass on each occasion. The number of stems per plant was determined, and then a subsample was used for estimation of the moisture content. The stems (S), green leaves (GL) and dead leaves (DL) were separated from a second subsample, in order to estimate the proportion of each organ. The first and second subsamples were dried for four days at 65°C and then weighed again in order to determine dry matter weight. In order to take better account of canopy variability, the number of stems per plant was counted in an undisturbed area (hereafter referred to as area A) of 25 m² (40 plants) in all blocks, to determine the number of stems per hectare (NS). A nylon net (mesh size 1 cm x 1 cm) was placed on the soil surface before leaf abscission in order to collect abscised leaves during senescence from six plants per block (corresponding to a 3.84 m² area) in late harvest treatments. Abscised leaves that had fallen to the ground were collected regularly (every two weeks) and analysed to quantify the nitrogen lost during winter by this process. The leaves were dried for four days at 65°C, and then nitrogen concentration was determined (section 2.3). The aboveground biomass at each harvest was calculated as:

$$W_A = [(dm_A) / ns] * NS \quad (1)$$

where W_A is the aboveground biomass production ($tDM.ha^{-1}$), dm_A the aboveground dry matter of the six plants (kg), ns the number of stems of the six plants and NS the number of stems per hectare determined in area A.

The dry weight per hectare of stems (W_S), green leaves (W_{GL}) and dead leaves (W_{DL}) was then determined by multiplying the aboveground biomass production ($tDM.ha^{-1}$) by the proportion of each respective organ (%).

According to Midorikawa *et al.* (1975), there is a linear relationship between aboveground biomass of a *Miscanthus sinensis* plant and its rhizome biomass. We also observed a linear relationship between aboveground and belowground biomass, but this relationship varied as a function of sampling date (data not shown). Therefore, in order to determine the belowground

biomass, we extracted the rhizome of one plant, the closest to the median among the six harvested plants. The median plant was determined for each block of each treatment on a number of stems per plant basis, after counting the stems of each plant in A. On each sampling occasion except May 2008, the rhizome and associated roots were extracted at a depth of 25 cm. After extraction, belowground biomass was washed and divided into rhizome (Rh) and roots (Ro). All organs were dried for four days at 65°C until constant weight and then weighed in order to determine their dry matter weight. Belowground biomass was calculated as:

$$W_B = (dm_{Rh} + dm_{Ro}) * NP \quad (2)$$

where W_B is the belowground biomass ($tDM.ha^{-1}$), dm_{Rh} the rhizome biomass ($tDM.ha^{-1}$), dm_{Ro} the root biomass ($tDM.ha^{-1}$) and NP the number of plants per hectare determined in area A.

Unfortunately, the median plant had not been determined before harvest in October 2007 and belowground biomass in February 2008 in early harvest treatments was not sampled according to this protocol, so these data were removed from the analysis to avoid random variability.

The cumulated degree-days (CDD) during each year of growth were calculated on a 6°C basis from emergence, as suggested by Clifton-Brown and Jones (1997). Emergence was determined as the date on which 50% of plants had sprouted.

2.3 Nitrogen analysis

The total nitrogen (N) concentration in plant organs was determined using the Dumas method.

2.4 Calculation of nitrogen content

i) The nitrogen content of plant organs, NC ($kgN.ha^{-1}$), was calculated as the product of nitrogen concentration ($g.kg^{-1}$) and biomass weight ($tDM.ha^{-1}$):

$$NC = W * N \quad (3)$$

where W is the dry matter weight of the plant organ and N its nitrogen concentration.

ii) The nitrogen content ($kgN.ha^{-1}$) of aboveground biomass (NC_A) was calculated as the sum of the individual values of NC for stems, green leaves and dead leaves and that of belowground (NC_B) biomass as the sum of the individual values of NC for rhizome and roots.

iii) Sigmoidal curves were used to fit the data of aboveground biomass nitrogen accumulation as a function of the cumulated degree-days:

$$NC_A = NC_Amax / [1 + \exp(-(X - X0) / b] \quad (4)$$

where NC_Amax is the asymptote and corresponds to the maximum aboveground biomass nitrogen accumulation ($kgN.ha^{-1}$), X is degree-days (°C), X0 the number of degree-days (°C) necessary to reach the half of NC_Amax and b the number of degree-days (°C) required to get between 5% and 95% of the NC_Amax.

This allowed us to calculate the maximum aboveground biomass nitrogen accumulation rate as:

$$NAR\ (kgN.ha^{-1}.°Cd^{-1}) = NC_Amax /b * 1/e \quad (5)$$

with LN(e) = 1

2.5 Calculation of apparent nitrogen fluxes

The apparent nitrogen fluxes within the plant were calculated from source to sink organs. Spring remobilisation, defined as the N flux from belowground to aboveground biomass during spring re-growth, was calculated as the difference between the nitrogen content in belowground biomass (source) in February and the minimum nitrogen content in

belowground biomass (min NC_B), according to Beale and Long (1997) and Himken et al. (1997):

$$\Delta N_{B/A} = NC_B \text{ (February)} - \min NC_B \quad (6)$$

where $\Delta N_{B/A}$ is the amount of nitrogen mobilised from belowground biomass ($kgN.ha^{-1}$) to aboveground biomass and NC_B the nitrogen content in belowground biomass ($kgN.ha^{-1}$).

Autumn remobilisation, defined as the N flux from aboveground to belowground biomass during senescence, was calculated as the change in aboveground biomass nitrogen content (source) between the maximum aboveground biomass nitrogen content and harvest. Beale and Long (1997) calculated autumn remobilisation as the difference in rhizome (sink) nitrogen content between the end of spring remobilisation and harvest in the following February, thus neglecting nitrogen uptake from soil after the end of spring remobilisation. Himken *et al.* (1997) calculated autumn remobilisation as the difference in rhizome (sink) nitrogen content between September (at maximum aboveground biomass nitrogen content) and March (harvest), since Greef (1996) had shown that nitrogen uptake by the crop is negligible at that time of year. In our experimental conditions, the maximum aboveground biomass nitrogen content was reached earlier in the season. To avoid possible nitrogen uptake from the soil and storage in belowground biomass during this period, we calculated autumn remobilisation from the decrease in aboveground nitrogen content (source). Nitrogen remobilisation was calculated as:

$$\Delta N_{A/B} = (NC_A max - NC_{Ah}) - NC_{AL} \quad (7)$$

where $\Delta N_{A/B}$ is the amount of nitrogen remobilised from aboveground biomass ($kgN.ha^{-1}$) to belowground biomass, $NC_A max$ the maximum nitrogen content in aboveground biomass ($kg.ha^{-1}$), NC_{Ah} the nitrogen content in aboveground biomass at harvest ($kg.ha^{-1}$) and NC_{AL} the nitrogen losses due to abscised leaves ($kg.ha^{-1}$).

The apparent nitrogen uptake by the whole crop (NT_{abs}) was calculated as the difference between the maximum nitrogen content in the whole crop (aboveground and belowground biomass) and the nitrogen content in the belowground biomass in February, as follows:

$$NT_{abs} = NC_T \text{ max} - NC_B \text{ (February)} \quad (8)$$

where NC_T max is the maximum nitrogen content in the whole crop ($kgN.ha^{-1}$) and NC_B the nitrogen content in belowground biomass ($kgN.ha^{-1}$) in February.

The apparent nitrogen uptake by aboveground biomass of the crop (NA_{abs}) was calculated as the difference between the maximum nitrogen content in the aboveground biomass and the spring remobilisation ($\Delta N_{B/A}$), as follows:

$$NA_{abs} = NC_A \text{ max} - \Delta N_{B/A} \quad (9)$$

where NC_A max is the maximum nitrogen content in the aboveground biomass ($kgN.ha^{-1}$) and $\Delta N_{B/A}$ the spring remobilisation from belowground to aboveground biomass ($kgN.ha^{-1}$).

2.6 Data analysis

ANOVA was used for statistical evaluations of year effects (corresponding to crop age), treatment effects (harvest date and nitrogen fertilisation) and their interactions. Differences were then tested by the Neuman-Keuls test at the 5% level. When ANOVA was not possible because of non variance homogeneity or non normality of data, a Kruskal-Wallis test at the 5% level was used for statistical evaluations of year and treatment effects.

In order to determine the variables influencing the aboveground biomass nitrogen accumulation, we used the simple correlation method to search for relationships between aboveground biomass nitrogen accumulation and nitrogen availability in the environment (SMN, available before crop regrowth and before N fertilisation; N fertilisation; and belowground biomass nitrogen stocks). The significance level for adding or retaining variables in the multiple regression model was $p<0.01$. Stepwise multiple linear regressions

were then performed to examine the relative importance of different variables in determining aboveground biomass nitrogen accumulation. Both B and ß coefficients of regression outputs were considered: B-coefficients, which dealt with raw values, were used to construct the prediction equation, while ß-coefficients, which were standardised according to the variable variances, were used to compare the ‘effects’ of variables within equations (Niknahad Gharmakher *et al*., 2009).

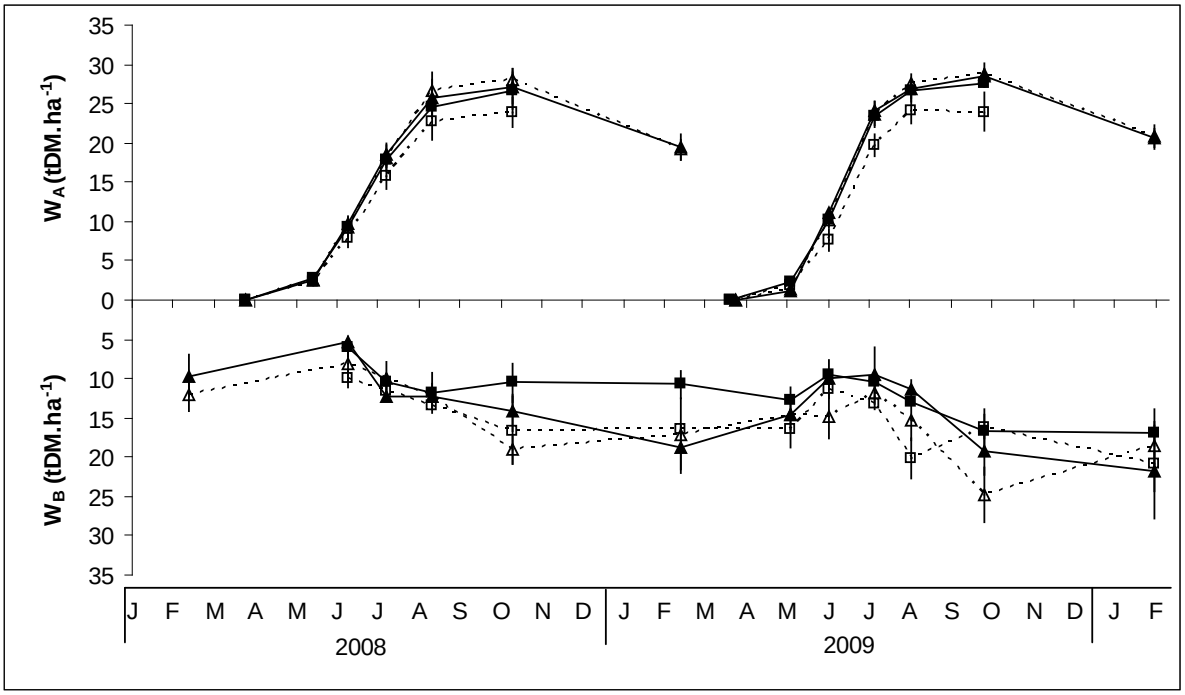


Figure 2: Effect of harvest date and fertilisation on aboveground and belowground biomass production of *Miscanthus* x *giganteus* in the third and fourth years of growth. Bars represent standard error. Non-fertilised treatments (N0) are represented by open symbols and broken lines and fertilised treatments (N1) by black symbols and continuous lines. Triangles correspond to late harvest (L) and squares to early harvest treatments (E).

3. Results

3.1 Biomass production of *Miscanthus x giganteus*: Effect of harvest date and nitrogen fertilisation

During the third year of growth (2008), emergence occurred on 1 April. An increase in aboveground biomass was observed until mid-October, corresponding to 1718 CDD (Figure 2). During the growing season (May to October), harvest date had a significant effect on aboveground biomass production ($p<0.05$), while fertilisation had no effect. Peak yield was 25 tDM.ha^{-1} in early harvest (E) treatments and 28 tDM.ha^{-1} in late harvest (L) treatments. During winter, aboveground biomass declined by 30% in L treatments, due to abscised leaves and stem senescence. In L treatments, final yield was 19 tDM.ha^{-1} and no effect of fertilisation was observed.

Harvest date and nitrogen fertilisation had no significant effect on belowground dry matter in the third year of growth. On average, belowground biomass before emergence was 11 tDM.ha^{-1} in late harvest treatments and declined significantly ($p<0.05$) by 37% until mid-June, corresponding to 537 CDD. Thereafter, belowground biomass increased significantly ($p<0.01$) up to 15 t DM.ha^{-1} in mid-October (Figure 2).

During the fourth year of growth (2009), emergence was affected by harvest date and occurred on 3 April (± 1 day) and 7 April (± 1 day) in E and L treatments, respectively. The delayed emergence in L treatments was attributed to the mulching effect of abscised leaves, which retarded the soil temperature increase. An increase in aboveground biomass was observed until mid-October, corresponding to 1871 and 1888 CDD in L and E treatments, respectively (Figure 2). During the growing season (May-October), there was a significant effect of harvest date ($p<0.01$), nitrogen fertilisation ($p<0.01$) and their interaction ($p<0.01$) on aboveground biomass production. Peak yield was 24 tDM.ha^{-1} in EN0 treatment, and 28 DM.ha^{-1} on average for L and EN1 treatments (Figure 2). During winter, aboveground

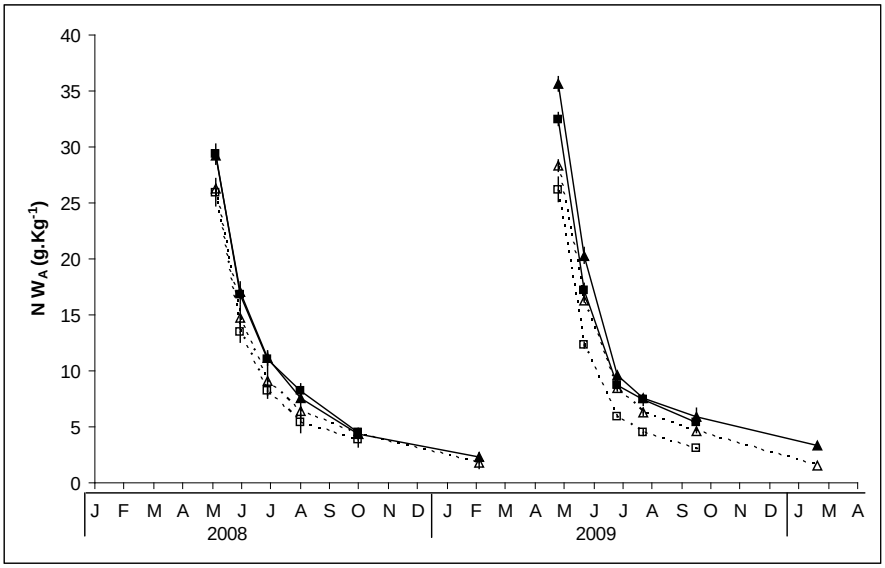


Figure 3a.

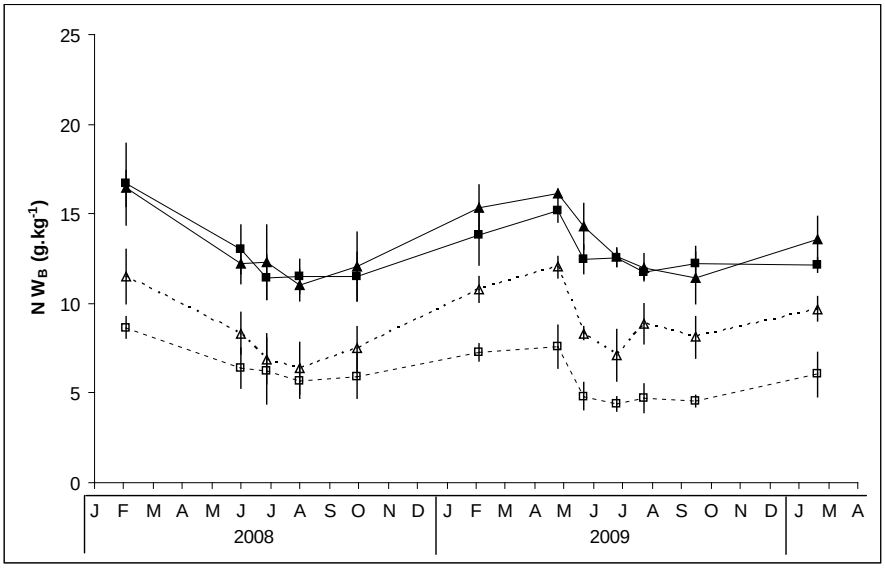


Figure 3b.

Figure 3: Nitrogen concentration (a) in aboveground biomass and (b) in belowground biomass during the two years of growth. Bars represent standard error. Non-fertilised treatments (N0) are represented by open symbols and broken lines and fertilised treatments (N1) by black symbols and continuous lines. Triangles correspond to late harvest (L) and squares to early harvest treatments (E).

biomass declined by 28% in L treatments, due to shed leaves and stem senescence. Final yield was 21 tDM.ha^{-1} and no effect of fertilisation was observed.

In the fourth year of growth, harvest date and nitrogen fertilisation again had no effect on belowground dry matter. On average, belowground biomass before emergence was 16 tDM.ha^{-1} and declined by 30% between emergence and mid-July, corresponding to 934 and 951 CDD in L and E treatments respectively. Thereafter, belowground biomass increased up to 19 tDM.ha^{-1} in mid-October (Figure 2).

3.2 Nitrogen concentration in aboveground and belowground biomass: Effect of harvest date and nitrogen fertilisation

During the two years of growth, the aboveground biomass nitrogen concentration declined during biomass accumulation due to the continuous increase in the stem fraction, which is poorer in nitrogen than the leaf fraction within the aerial biomass (data not shown), and then declined further as the canopy senesced (Figure 3a). In 2008, during the growing season (May-October), aboveground biomass nitrogen concentration was significantly higher in fertilised treatments ($p<0.01$) than in non-fertilised. In 2009, during the growing season (May-October), there was a significant effect of harvest date ($p<0.01$), nitrogen fertilisation ($p<0.01$) and an interaction between harvest date and nitrogen fertilisation ($p<0.01$) on aboveground biomass nitrogen concentration. Aboveground biomass nitrogen concentrations were lowest in the EN0 treatment and highest in the LN1 treatment. Moreover, during the two years of growth, aboveground biomass nitrogen concentration at harvest in L and E treatments was significantly higher in fertilised treatments than in non-fertilised ($p<0.05$).

During the two years of growth, belowground biomass nitrogen concentrations declined until mid-summer (Figure 3b). Thereafter, belowground biomass nitrogen concentration increased until harvest. In 2008, belowground biomass nitrogen concentration was significantly higher

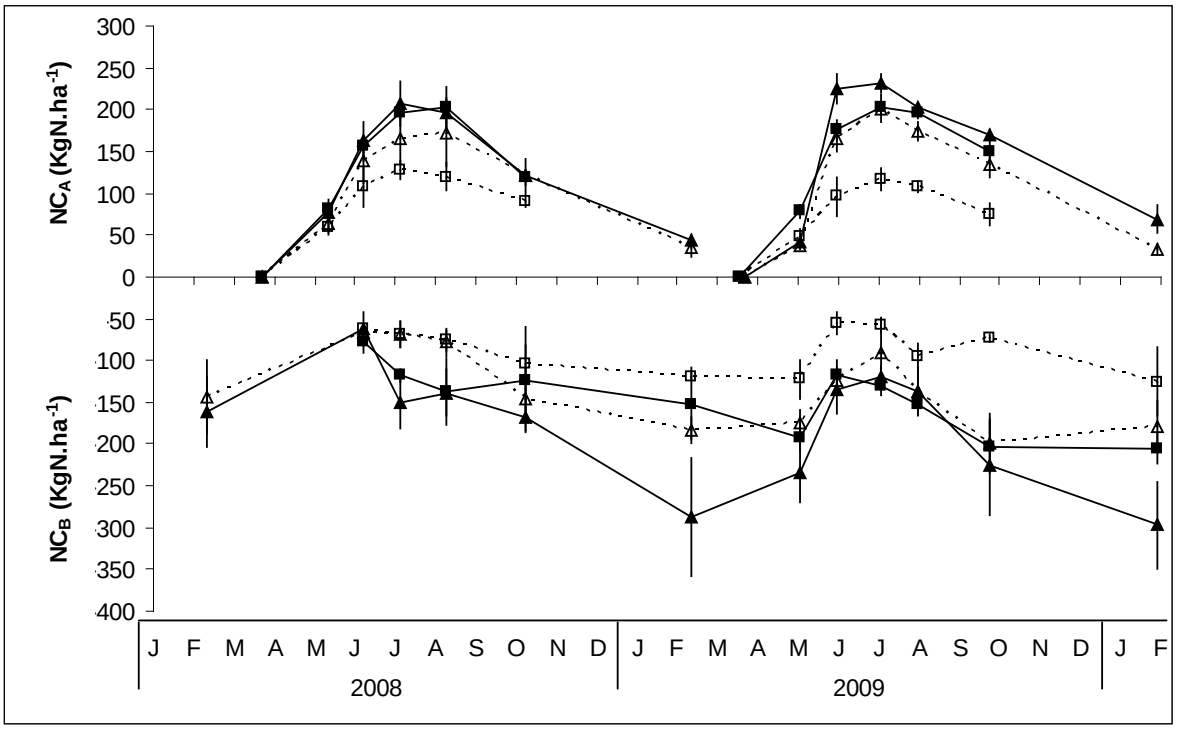


Figure 4: Effect of harvest date and fertilisation on aboveground and belowground nitrogen content of *Miscanthus* x *giganteus* in the third year and fourth years of growth. Bars represent standard error. Non-fertilised treatments (N0) are represented by open symbols and broken lines and fertilised treatments (N1) by black symbols and continuous lines. Triangles correspond to late harvest (L) and squares to early harvest treatments (E).

in fertilised treatments ($p<0.01$) than in non-fertilised. In 2009, there was a significant effect of harvest date ($p<0.01$), nitrogen fertilisation ($p<0.01$) and an interaction between harvest date and nitrogen fertilisation ($p<0.01$) on belowground biomass nitrogen concentration. Belowground biomass nitrogen concentrations were lowest in the EN0 treatment, intermediate in the LN0 treatment and highest in the LN1 and EN1 treatments.

3.3 Nitrogen accumulation and partitioning between aboveground and belowground plant parts: Effect of harvest date and nitrogen fertilisation

During the third year of growth, aboveground nitrogen content increased from emergence to July, corresponding to 835 CDD, and remained stable until the end of August, corresponding to 1268 CDD (Figure 4). The maximum aboveground biomass nitrogen content was significantly higher in fertilised treatments ($p<0.05$) than in non-fertilised. The maximum aboveground biomass nitrogen content was 150 and 205 $kgN.ha^{-1}$ in N0 and N1 treatments, respectively. This was followed by a significant decline in aboveground biomass nitrogen content until harvest in October or February for E and L treatments, respectively. Nitrogen removal at harvest was significantly higher ($p<0.01$) in E treatments (105 $kgN.ha^{-1}$) than in L treatments (40 $kgN.ha^{-1}$) and was not affected by nitrogen fertilisation.

During the third year of growth, no significant effect of nitrogen fertilisation was observed on the belowground nitrogen content of L treatments before regrowth. On average, belowground nitrogen content was 152 $kgN.ha^{-1}$ (Figure 4). A significant decline ($p<0.01$) in belowground nitrogen content, of 57% on average, was observed between emergence and mid-June, corresponding to 537 CDD. Belowground biomass nitrogen content then increased until harvest and varied between 120 and 287 $kgN.ha^{-1}$ in February 2009. In July and August, belowground nitrogen content was significantly higher ($p<0.05$) in fertilised treatments, suggesting that a part of the nitrogen taken up was stored in these organs.

During the fourth year of growth, aboveground nitrogen content increased from emergence to July, corresponding to 934 and 951 CDD in L and E treatments, respectively, and then decreased until harvest (Figure 4). Maximum nitrogen accumulation in aboveground biomass was affected both by nitrogen fertilisation ($p<0.01$) and harvest date ($p<0.01$) and their interactions ($p<0.05$). Maximum nitrogen accumulation in aboveground biomass was 116, 202, 202 and 232 kgN.ha^{-1} in the EN0, EN1, LN0 and LN1 treatments, respectively. Thereafter, there was a significant decline in aboveground biomass nitrogen content until harvest in October or February in E and L treatments, respectively. Nitrogen removal at harvest was significantly higher in E treatments than in L treatments ($p<0.01$) and, unlike in the third year of growth, it was significantly higher in fertilised treatments ($p<0.05$), regardless of harvest date. Nitrogen removal at harvest was 75, 151, 33 and 69 kgN.ha^{-1} in the EN0, EN1, LN0 and LN1 treatments, respectively.

In the fourth year of growth, belowground nitrogen content before emergence was significantly higher ($p<0.05$) in L treatments (235 kgN.ha^{-1}) than in E treatments (136 kgN.ha^{-1}) (Figure 4). Thereafter, belowground nitrogen content was affected by nitrogen fertilisation ($p<0.05$) and harvest date ($p<0.05$). A significant decline in belowground biomass nitrogen content, of 47% on average, was observed between emergence and mid-July, corresponding to 934 and 951 CDD in L and E treatments respectively. Belowground biomass nitrogen content then increased until harvest and was 125, 205, 180 and 297 kgN.ha^{-1} in the EN0, EN1, LN0 and LN1 treatments, respectively.

Nitrogen removal at harvest was two- to three-fold higher in early harvested plants than in late harvested. This led to a decrease in nitrogen content in aboveground and belowground biomass during the fourth year of growth in the EN0 treatment.

Year of growth		LN0	LN1	EN0	EN1
2008	$\Delta N_{B/A}$	84 ± 35	98 ± 61		
	% NC_A max	45% ± 12	44% ± 16		
2009	$\Delta N_{B/A}$	107 ± 24	175 ± 65	68 ± 10	36 ± 58
	% NC_A max	54% ± 9	71% ± 15	59% ± 8	22% ± 27

Table 1: Spring remobilisation ($\Delta N_{B/A}$ = kgN.ha^{-1}) from belowground to aboveground biomass calculated in two successive years (± standard error) and its relative contribution to aboveground biomass nitrogen accumulation (%). LN0 = late harvest without fertilisation, LN1 = late harvest with 120 kgN.ha^{-1}.year^{-1}, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 kgN.ha^{-1}.year^{-1}.

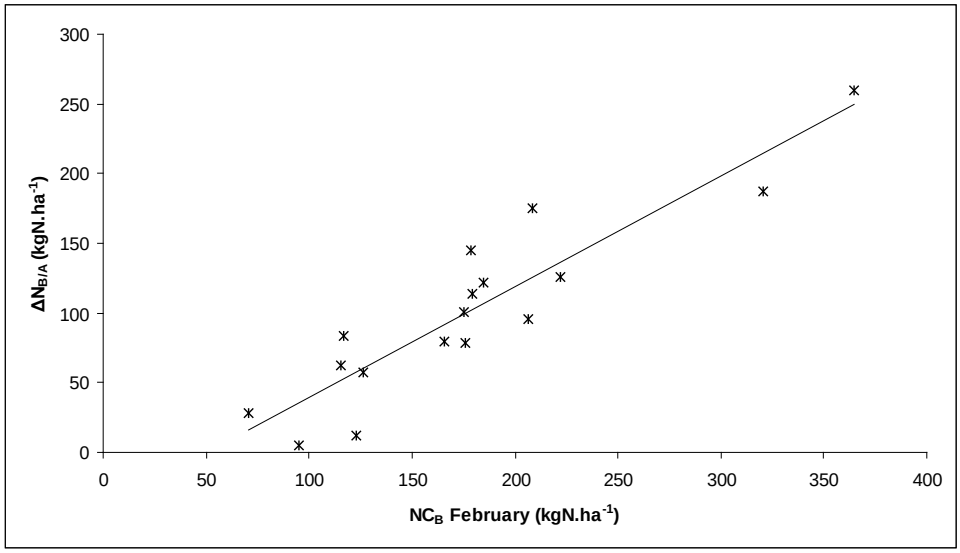


Figure 5: Relationship between spring remobilisation ($\Delta N_{B/A}$ = kgN.ha^{-1}) calculated with equation (6) and initial nitrogen stocks in belowground biomass (kg N ha^{-1}). Y = 0.79 * X – 39.34; R^2 = 0.92; p<0.01.

3.4 Nitrogen fluxes within the plant: Spring and autumn remobilisation

Spring remobilisation from belowground to aboveground biomass calculated with equation (6) averaged 91 kgN.ha^{-1} in the third year of growth in L treatments and 52 and 141 kgN.ha^{-1} in the fourth year of growth in E and L treatments, respectively (Table 1). In 2009, the difference in spring remobilisation between E and L treatments was significant ($p<0.05$). A strong linear relationship was found between remobilised nitrogen and initial nitrogen stocks in belowground biomass (Figure 5). A threshold of 50 kgN.ha^{-1} appeared to be unavailable for spring remobilisation. Therefore, 79% of the available nitrogen stocks in belowground biomass were remobilised during spring. The variability in spring remobilisation was thus mainly due to differences in initial nitrogen stocks in belowground biomass.

On average, 49% of the aboveground nitrogen content originated from spring remobilisation, (Table 1). This proportion varied widely between treatments and years of growth (from 22% to 71%), but no significant difference was observed due to the high variability in the belowground data. The other fraction of the aboveground nitrogen content, 51% on average, originated from external supply.

Autumn remobilisation, calculated with equation (7), varied between 39 and 145 kgN.ha^{-1} (Table 2). Autumn remobilisation was significantly lower in E treatments ($p<0.01$) due to harvest before total senescence of aboveground biomass and leaf abscission. Furthermore, autumn remobilisation was significantly higher in fertilised treatments than in non-fertilised ($p<0.05$). No effect of crop age was observed. On average, autumn remobilisation was 42, 72, 125 and 140 kgN.ha^{-1} in the EN0, EN1, LN0 and LN1 treatments, respectively. Abscised leaves represented 25-32 kgN.ha^{-1} in L treatments and were considered negligible in E treatments. For each harvest date, the amount of nitrogen remobilised during autumn was closely and linearly linked with maximum nitrogen content in aboveground biomass (Figure

		LN0	LN1	EN0	EN1
2008	NC_A max - NC_{Ah} (kgN.ha^{-1})	139 ± 29	165 ± 29	39 ± 15	93 ± 18
	Nitrogen lost by abscised leaves (kgN.ha^{-1})	32 ± 4	31 ± 3	0	0
	Net autumn remobilisation (kgN.ha^{-1})	107 ± 25	134 ± 30	39 ± 15	93 ± 18
2009	NC_A max - NC_{Ah} (kgN.ha^{-1})	168 ± 13	175 ± 7	45 ± 6	51 ± 13
	Nitrogen lost by abscised leaves (kgN.ha^{-1})	25 ± 2	30 ± 3	0	0
	Net autumn remobilisation (kgN.ha^{-1})	143 ± 12	145 ± 10	45 ± 6	51 ± 13

Table 2: Autumn remobilisation from aboveground to belowground biomass ($\Delta N_{A/B}$ = kgN.ha^{-1}) calculated in two successive years (± standard error). LN0 = late harvest without fertilisation, LN1 = late harvest with 120 kgN.ha^{-1}.year^{-1}, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 kgN.ha^{-1}.year^{-1}.

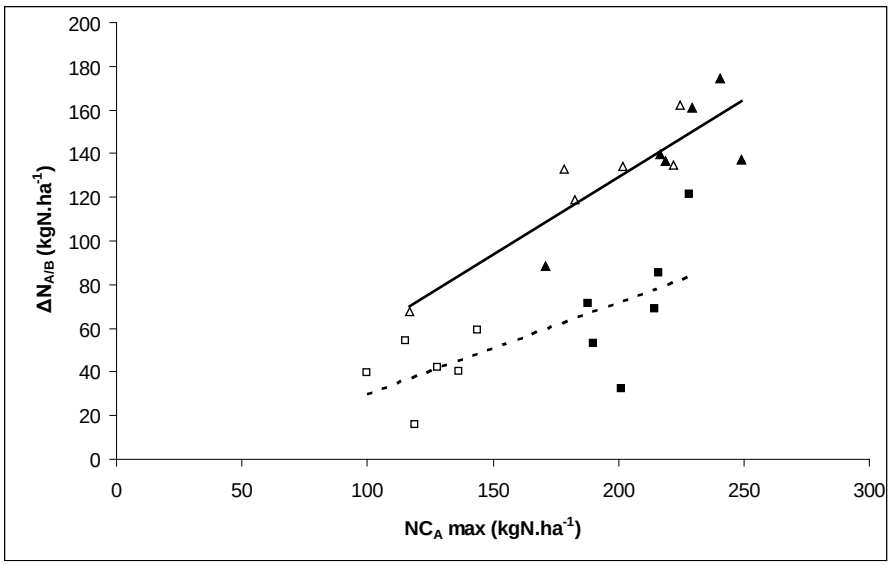


Figure 6: Relationship between autumn remobilisation ($\Delta N_{A/B}$ = kgN.ha^{-1}) calculated with equation (7) and maximum nitrogen accumulation in aboveground biomass (kgN.ha^{-1}). Non-fertilised treatments (N0) are represented by open symbols and broken lines and fertilised treatments (N1) by black symbols and continuous lines. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). In L treatments (triangles and continuous line), Y = 0.71 * X - 13.34; R^2 = 0.86; $p<0.05$. In E treatments (squares and broken line), Y = 0.42 * X – 12.06; R^2 = 0.69; $p<0.05$.

6). In L and E treatments, 19 and 29 kgN.ha^{-1} respectively was not available for autumn remobilisation. 71% of the nitrogen accumulated in aboveground biomass in L treatments, and only 42% in E treatments, was remobilised during autumn remobilisation. In our experimental conditions, N remobilisation made a much higher contribution than abscised leaves to nitrogen recycling between October and February.

3.5 Soil-crop relationship

The maximum nitrogen accumulation in the aboveground biomass was highly correlated (R^2 = 0.86) to the belowground nitrogen content in February (β = 0.83) and the nitrogen uptake by the whole crop (β = 0.57). The predictive equation found with the multiple regression was:

$$NC_A \text{ max} = 37.2 + 0.52\ (NC_{Bi}) + 0.42\ (NT_{abs})$$

where NC_A max is the maximum aboveground nitrogen content (kgN.ha^{-1}), NC_{Bi} the belowground nitrogen stocks in February (kgN.ha^{-1}) and NT_{abs} the nitrogen uptake by the whole crop (kgN.ha^{-1}).

The high dependency of the maximum nitrogen accumulation in the aboveground biomass on the belowground biomass nitrogen content in February was due to the strong linear relationship between the aboveground biomass nitrogen accumulation rate and the belowground biomass nitrogen stocks before regrowth (Figure 7). Moreover, a significant relationship (R^2 = 0.90) was found between the aboveground biomass nitrogen uptake and the whole crop nitrogen uptake (Figure 8). This relationship indicates that a stable fraction, around 60% of the total nitrogen uptake, is used for the aerial organs, while the other part is trapped by the belowground biomass. This phenomenon led to a lower dependency of the maximum nitrogen accumulation in the aboveground biomass on whole crop nitrogen uptake. Hence, it appeared that the whole crop nitrogen uptake had a direct effect on the maximum

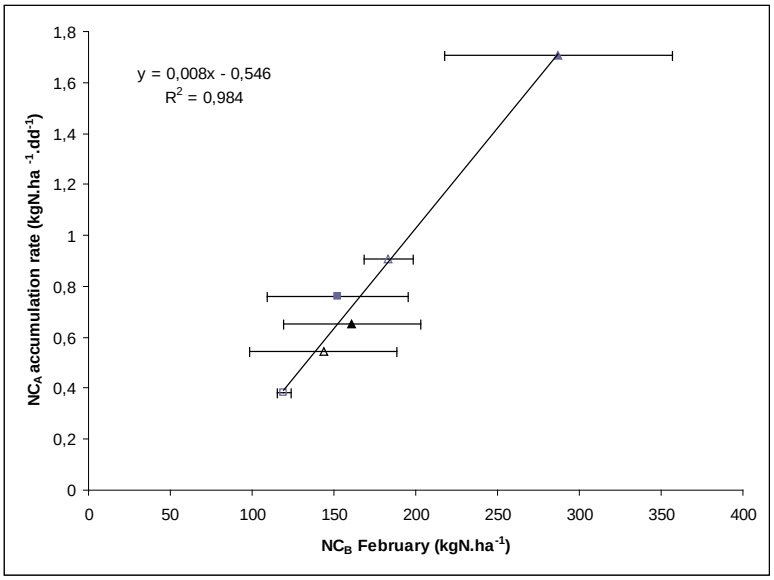


Figure 7: Relationship between the aboveground biomass nitrogen accumulation rate ($kgN.ha^{-1}.dd^{-1}$) of the crop and the belowground biomass nitrogen stocks before regrowth ($kgN.ha^{-1}$). Bars represent standard error. Non-fertilised treatments (N0) are represented by open symbols and fertilised treatments (N1) by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). Black symbols correspond to the third year of growth (2008) and grey symbols to the fourth year of growth (2009).

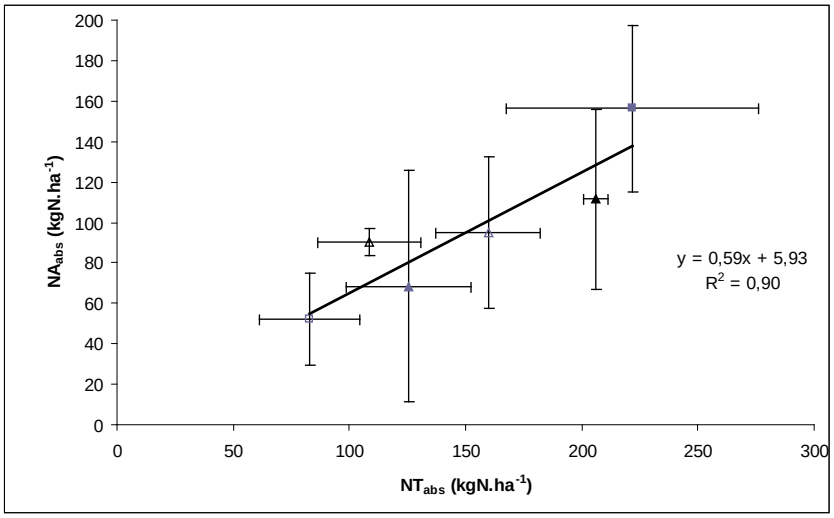


Figure 8: Relationship between the nitrogen taken up recovered in aboveground plant parts (NA_{abs} = $kgN.ha^{-1}$) and nitrogen uptake by the whole crop (NT_{abs} = $kgN.ha^{-1}$). Bars represent standard errors. Non-fertilised treatments (N0) are represented by open symbols and fertilised treatments (N1) by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). Black symbols correspond to the third year of growth (2008) and grey symbols to the fourth year of growth (2009).

nitrogen accumulation in the aboveground biomass *via* nitrogen allocation to the aboveground biomass, and an indirect effect *via* nitrogen storage in the belowground biomass.

In 2008, the whole crop nitrogen uptake in L treatments was significantly higher ($p<0.05$) in fertilised treatments (206 $kgN.ha^{-1}$) than in non-fertilised (124 $kgN.ha^{-1}$). In 2009, the nitrogen uptake by the whole crop was lowest in the EN0 treatment (80 $kgN.ha^{-1}$), intermediate in the LN0 and LN1 treatments (143 $kgN.ha^{-1}$ on average) and highest in the EN1 treatment (222 $kgN.ha^{-1}$) ($p<0.05$).

The SMN content in the 0-150 cm layer at the end of the winter, was significantly higher ($p<0.01$) in fertilised treatments than in non-fertilised (Table 3). Soil mineral nitrogen content decreased significantly ($p<0.01$), by 24 $kg.ha^{-1}$ on average, between the third and fourth year of growth, suggesting possible N absorption by the crop.

	Soil mineral nitrogen March 2008 (0-150 cm) ($kgN.ha^{-1}$)	Soil mineral nitrogen March 2009 (0-150 cm) ($kgN.ha^{-1}$)
LN0	51 ± 15	32 ± 3
LN1	74 ± 18	47 ± 7
EN0	47 ± 10	29 ± 6
EN1	70 ± 13	40 ± 4

Table 3: Soil mineral nitrogen in the 0-150 cm layer (± standard error) at the end of winter in the third (2008) and fourth (2009) years of *Miscanthus* x *giganteus* growth. LN0 = late harvest without fertilisation, LN1 = late harvest with 120 $kgN.ha^{-1}.year^{-1}$, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 $kgN.ha^{-1}.year^{-1}$.

4. Discussion

4.1 Nitrogen accumulation and partitioning in *Miscanthus* x *giganteus*: Effect of nitrogen stocks and fertilisation

Maximum nitrogen content in aboveground biomass of L treatments in the present study was 208 kgN.ha^{-1} in the LN1 treatment and 202 kgN.ha^{-1} in the LN0 treatment in the third and fourth years of growth respectively. These values were slightly lower than the 253 kgN.ha^{-1} reported by Beale and Long (1997) and the 229 kgN.ha^{-1} reported by Himken *et al.* (1997) for the third and fourth years of growth, respectively. This is probably due to differences in nitrogen availability in the soil. Moreover, in contrast to Himken *et al.* (1997), we observed a significant effect of nitrogen fertilisation on aboveground and belowground biomass nitrogen content (section 3.2). This difference could be due to very high soil mineral nitrogen content (185 kg N.ha^{-1}) at the beginning of their experiments, compared with 29-47 kgN.ha^{-1} at the beginning of the fourth year of growth in our study (Table 3), if we assume the soil N mineralisation did not differ greatly. Furthermore, in the fourth year of growth, nitrogen accumulation was lowest in early harvest treatments without fertilisation due to lower nitrogen availability in belowground biomass and lower nitrogen uptake. Indeed, the linear relationship between the aboveground biomass nitrogen accumulation rate and the belowground biomass nitrogen stocks before regrowth (Figure 7) underlines the importance of belowground biomass nitrogen stocks for nitrogen accumulation in aboveground biomass during the growing season. The higher the belowground biomass nitrogen stocks, the higher the aboveground biomass nitrogen accumulation rate. This is in agreement with findings reported by Ourry *et al.* (1994), who showed that in *Medicago sativa* L., the aboveground biomass nitrogen accumulation was dependent on the belowground biomass nitrogen stocks before regrowth. This result is also consistent with Thornton and Millard (1997), who showed in their study that nitrogen accumulation rate in *F. rubra* and *L. perenne* decreased with the

frequency of defoliation, due to lower nitrogen remobilisation during regrowth and so higher dependency on nitrogen supply from the soil.

The various experimental treatments led to different belowground biomass nitrogen stocks. Initial belowground biomass nitrogen stocks in our study were 161 kgN.ha^{-1} in the LN1 treatment and 183 kgN.ha^{-1} in the LN0 treatment in the third and fourth years of growth, respectively. This was higher than the 108 kgN.ha^{-1} reported by Beale and Long (1997) in the third year of *Miscanthus* x *giganteus* growth and comparable to the 187 kgN.ha^{-1} reported by Himken *et al.* (1997) in the fourth year of growth.

4.2 Soil-crop relationship

The nitrogen taken up by the whole crop from the soil had different origins which cannot be estimated separately without using ^{15}N tracing (Christian *et al.*, 2006; Constantin *et al.*, in press). This nitrogen originated from: i) SMN, which was low and tended to decrease with time, ii) atmospheric deposition, which is low in our experimental conditions (around 13 kgN.ha^{-1}.y^{-1}, J-M. Machet personal communication) iii) N from fertiliser applied to the crop, with an unavailable fraction due to N immobilization and gaseous N losses and iv) N from soil mineralisation, which can have reached roughly the half of previous values estimated at our experimental site during 13 months for a bare soil (163 and 188 kgN.ha^{-1} with wheat straw respectively incorporated or removed) (Garnier *et al.*, 2003). Several factors can affect the nitrogen mineralisation under *M. giganteus* cropping systems, such as soil structure, biomass residues returned to soil, or the mulch effect in late harvested treatments. Amougou *et al.* (2010) showed that *M. giganteus* residues in late harvested plants allowed an important net input of organic matter high in carbon and nitrogen to the soil but did not allow higher nitrogen mineralisation in the soil in the short term. Many studies have shown that organic

mulch improves soil moisture through reducing soil surface evaporation and so increases nitrogen mineralisation in soil (Tu *et al*., 2006; Martens, 2001; Pinamonti, 1998).

The whole crop nitrogen uptake in the LN1 treatment decreased in 2009 compared with 2008. That was probably due to the inhibition of nitrogen uptake during spring remobilisation. We found a negative correlation between N uptake and N spring remobilisation (r=-0.57), although this was not statistically significant ($p>0.05$). Some studies with ^{15}N-labelled fertiliser have shown that nitrogen uptake by the crop decreases with an increase in nitrogen stocks (Thornton and Millard, 1997; Louahlia *et al.*, 1999) due to inhibition of uptake by the plant during nitrogen remobilisation.

4.3 Spring remobilisation

Our results showed that a high proportion of the belowground nitrogen stocks was remobilised in spring. Furthermore, there was a linear relationship between nitrogen remobilisation in spring and belowground biomass nitrogen stocks before regrowth (section 3.3.1), indicating that spring remobilisation was proportional to nitrogen stocks. A non-available nitrogen fraction was observed, which could correspond to the minimum nitrogen content necessary for belowground biomass metabolism and structural nitrogen in an established stand of *M. giganteus*. Beale and Long (1997) reported that 25 $kgN.ha^{-1}$ were remobilised for initial belowground biomass nitrogen stocks of 108 $kgN.ha^{-1}$ and Himken *et al.* (1997) reported that 61 $kgN.ha^{-1}$ were remobilised for initial belowground biomass nitrogen stocks of 187 $kgN.ha^{-1}$. These data are close to our relationship, which seems sufficiently robust to estimate the contribution of the rhizome to nitrogen nutrition of the crop. This relationship has already been described during nitrogen remobilisation from taproots and lateral roots in *Medicago sativa* L. after cutting (Ourry *et al.*, 1994). Furthermore, nitrogen stocks in the form of vegetative storage proteins have been described as playing a key role in

regrowth of the plant after cutting and thus in aboveground biomass production (Ourry *et al.*, 1994).

Our results showed that 49% of nitrogen accumulated in aboveground biomass originated from spring remobilisation. This is far higher than the results from Beale and Long (1997) and Himken *et al.* (1997), who reported that nitrogen remobilisation represented 9 or 21% of nitrogen accumulated in aboveground biomass, respectively. These differences are due to lower nitrogen accumulation in aboveground biomass and higher amount of nitrogen coming from belowground biomass in our study. Moreover, the higher belowground biomass nitrogen stocks in the third year of growth compared with Beale and Long (1997) could also explain the difference observed between the studies.

4.4 Autumn remobilisation

The linear relationship found between nitrogen remobilisation in autumn and maximum nitrogen accumulation in aboveground biomass (section 3.3.1) indicates that autumn remobilisation is function of maximum nitrogen accumulation in aboveground biomass. In this study, nitrogen storage from aboveground to belowground biomass in late harvested plants represented 71% of the maximum nitrogen content in aboveground biomass. A non-available nitrogen fraction was observed, which could correspond to structural nitrogen. This was higher than values reported by Himken *et al.* (1997) and Beale and Long (1997), who found that 46% and 18% of nitrogen was remobilised, respectively. In our study, the aboveground biomass nitrogen concentration at harvest in February (between 2.3 and 3.3 $mg.g^{-1}$) was lower than the 5.0 $mg.g^{-1}$ reported by Beale and Long (1997). The higher aboveground biomass nitrogen concentrations can explain the lower remobilisation observed by those authors. The difference observed compared with the study by Himken *et al.* (1997) was due to higher nitrogen losses by abscised leaves in their study (60 $kgN.ha^{-1}$) compared

with ours (30 kgN.ha^{-1}). Hence, differences observed between studies may be due to different climate conditions. Further studies are needed to determine the effects of environmental factors (photoperiod, temperatures) and phenological stage of the crop on autumn remobilisation in *M. giganteus.*

In early harvested plants, only 42% of aboveground nitrogen was remobilised, suggesting that autumn remobilisation was not complete in mid-October. This limitation in autumn remobilisation led to a reduction in the nitrogen stocks available in belowground biomass for the subsequent year compared with late harvest treatments and thus increased the need for an external nitrogen supply. Indeed, in the fourth year of growth, an interactive effect of nitrogen fertilisation and harvest date on aboveground biomass nitrogen accumulation was observed, whereas there was no effect of harvest date in the third year of growth. This is probably due to high nitrogen stocks in belowground biomass at the beginning of the experiment and nitrogen remobilisation before harvest.

4.5 Dry matter production of *Miscanthus x giganteus*: Effects of nitrogen availability and crop age

4.5.1 Aboveground biomass

The peak yields measured in October in the third and fourth years of growth (28-29 t DM ha^{-1}; section 3.1) are of the same order of magnitude as the maximum biomass production of 30 tDM.ha^{-1} for those years described by Beale and Long (1997) and Himken *et al.* (1997). When the crops were harvested after winter, yield had declined by 28-30% due to abscised leaves and remobilisation of nutrients from aboveground to belowground biomass during stems and leaves senescence. As reported by many authors (Christian *et al*., 2008; Clifton-Brown *et al.*, 2007; Danalatos *et al.*, 2007; Himken *et al.*, 1997), nitrogen fertilisation had no effect on aboveground biomass production when the crops were harvested after winter,

suggesting that nitrogen originating from belowground biomass and soil was sufficient to give maximum aboveground biomass production in the experimental conditions, as discussed in section 4.1 and 4.2. Other authors have concluded that nitrogen fertilisation is needed in order to achieve maximum biomass production (Cosentino *et al.*, 2007; Ercoli *et al.*, 1999). In the latter studies, nitrogen fertilisation had a great impact on aboveground biomass nitrogen accumulation, and this was most likely due to low nitrogen availability in soil and/or in belowground biomass, but these data are not available. In our study, aboveground biomass production and nitrogen accumulation in the EN0 treatment was limited compared with the others, due to lower belowground biomass nitrogen stocks and nitrogen availability from soil. Therefore, fertilisation will enhance aboveground biomass production when crops are harvested early, due to higher nitrogen removal from the field at harvest and thus lower belowground biomass nitrogen stocks available for subsequent years. Moreover, it appeared that in our experimental conditions, nitrogen fertilisation in late harvest treatments allowed the crop to have a luxury uptake of nitrogen because no benefit in terms of aboveground biomass production was observed. Unfortunately, there is no critical nitrogen dilution curve available for *Miscanthus* x *giganteus,* and hence we cannot determine the nitrogen nutrition index of our crops.

4.5.2 Belowground biomass

Similarly to Beale and Long (1997), we observed a significant increase in belowground biomass during the third year of growth, suggesting that the crop was not fully established. During the fourth year of growth, belowground biomass dry matter (16-19 $tDM.ha^{-1}$) was comparable to the 16 t ha^{-1} reported by Himken *et al.* (1997). No increase in belowground biomass was observed during the fourth year of growth, suggesting that the crop had reached a steady state. Furthermore, nitrogen fertilisation had no effect on belowground

biomass production, as reported previously by Himken *et al.* (1997). No effect of harvest date on belowground biomass dry matter was observed during the two years of growth studied, suggesting that there is little or no dry matter allocation to belowground biomass after mid-October in our climate conditions.

5. Conclusions

The experimental design and procedure allowed us i) to obtain variable amounts of belowground biomass nitrogen stocks in *M. giganteus* ii) to determine apparent N fluxes between organs, despite the belowground biomass variability, and iii) to understand the determinants of the spring N remobilisation. Hence, the study was able to illustrate the key role of belowground biomass nitrogen stocks for aboveground biomass nitrogen accumulation. Nitrogen remobilisation was shown to be a function of initial belowground biomass nitrogen stocks and allowed a higher aboveground biomass nitrogen accumulation rate. A strong relationship was found between maximum nitrogen accumulation in aboveground biomass and initial belowground biomass nitrogen stocks and whole crop nitrogen uptake. This study also showed that nitrogen storage is a function of the maximum aboveground biomass nitrogen content, but it may depend on other factors such as environment or phenological stage of the crop.

Although biomass yields are enhanced by early harvest, e.g. when the crop is intended for ethanol production, this practice can deplete belowground biomass nitrogen stocks and soil mineral nitrogen content. In early harvested crops, biomass production will decline if nitrogen removed from the field at harvest is not replaced by fertilisation in subsequent years, due to lower spring remobilisation. In late harvested crops, e.g. those intended for heat combustion or ethanol production, nitrogen removal from the field at harvest is very low and thus the nitrogen fertiliser requirement will be considerably decreased, or perhaps even zero, as long as belowground biomass nitrogen stocks and nitrogen availability from the soil are sufficient. Therefore, it is essential to take into account belowground biomass nitrogen stocks and soil nitrogen availability in determining the nitrogen fertiliser requirement of *M. giganteus*. Assessing actual flux as opposed to apparent flux would provide a better understanding of the soil-crop relationship, especially fertiliser utilisation. Thus, nitrogen stocks and nitrogen

fluxes within the plant need to be integrated into a soil-crop-atmosphere model, in order to take account of long-term crop nitrogen requirements in different pedo-climatic conditions and cultural practices.

Acknowledgements

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Chapitre 2

Influence of belowground nitrogen stocks on light interception and conversion by *Miscanthus x giganteus*

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Abstract

In perennial plants such as forage crops, nitrogen reserves play a key role in regrowth rate and crop productivity. This study assessed the impact of *Miscanthus* x *giganteus* management (harvest date and nitrogen fertilisation) on subsequent regrowth of the crop *via* their effects on the nitrogen stocks of belowground organs. Crop growth was analysed by studying the radiation use efficiency of the crop and the kinetics of leaf area index development. The results showed that decreasing nitrogen stocks through early harvesting of the crop and no fertilisation had a negative effect on subsequent regrowth. A linear relationship was observed between leaf area index and aboveground nitrogen accumulation in the crop. The growth rate of the leaf area index depended on belowground nitrogen stocks before regrowth. There was also a correlation between aerial radiation use efficiency of *Miscanthus* x *giganteus* and belowground nitrogen stocks before regrowth, as observed in other perennial crops. Low nitrogen availability was found to affect resource conversion by *M. giganteus* more than resource capture.

Keywords

Leaf area index; radiation use efficiency; nitrogen fertilisation; harvest date; energy crop

1. Introduction

Miscanthus x *giganteus* is a perennial rhizomatous grass that uses the C4 photosynthetic pathway. It originates from Asia and has been described as having high potential biomass production with a low nitrogen requirement [1]. For a large number of crops, biomass production has been shown to be linearly related to intercepted (or absorbed) radiation in the case of no biotic or abiotic stress. The slope of this relationship is defined as the radiation use efficiency (RUE) of the crop [2]. Radiation interception by the crop is determined by leaf area index (LAI) development and by the radiation extinction coefficient of the canopy (*k*). This coefficient depends on canopy architecture, and thus species, and row spacing [3]. In many crops, nitrogen deficiency alters radiation interception (resource capture), through reduction of LAI, and/or RUE (resource conversion) [4, 5, 6]. Either or both these response patterns are species-specific, although C4 crops generally maintain resource capture instead of resource use efficiency [7].

Nitrogen uptake and partitioning between organs influences light interception and the photosynthetic capacity of the crop, with both being increased if nitrogen is allocated to upper organs in the crop [8]. In perennial crops such as *Medicago sativa* L., aboveground nitrogen accumulation is reported to be dependent on the belowground nitrogen stocks before regrowth [9]. Hence, the amount of nitrogen reserves before regrowth has a direct impact on the kinetics of LAI development [10, 11, 12] or on RUE [10].

In *M. giganteus*, the belowground organs (*i.e.* rhizomes and roots) are storage organs, and source of nitrogen during regrowth in spring *via* remobilisation processes [13, 14, 15, 16]. The amount of nitrogen reserves before regrowth has a direct impact on remobilisation and thus nitrogen accumulation (amount and rate) in the aboveground biomass [16]. In addition, Strullu *et al.* [16] showed that nitrogen absorption from soil mineral nitrogen also has an effect on aboveground biomass nitrogen accumulation, either directly through allocation to

aerial biomass or indirectly through nitrogen storage in belowground biomass during the vegetative period.

In a study in Sicily, Cosentino *et al.* [17] showed that in the second and third years of growth, the maximum leaf area index reached by *M. giganteus* and the radiation use efficiency were higher in fertilised treatments than in non-fertilised. Strullu *et al.* [16] showed that early harvest (in October) had a negative impact on aboveground biomass production and nitrogen accumulation by *M. giganteus* when no nitrogen fertiliser was applied, due to soil and rhizome N depletion in this system. However, they did not consider the crop physiological processes explaining this lower biomass production and nitrogen accumulation.

In this study, we investigated the response pattern of *M. giganteus* to readily available nitrogen in terms of light interception and radiation use efficiency. In particular, we analysed the effect of belowground nitrogen stocks and nitrogen absorption on LAI development and RUE of *M. giganteus*.

2. Materials and methods

Study site and trial set-up

The experimental site was located in the Picardie region of Northern France (49°52'N, 3°00'E). The soil was a deep silt loam (Ortic luvisol) and was characterised by pH 7.6, 19% clay, 74% silt, 5% sand. The climate at the site is oceanic, with mean rainfall of 625 mm per year and mean temperature of 10.7°C for the 10 past years. Treatments varied in terms of nitrogen (N) fertiliser rate, applied as ammonium nitrate in late April: 0 $kgN.ha^{-1}$ (N0) or 120 $kgN.ha^{-1}$ (N1), and harvest date: early harvest (E) in October or late harvest (L) in February. For early harvest (E), the whole plots were harvested each year in October, while for late harvest (L) the whole plots were harvested each year in February. The study site and trial set-up are described in more detail by Strullu *et al.* [16].

Biomass sampling

Full details of the methodology used to determine aboveground and belowground biomass and dry weight of organs are given in Strullu *et al.* [16]. In brief, in each of the four treatments, aboveground biomass production was estimated on six occasions in 2008 (third year of growth) and 2009 (fourth year of growth). Six adjacent plants per replicate were harvested to measure the aboveground biomass on each occasion. A first subsample was used for estimation of the moisture content. The proportion of stems (S), green leaves (GL) and dead leaves (DL) were estimated from a second subsample. The surface area of green leaves was determined with a LI-3100C Area Meter (LI-COR®) and the green leaves were then weighed. This allowed the specific leaf area ($m^2.g^{-1}$) to be calculated.

The aboveground biomass at each harvest was calculated as:

$$W_A = [(dm_A * 10) / ns] * NS$$

where W_A is the aboveground biomass production ($tDM.ha^{-1}$), dm_A the aboveground dry matter of the six plants (kg), ns the number of stems of the six plants and NS the number of stems per hectare determined in area A.

The LAI was calculated on each sampling occasion as:

$$LAI = SLA * (W_{GL} * 100)$$

where LAI is the leaf area index ($m^2.m^{-2}$), SLA the specific leaf area ($m^2.g^{-1}$) of the second subsample and W_{GL} the green leaves weight ($kgDM.ha^{-1}$).

The belowground biomass was determined after extraction of a median plant in each treatment. The median plant was determined for each block of each treatment, on a number of stems per plant basis, after counting the stems of each plant in the 25 m^2 area (area A). Belowground biomass was calculated as:

$$W_B = (dm_{Rh} + dm_{Ro}) * NP$$

where W_B is the belowground biomass ($tDM.ha^{-1}$), dm_{Rh} the rhizome biomass ($tDM.ha^{-1}$), dm_{Ro} the root biomass ($tDM.ha^{-1}$) and NP the number of plants per hectare determined in area A.

Unfortunately, the median plant had not been determined before harvest in October 2007, so belowground biomass in February 2008 in early harvested treatments was not sampled according to this protocol. These data were therefore removed from the analysis to avoid random variability.

The cumulated degree-days during each year of growth were calculated on a 6°C basis from emergence, as suggested by Clifton-Brown and Jones [18]. Emergence was determined as the date on which 50% of plants had sprouted.

Kinetics of LAI development

A Gompertz curve with three parameters was used to fit the data on LAI development as a function of the cumulated degree-days:

$LAI = LAI_{max} * \exp(-\exp(-(X- X0) / b))$

where LAI_{max} is the asymptote and corresponds to the maximum LAI reached by the crop ($m^2.m^{-2}$), X is the cumulated degree-days (degree-days), X0 the degree-days necessary to reach the half of the maximum LAI (LAI_{max}), and b is the degree-days between 5% and 95% of LAI_{max}.

A Gompertz curve was chosen because it provided a better fit (higher R^2) than logistic curves. Data on LAI measured during canopy senescence were not used for the fit.

The maximum LAI growth rate (MLGR, $m^2.m^{-2}.dd^{-1}$) was then calculated as:

$MLGR = LAI_{max} /b * 1/e$

$LN(e) = 1$

Determination of the extinction coefficient and ε_i or ε_a max

In order to determine the extinction coefficient of *M. giganteus*, light interception and absorption by the crop was measured using PAR-LE sensors (Solems®) and an LI-190SZ quantum sensor (LI-COR®) in 2009. The LI-190SZ quantum sensor was used to measure the incident photosynthetically active radiation (PAR) and PAR-LE sensors were used to measure transmitted and reflected PAR. PAR-LE sensors were calibrated before the experiment using the LI-190SZ quantum sensor as reference. All PAR-LE sensors read within ± 2% of the reference instrument. In four plots, corresponding to the four treatments, 36 PAR-LE sensors were used to measure the transmitted PAR (PARt) through the canopy. In two plots corresponding to the EN1 and LN1 treatments, four PAR-LE sensors were used to measure the reflected PAR (PARrc) by the canopy and four others to measure the transmitted PAR

reflected by the soil (PARrs). PAR-LE sensors were placed perpendicular to the row and orientated in an east-west direction. The LI-190SZ quantum sensor and the PAR-LE sensors were washed weekly. PAR-LE and LI-190SZ outputs were read every 15 minutes, averaged over an hourly interval, and recorded using CR10X data loggers (Campbell Scientific®). These data were used to calculate the daily PAR interception efficiency (ε_i, %) and the daily PAR absorption efficiency (ε_a, %) as follows:

$\varepsilon_i = (PAR_I - PAR_t) / PAR_I$

$\varepsilon_a = [(PAR_I + PAR_{rs}) - (PARt + PAR_{rc})] / PAR_I$

where PAR_I is the incident photosynthetic active radiation ($MJ.m^{-2}$), PAR_t the PAR transmitted to the soil through the canopy, PAR_{rs} the transmitted PAR reflected by the soil and PAR_{rc} the PAR reflected by the canopy.

In parallel, the LAI was measured three times per week at dawn with a LAI-2000 Plant Canopy Analyser (LI-COR®) until LAI reached 5 $m^2.m^{-2}$. This allowed the extinction coefficient of the canopy to be determined according to the formula of Monsi and Saeki [19]:

$\varepsilon_i = \varepsilon_{i\ max} (1 - \exp(-k_i * LAI))$

where ε_i is the radiation interception efficiency, k_i the radiation extinction coefficient for the intercepted PAR and $\varepsilon_{i\ max}$ the maximum radiation interception efficiency by the crop;

and:

$\varepsilon_a = \varepsilon_{a\ max} (1 - \exp(-k_a * LAI))$

where ε_a is the radiation absorption efficiency, k_a the radiation extinction coefficient for the absorbed PAR and $\varepsilon_{a\ max}$ the maximum radiation absorption efficiency by the crop.

Determination of radiation use efficiency, RUE

The RUE, corresponding to the slope of the linear relationship between the aboveground biomass and the intercepted or absorbed PAR, was determined during the linear phase of

aboveground biomass accumulation. Data were excluded from the relationship when nitrogen remobilisation from aboveground to belowground biomass occurred. Hence, data corresponding to the sampling occasion in October 2008 and the sampling occasions in mid-August and October 2009 were excluded from the relationship.

Nitrogen analysis and calculation of N content

The total nitrogen concentration in plant aboveground biomass was determined using the Dumas method. The nitrogen accumulated in the different organs, NC ($kgN.ha^{-1}$), was calculated as the product of nitrogen content in the organ (N in $g.kg^{-1}$) and dry weight of the organ (W in $tDM.ha^{-1}$):

$$NC = W * N$$

The nitrogen accumulated in the aboveground biomass (NC_A = $kgN.ha^{-1}$) was calculated as the sum of the individual values of NC for stems, green leaves and dead leaves.

Data analysis

ANOVA (Statistica®) was used for statistical analysis of year effects (corresponding to crop age and climate), treatment effects (harvest date and nitrogen fertilisation) and their interactions. Differences were then tested by the Neuman-Keuls test at the 5% level.

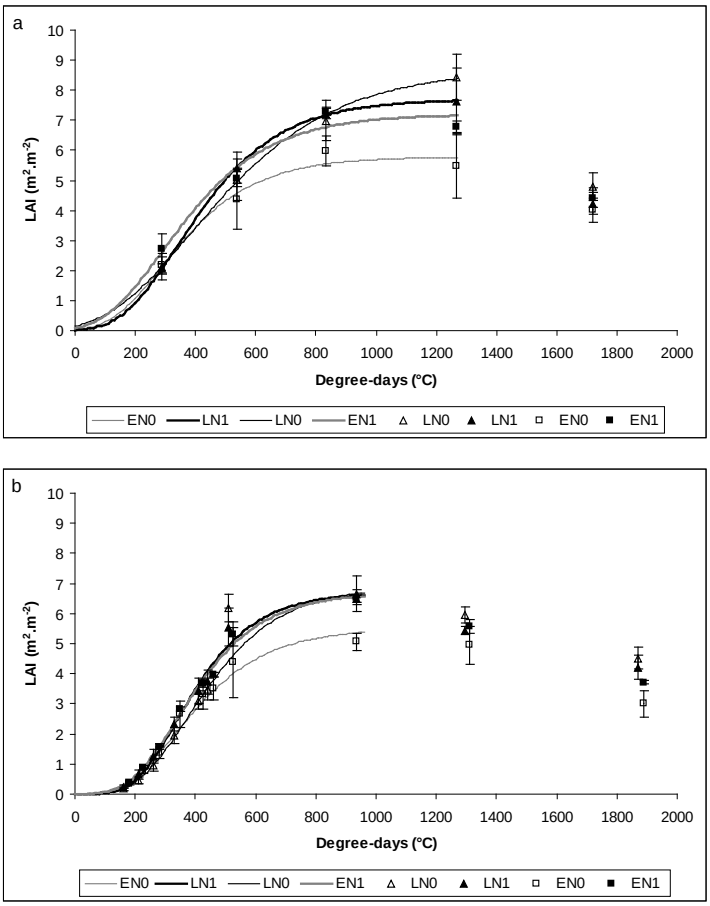


Figure 1: Leaf area index (LAI) development of *M. giganteus* as a function of degree-days (^{o}C) in a) the third year of growth (2008) and b) the fourth year of growth (2009) in the various treatments. Bars represent standard errors. Curves correspond to the fit with a Gompertz curve until maximum LAI development.

	$P - ET_0$ (mm) 2008	$P - ET_0$ (mm) 2009
January	32	36
February	15	8
March	49	-4
April	-16	-38
May	-44	-17
June	-66	-93
July	-78	-117
August	-4	-133
September	32	-65
October	39	29
November	27	60
December	32	56
April to August	-208	-397

Table 1: Monthly water balance ($P - ET_0$, mm) and cumulative deficit between April and August in 2008 and 2009.

3. Results and discussion

3.1 Radiation interception

Leaf Area Index development

The leaf area index (LAI) reached its maximum value in mid-July in 2008 and 2009, corresponding to 834 degree-days in 2008 and 934 and 951 degree-days in the L and E treatment, respectively, in 2009 (Figure 1). The maximum LAI reached by the crop was significantly lower in 2009 than in 2008 ($p<0.05$).

This could be due to the greater difference observed between rainfall (P) and evapotranspiration (ET_0) in the period April-August, the parameter used as a water stress indicator, in 2009 compared with 2008 (Table 1).

In both years, the maximum LAI reached by the crop was significantly lower (6.0 and 5.4 $m^2.m^{-2}$) in the EN0 treatment ($p=0.02$) compared with the other treatments (7.6 and 6.6 $m^2.m^{-2}$ on average for 2008 and 2009, respectively). Thereafter, the LAI declined in all treatments until harvest.

The maximum LAI reached by the crop in this study was of the same order of magnitude as that reported by other authors [17, 20, 21]. As observed by Cosentino *et al.* [17], nitrogen fertilisation had a positive effect on the maximum LAI reached by the crop. However, this positive effect was only observed in early harvest treatments in our experimental conditions. Strullu *et al.* [16] showed that the EN0 treatment accumulated less nitrogen than other treatments due to lower belowground nitrogen stocks and lower nitrogen supply from the soil. Hence, nitrogen fertilisation had a positive effect on the maximum LAI reached by the crop when belowground nitrogen stocks before regrowth and nitrogen availability in the soil were low [16].

		$Y = LAI_{max} * \exp(-\exp(-(X-X0)/b))$	
Year	Treatment	R^2	MLGR ($m^2.m^{-2}.dd^{-1}$)
2008	LN0	0.98	0.0117 a
	LN1	0.98	0.0152 a
	EN0	0.93	0.0124 a
	EN1	0.99	0.0135 a
2009	LN0	0.98	0.0149 b
	LN1	0.98	0.0176 b
	EN0	0.98	0.0117 a
	EN1	0.99	0.0151 b

Table 2: Maximum leaf area index growth rate (MGLR = $m^2.m^{-2}.dd^{-1}$) calculated in the different treatments during the two years of growth. LN0 = late harvest without fertilisation, LN1 = late harvest with 120 $kgN.ha^{-1}.year^{-1}$, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 $kgN.ha^{-1}.year^{-1}$.

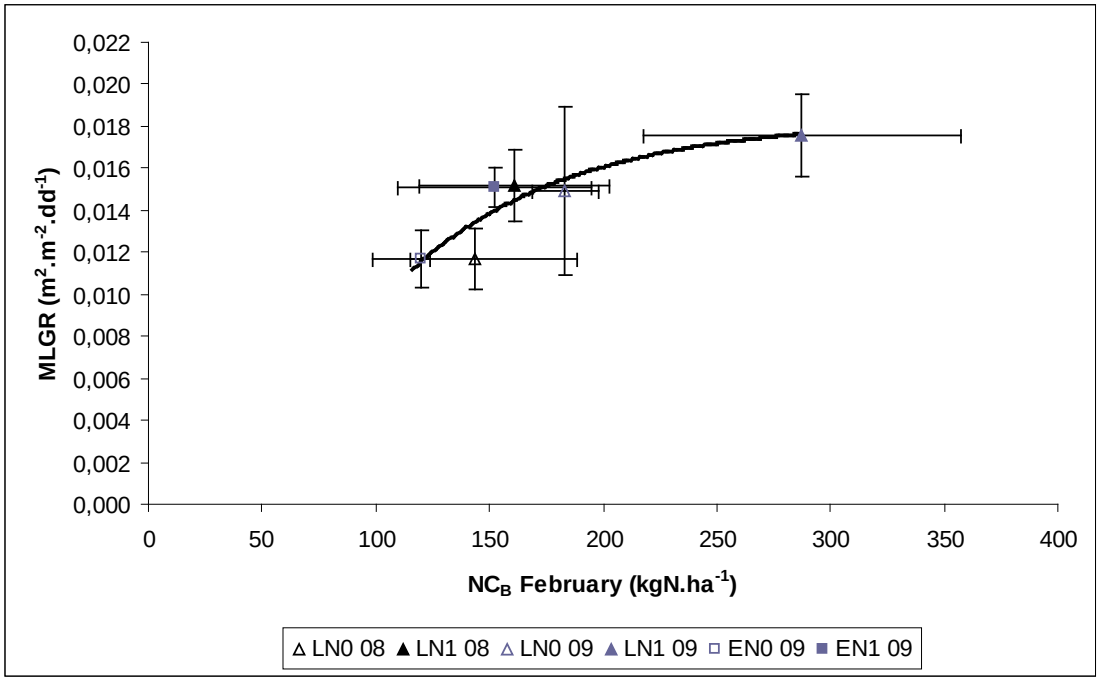


Figure 2: Relationship between maximum leaf area index growth rate (MLGR) and belowground nitrogen stocks before regrowth (NC_B, $kgN.ha^{-1}$). Non-fertilised treatments (N0) are represented by open symbols and fertilised (N1) treatments by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). Black symbols correspond to the third year of growth (2008) and grey symbols to the fourth year of growth (2009). Bars represent standard-error. $Y = -0.017 + 0.0353 * (1 - \exp(-0.0138 * X))$; $R^2 = 0.90$.

Leaf Area Index growth rate

In order to study the kinetics of LAI development and determine the cumulative PAR intercepted and absorbed by the crop, the data were fitted to a Gompertz curve until the maximum LAI development was achieved. This gave good results, as shown by the correlation coefficient ranging from 0.93 to 0.99 (Table 2). The maximum LAI growth rate (MLGR) varied between treatments and years of growth. In 2008, there were no significant differences in MLGR between treatments. In 2009, there was an effect of harvest date ($p=0.02$) and nitrogen fertilisation ($p<0.05$) on MLGR, with MLGR being higher in late harvest treatments than in early harvest and in fertilised treatments than in non-fertilised.

An exponential relationship was found between MLGR and belowground N stocks before regrowth (Figure 2).

M. giganteus has been described in many studies as having rapid canopy growth in spring and reaching high LAI early in the season [17, 20, 21]. The time required to reach the maximum LAI has been reported to decrease with crop age during the establishment phase of *M. giganteus* [17, 20]. Those studies found that in first year of growth, the maximum LAI of the crop was reached at the end of September, while in second year of growth it was reached from mid-July. This was confirmed by our study, where the maximum LAI was reached from mid-July during the two years of growth (see section *Leaf Area Index development*). Perennial plants use nitrogen reserves for regrowth in spring or after cutting and the amount of remobilised nitrogen is dependent on nitrogen stocks before regrowth [9, 16]. The LAI growth rate has been shown to be dependent on the amount of nitrogen reserves before regrowth in *Medicago sativa L.* [10, 12] or grasses such as *Festuca rubra* and *Lolium perenne* [11]. Strullu *et al.* [16] showed that the aboveground nitrogen accumulation rate was highly dependent on the belowground nitrogen stocks before regrowth. Hence, the crop age effect on LAI development observed by Beale and Long [20] and Cosentino *et al.* [17] is likely to be an

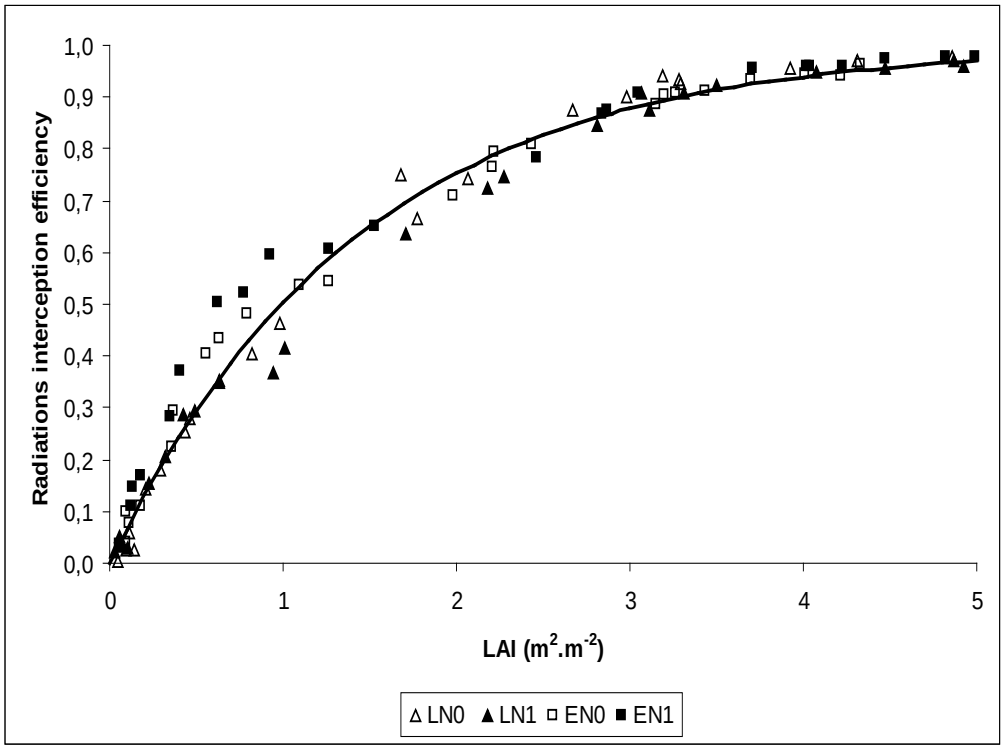


Figure 3: Relationship between leaf area index (LAI) and radiation interception efficiency by the canopy. Non-fertilised treatments (N0) are represented by open symbols and fertilised (N1) treatments by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). $\varepsilon_i = 0.97 * (1 - \exp(-0.70 * LAI)$, ($R^2 = 0.99$).

Year	Treatment	Intercepted PAR ($MJ.m^{-2}$)
2008	LN0	916 c
	LN1	884 c
	EN0	873 c
	EN1	927 c
2009	LN0	675 a
	LN1	702 a
	EN0	713 b
	EN1	739 b

Table 3: Photosynthetically active radiation (PAR) intercepted by *Miscanthus* x *giganteus* determined before nitrogen remobilisation between aboveground and belowground biomass. LN0 = late harvest without fertilisation, LN1 = late harvest with 120 $kgN.ha^{-1}.year^{-1}$, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 $kgN.ha^{-1}.year^{-1}$.

effect of the belowground nitrogen stocks, which are reported to increase with crop age [15]. The effect of aboveground N accumulation rate on MLGR could be due to the effect of aboveground N content on cell division and elongation in aboveground organs [8].

PAR interception and absorption by the crop

According to the close relationship (R^2 = 0.99) between LAI and radiation interception efficiency, the radiation extinction coefficient (k_i) of *M. giganteus* canopy was calculated to be 0.70 ± 0.028 for the intercepted radiation (Figure 3). The radiation extinction coefficient for the absorbed radiation (k_a) was estimated to be 0.65 ± 0.038 (R^2 = 0.99) (data not shown). The maximum radiation interception was 97% and maximum absorption efficiency was 92%. The kinetics of LAI development and the extinction coefficient were used to determine the PAR intercepted or absorbed by the crop.

On average, the cumulated intercepted PAR was 900 $MJ.m^{-2}$ in mid-August 2008 and 707 $MJ.m^{-2}$ in mid-July 2009 (Table 3). In 2008, there was no significant difference in intercepted PAR between treatments. In 2009, the early harvest treatments intercepted more PAR than the late harvest treatments ($p<0.05$).

The rapid canopy growth and high LAI reached by *M. giganteus* allowed the crop to intercept a high amount of incident PAR during the growing season, *i.e.* from emergence to peak yield. This early and rapid canopy growth allowed *M. giganteus* to intercept higher amounts of PAR than other C4 crops such as maize or sorghum [22, 23]. For example, Tayot *et al.* [22] reported that *M. giganteus* intercepted twice as much PAR as sorghum, while Dohleman *et al.* [23] showed that *M. giganteus* intercepted 1.5 to 1.8 times more PAR than maize. We found that *M. giganteus* intercepted on average 77% of incident PAR during the growth season between emergence and peak yield in mid-October. This value was similar to the 80% reported by Beale and Long [20]. In our experiment, the maximum LAI reached by

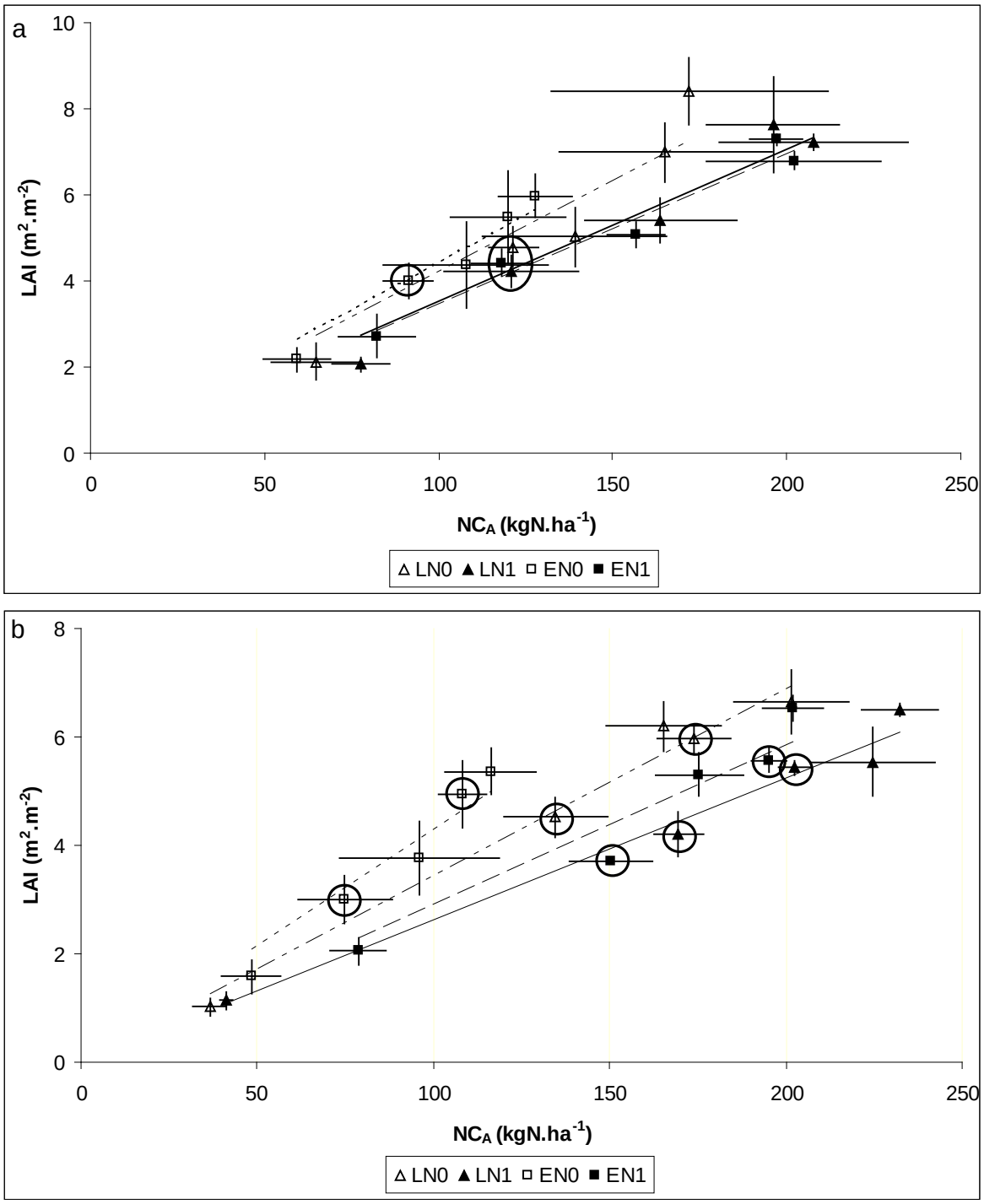


Figure 4: Relationship between leaf area index (LAI) and aboveground N uptake (NC_A) in a) the third year of growth (2008) and b) the fourth year of growth (2009). Non-fertilised treatments (N0) are represented by open symbols and fertilised (N1) treatments by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). Surrounded symbols correspond to the LAI decline during nitrogen remobilisation between aboveground and belowground biomass.

the crop in all treatments was sufficient to have maximum interception efficiency during summer, when incident PAR is maximal. The earlier emergence of early harvest treatments compared with late harvest in 2009 could explain the slightly higher PAR accumulated by the early harvest treatments [16]. Hence, we did not observe a significant effect of maximum LAI growth rate on the amount of PAR intercepted by the crop.

Effect of nitrogen on LAI development

During the two years of growth, LAI was linearly correlated to aboveground N accumulation (Figure 4).

The slope of the relationship was defined here as the nitrogen use efficiency for LAI development (NUE-LAI, Table 4). There was an effect of year of growth ($p<0.01$), nitrogen fertilisation ($p<0.01$) and harvest date ($p<0.05$) on NUE-LAI. In 2008, NUE-LAI was significantly higher ($p<0.05$) in the non-fertilised treatments than in the fertilised. In 2009, the nitrogen use efficiency for LAI development was highest in the EN0 treatment, intermediate in the LN0 treatment and lowest in the LN1 and EN1 treatments ($p<0.05$).

The maximum LAI reached by the crop was limited by aboveground nitrogen accumulation in the EN0 treatment during the two years of growth (*cf* section 3.1), while this was not the case in the other treatments. This suggests that when available nitrogen decreases, the crop increases its NUE-LAI to a maximum value ranging from 0.42 to 0.44 $m^2.g^{-1}$ N. Using the value of maximum NUE-LAI, we determined that a minimum aboveground nitrogen accumulation of 116 $kgN.ha^{-1}$ is required in order to reach a LAI of 5 $m^2.m^{-2}$ and thus have maximum radiation interception efficiency by the crop. The highest maximum aboveground nitrogen accumulation observed in fertilised treatments in 2008 and 2009 and in the LN0 treatment in 2009 did not allow a higher maximum LAI to be achieved (*cf* section 3.1) and so decreased the NUE-LAI. The minimum NUE-LAI for *M. giganteus* varied

Year	Treatment	NUE LAI (m^2. $g^{-1}N$.)	R^2
2008	LN0	0.42 c	0.89
	LN1	0.35 b	0.95
	EN0	0.44 c	0.94
	EN1	0.35 b	0.96
2009	LN0	0.34 b	0.98
	LN1	0.26 a	0.98
	EN0	0.43 c	0.93
	EN1	0.29 a	0.92

Table 4: Nitrogen use efficiency for leaf area index development (NUELAI) in the different treatments during two years of growth and correlation coefficient for the linear regression. LN0 = late harvest without fertilisation, LN1 = late harvest with 120 $kgN.ha^{-1}.year^{-1}$, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 $kgN.ha^{-1}.year^{-1}$.

between 0.26 and 0.29 $m^2.g^{-1}$ N in 2009 in the LN1 and EN1 treatments, respectively, in our experimental conditions. In all treatments and during the two years of growth, LAI decreased at the end of the growing season when nitrogen remobilisation from aboveground to belowground organs began, as shown by Strullu *et al.* [16]. This decline in LAI followed the same linear relationship as during LAI growth, so no hysteresis was observed. Nitrogen accumulation in aboveground biomass should thus be a robust tool to model the development of LAI in *M. giganteus.*

Lemaire *et al.* [7] also observed that nitrogen use efficiency for LAI development increases as aboveground biomass nitrogen accumulation decreases for a given biomass production, *i.e.* when the nitrogen nutrition index of the crop declines. They reported values of maximum NUE-LAI that varied between 0.32 $m^2.g^{-1}N$ for wheat and 0.62 $m^2.g^{-1}N$ for maize. *M. giganteus* had an intermediate maximum NUE-LAI of around 0.43 $m^2.g^{-1}N$.

Lemaire *et al.* [7] reported that in treatments without nitrogen stress, the value of minimum nitrogen use efficiency for LAI development varied between species, being 0.28 $m^2.g^{-1}N$ in wheat, 0.35 $m^2.g^{-1}N$ in maize and 0.45 $m^2.g^{-1}N$ sorghum. Thus it appears that *M. giganteus* has minimum nitrogen use efficiency for LAI development without nitrogen stress close to that observed for wheat and maize.

As observed in other crops by Lemaire *et al.* [7], the LAI development of *M. giganteus* was linearly correlated to the aboveground nitrogen accumulation. The variation in NUE-LAI observed in our experiment was due to the effect of the different treatments on the aboveground nitrogen accumulation *via* their effect on belowground nitrogen stocks and nitrogen absorption by the crop [16]. The nitrogen requirements of *M. giganteus* for LAI development seemed to be higher than those for maize and lower than those for wheat. Moreover, it appeared that, contrary to wheat and to a lesser extent to maize, *M. giganteus* is

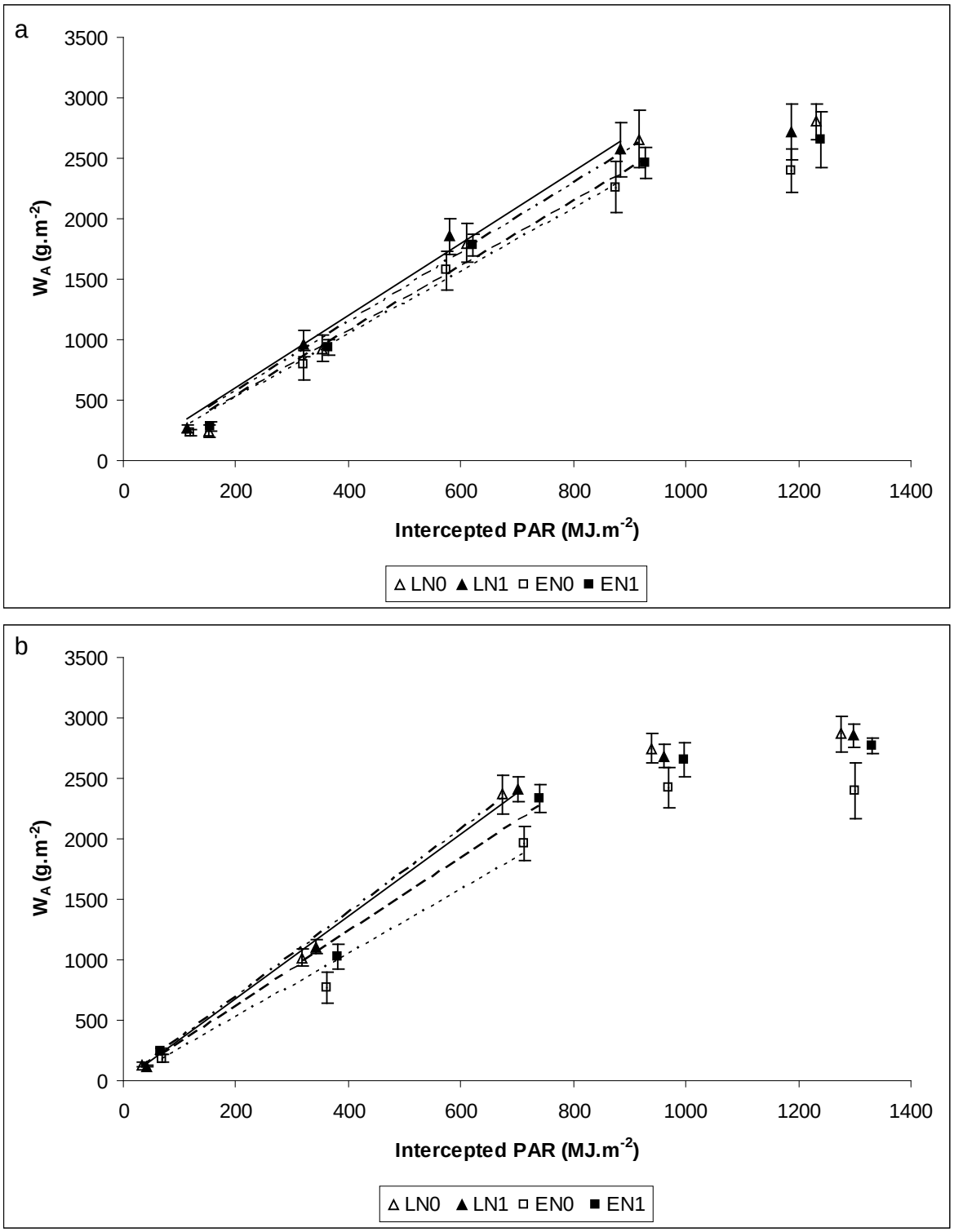


Figure 5: Relationship between aboveground biomass (W_A) and cumulative photosynthetically active radiation (PAR) intercepted by the crop from emergence to the beginning of senescence in a) the third year of growth (2008) and b) the fourth year of growth (2009). Non-fertilised treatments (N0) are represented by open symbols and fertilised (N1) treatments by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E).

able to increase its NUE for LAI development in order to maintain its resource capture when belowground nitrogen stocks and nitrogen absorption by the crop are low.

3.2 Radiation use efficiency of *M. giganteus*

RUE varied between treatments and years of growth (Figure 5 and Table 5).

There was an increase in RUE between the two years of growth, so it was significantly higher ($p<0.01$) in 2009 (3.1 $g.MJ^{-1}$ on average) than in 2008 (2.8 $g.MJ^{-1}$ on average). There was also a significant effect of the treatments on RUE of the crop, with it being lowest in the EN0 treatment (2.6 $g.MJ^{-1}$ on average), intermediate in the EN1 treatment (2.9 $g.MJ^{-1}$ on average) and highest in the late harvest treatments (3.2 $g.MJ^{-1}$ on average). The radiation use efficiency for absorbed radiation was 7% higher than that for intercepted radiation (Table 5), due to lower accumulated PAR.

There was a linear relationship between RUE and initial belowground N stocks (Figure 6), until a plateau was reached at a maximum RUE of 3.4 $g.MJ^{-1}$. Hence, it seems that there was critical nitrogen content in belowground biomass of between 150 and 187 $kgN.ha^{-1}$. Below this critical nitrogen content, RUE was significantly reduced.

The radiation use efficiency reported for *M. giganteus* varies widely, from 1.1 to 4.1 $g.MJ^{-1}$ [17, 20, 21, 23, 24]. Several authors have shown that RUE of *M. giganteus* increases with crop age during the establishment phase [20, 24]. The maximum RUE determined during our experiment was similar to that reported for *M. giganteus* by Beale and Long [20], but lower than the value of 4.1 $g.MJ^{-1}$ reported by Tayot *et al.* [23]. The difference compared with Tayot *et al.* [23] could be due to the warmer climate in their experimental sites.

Avice *et al.* [10] showed that RUE of *Medicago sativa* L. varies according to cultivar and shoot removal frequency, due to differences in nitrogen and starch reserves in taproots before

Year	Treatment	Intercepted PAR ($MJ.m^{-2}$)	RUE_i ($g.MJ^{-1}$)	R^2	Absorbed PAR ($MJ.m^{-2}$)	RUE_a ($g.MJ^{-1}$)	R^2
2008	LN0	916 c	2.87 ab	0.98	856 c	3.08 ab	0.99
	LN1	884 c	2.99 ab	0.99	816 c	3.20 ab	0.99
	EN0	873 c	2.61 a	0.99	829 c	2.81 a	0.99
	EN1	927 c	2.69 a	0.99	869 c	2.88 a	0.99
2009	LN0	675 a	3.45 b	0.99	629 a	3.71 b	0.99
	LN1	702 a	3.40 b	0.99	655 a	3.65 b	0.99
	EN0	713 b	2.62 a	0.97	662 b	2.83 a	0.98
	EN1	739 b	3.06 ab	0.99	690 b	3.29 ab	0.99

Table 5: Intercepted or absorbed photosynthetically active radiation (PAR) and radiation use efficiency (RUE) of *Miscanthus* x *giganteus*, determined before nitrogen remobilisation between aboveground and belowground biomass. LN0 = late harvest without fertilisation, LN1 = late harvest with 120 $kgN.ha^{-1}.year^{-1}$, EN0 = early harvest without fertilisation and EN1 = early harvest with 120 $kgN.ha^{-1}.year^{-1}$.

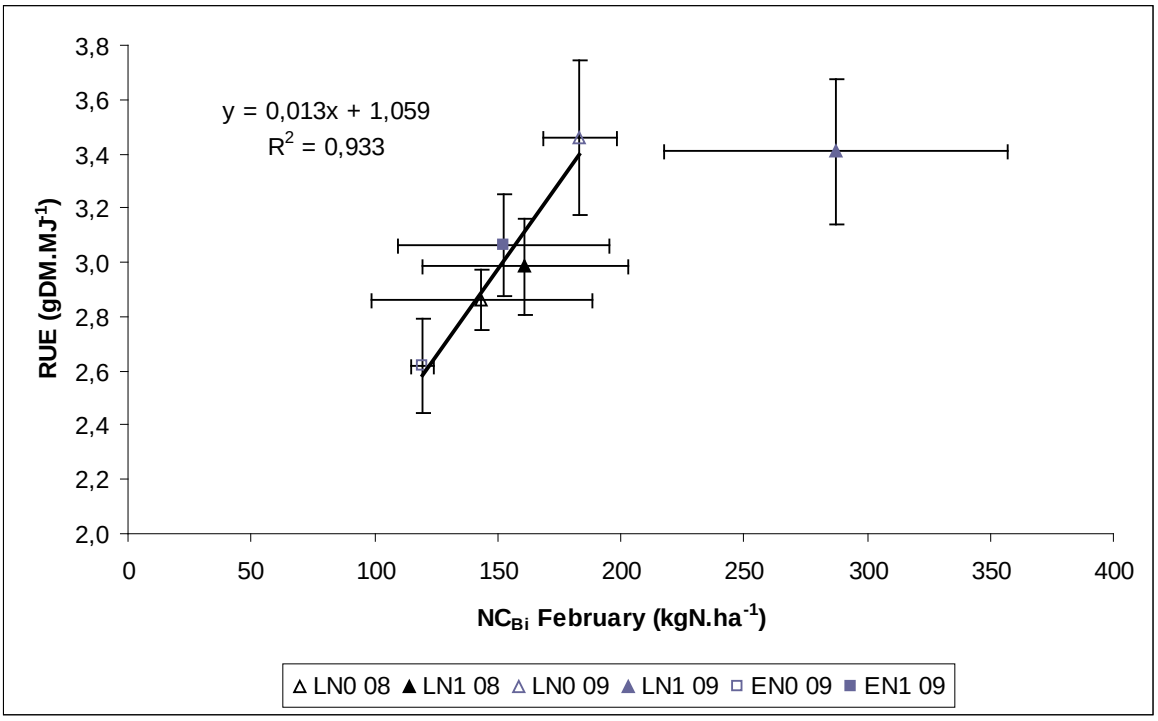


Figure 6: Relationship between radiation use efficiency (RUE, $g.MJ^{-1}$) determined from intercepted photosynthetically active radiation (PAR) and belowground nitrogen stocks (NC_{Bi}) before regrowth ($kgN.ha^{-1}$). Non-fertilised treatments (N0) are represented by open symbols and fertilised (N1) treatments by full symbols. Triangles correspond to late harvest (L) and squares to early harvest treatments (E). Black symbols correspond to the third year of growth (2008) and grey symbols to the fourth year of growth (2009).

regrowth. We did not examine the effect of starch reserves in this study, but the belowground nitrogen stocks before regrowth explained the variation in RUE between treatments. Moreover, Avice *et al.* [25] showed that in *Medicago sativa* L., most of carbon stored in organs before defoliation was mobilised during regrowth, but this carbon was mainly used by respiration and a small proportion was recovered in regrowing tissues. Hence, we assumed that carbon remobilised from belowground to aboveground organs had a negligible impact on the RUE of the crop. Moreover, we assumed that the relationship between RUE and belowground biomass N stocks was due to the effect of the latter on aboveground biomass N accumulation rate, as shown by Strullu *et al.* [16]. Aboveground N accumulation rate can affect crop RUE due to i) the effect of N content in leaves on photosynthesis and/or ii) the effect of aboveground N content on cell division and elongation in aboveground organs [8]. The crop age effect on RUE of *M. giganteus* observed by Jørgensen *et al.* [24] and Beale and Long [20] is probably an effect of belowground nitrogen stocks, which have been reported to increase with crop age [15].

We observed significant differences in RUE between treatments but light interception varied only slightly between treatments, suggesting that N availability mainly affected resource conversion rather than resource capture by *M. giganteus* in our experimental conditions.

4. Conclusions

This study showed that like other most C4 crops, *M. giganteus* is able to increase its nitrogen use efficiency for LAI development in order to maintain its resource capture efficiency. LAI development was dependent on the nitrogen accumulated in aboveground biomass and thus on belowground nitrogen stocks and nitrogen absorption by the crop. Depleting belowground nitrogen stocks, and thus reducing aboveground nitrogen accumulation rate, led to lower radiation use efficiency by the crop due to its high dependency on belowground nitrogen stocks before regrowth. Low nitrogen availability (in the case of early harvest and no fertilisation) affected resource conversion by *M. giganteus* more than resource capture in our experimental conditions. The reduction in RUE was mainly due to a reduction in belowground biomass nitrogen stocks and thus in nitrogen accumulation rate in the crop. The relationships described in this study could be used for modelling biomass production of the crop in the long term, taking into account the effect of belowground nitrogen stocks on radiation use efficiency by the crop and aboveground nitrogen accumulation on LAI development.

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Chapitre 3

Assessment of nitrogen absorption and remobilisation using ^{15}N-labelled in *Miscanthus x giganteus* with early or late harvest date

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En cours de soumission

Abstract

Nitrogen fertilizer requirements for energy crops production is a key point regarding to the negative environmental impacts of nitrogen *via* nitrate leaching or greenhouse gas emissions, and to the energetic balance of crops. This study aimed firstly to measure how much ^{15}N-labelled fertilizer was taken up by the crop and to which organs it was allocated within the crop. In a second part we calculated the rough fluxes of nitrogen remobilization and absorption thanks to ^{15}N tracing. The results showed that N fertilizer uptake by *M. giganteus* were low with maximum ^{15}N recovery of 41% in the whole crop. The nitrogen coming from fertilizer represented thus only 33% of the total N uptake from the soil. The proportion of N coming from external uptake and its partitioning within above and belowground organs depended on belowground biomass N stocks before regrowth. Finally we observed that *M. giganteus* is able to keep almost close its internal N cycle between years. Indeed, the high efficiency of N remobilisation from above to belowground organs allowed the crop to recover the amount of N remobilized from below to aboveground organs during regrowth in spring.

Further studies, on the effect of time and rate of N fertilizer applied to *M. giganteus*, are needed in order to increase the N recovery by the crop.

Keywords

Energy crop, N fertilisation, rough N fluxes, N cycling,. ^{15}N tracing

1. Introduction

Growing crops to produce biofuels is of increasing interest in order to substitute a part of energy derived from fossil fuels. Nitrogen fertilizer requirements for energy crops production is a key point regarding to the negative environmental impacts of nitrogen, *via* greenhouse gas emissions or nitrate leaching, and to the energetic balance of crops (Boehmel *et al.*, 2006). *Miscanthus* x *giganteus*, a rhizomatous perennial grass, originates from Asia and was introduced in Europe in 1930s. It has been described as one of the most interesting crops due to its low nitrogen requirements and high potential of biomass production (Lewandowski *et al.*, 2000). These low nitrogen requirements are due to the ability of the crop to store nitrogen in belowground organs and to remobilize it during regrowth (Beale and Long, 1997; Himken *et al.*, 1997; Strullu *et al.* submitted). However, these interesting net fluxes can result from the addition of antagonist rough fluxes. The latter can be more easily relied to environmental signals than the apparent fluxes (Mary and Recous, 1993).

Determination of N fluxes by using ^{15}N-labelled fertilizer has never been done in *M. giganteus,* despite N labeling is a strong tool to understand complex N fluxes which occurred in perennial crops (Millard and Grelet, 2010) or in soil (Mary and Recous, 1993). Two studies on ^{15}N-labelled fertilizer recovery have been realized for *M. giganteus* (Christian *et al.*, 1997; Christian *et al.*, 2006). The authors showed that nitrogen recovery from N fertilizer in the crop increased with crop age and that an important part of absorbed nitrogen was stored in the belowground organs at harvest. However, they did not determine absorption or remobilisation N fluxes because they only had one sampling date at crop harvest. Moreover, they only studied the late harvest conditions (over-winter) while miscanthus crops could be early harvested (in autumn) to improve its quality with regards to fermentation-based conversions (Le Ngoc Huyen *et al.* 2010). We propose here to study the dynamic of nitrogen absorption and nitrogen remobilisation within the plant by using ^{15}N tracing for both early (E) and late

(L) harvests. The objectives of this study were i) to measure how much ^{15}N-labelled fertilizer was taken up by the crop and to which organs it was allocated within the crop, and ii) to calculate the rough fluxes of nitrogen remobilization and absorption. The ^{15}N tracing method has been implemented in a 3 year old crop, for which the net N fluxes have already been assessed (Strullu *et al.*, submitted).

2. Materials and methods

Study site and trial set-up

The experimental site was located in the Picardie region of Northern France (49°52'N, 3°00'E). The soil was a deep silt loam soil (Ortic luvisol) and was characterised by pH 7.6, 19% clay, 74% silt, 5% sand. The climate at the site is oceanic, with mean rainfall of 625 mm per year and average temperature of 10.7°C for the ten past years. *Miscanthus* x *giganteus* was planted in May 2006 at a density of 15,625 plants ha^{-1} in a randomised block design. The previous crop was wheat, harvested in July 2005. The density after the first growth season was 14,941 plants ha^{-1}. During the second year (2007), four different treatments with three replicates were established. Treatments varied in terms of nitrogen (N) fertiliser rate: 0 $kgN.ha^{-1}$ (N0) or 120 $kgN.ha^{-1}$ (N1), and harvest date: early harvest (E) or late harvest (L). Plot size was 360 m² (12 m x 30 m), with 540 plants per plot. Each year, from 2007, nitrogen was applied as ammonium nitrate in late April. The study site and trial set-up are described in more detail by Strullu *et al.* (submitted). In this study, we studied only the N-fertilised treatments.

On 16 April 2009, ^{15}N-labelled fertilizer as $^{15}NH_4$ $^{15}NO_3$ solution (120 kgN ha^{-1}) was applied to the crop in LN1 and EN1 treatments, replacing the application of ammonium nitrate. Isotopic excess of ^{15}N-labelled fertilizer applied to the crops was 4.17%.

Biomass sampling

Full details of the methodology used to determine aboveground and belowground biomass and dry weight of organs are given in Strullu *et al.* (submitted). In brief, in each treatment, aboveground and belowground biomass production was estimated on six occasions in 2009 (fourth growth year). Six adjacent plants were harvested to measure the aboveground biomass on each occasion and the number of stems per plant was determined. A first subsample was

dried for four days at 65°C to estimate the moisture content. In order to better take into account of canopy variability, the dry mater yield was corrected by the number of stems determined in a wider undisturbed area of 25m² (hereafter referred to as area A). The stems (S), green leaves (GL) and dead leaves (DL) were separated from a second subsample, weighted after four days of drying at 65°C, in order to estimate the proportion of each organ. A nylon net was placed on the soil surface at the end of October, before leaf abscission, in order to collect abscised leaves during senescence from six plants per block (corresponding to a 3.84 m² area) in late harvest treatments. Abscised leaves that had fallen to the ground were collected regularly (every two weeks) and analysed to quantify the nitrogen lost during winter by this process. The leaves were dried for four days at 65°C, and then nitrogen concentration was determined (section 2.3).

The aboveground biomass at each sampling was calculated as:

$$W_A = [(dm_A) / ns] * NS \quad (1)$$

where W_A is the aboveground biomass production (tDM ha^{-1}), dm_A the aboveground dry matter of the six plants (kg), ns the number of stems of the six plants and NS the number of stems per hectare determined in area A.

The dry weight per hectare of stems (W_S), green leaves (W_{GL}) and dead leaves (W_{DL}) was then determined by multiplying the aboveground biomass production (tDM.ha^{-1}) by the proportion of each respective organ (%).

The belowground biomass was determined after extraction of a median plant in each treatment. The median plant was determined for each block of each treatment on a number of stems per plant basis, after counting the stems of each plant in the area A. On each sampling occasion, the rhizome and associated roots were extracted at a depth of 25 cm. After extraction, belowground biomass was washed and divided into rhizome (Rh) and roots (Ro).

All organs were dried for four days at 65°C until constant weight and then weighed in order to determine their dry matter weight. Belowground biomass was calculated as:

$$W_B = (dm_{Rh} + dm_{Ro}) * NP \quad (2)$$

where W_B is the belowground biomass (tDM ha^{-1}), dm_{Rh} the rhizome biomass (tDM ha^{-1}), dm_{Ro} the root biomass (tDM ha^{-1}) and NP the number of plants per hectare determined in area A.

N content

All samples were finely ground (500 µm). Then total N concentrations were determined by using an elemental analyser (FLASH EA 1112 series, Thermo Electron, Germany) and ^{15}N enrichment using an automatic N analyser linked to a mass spectrometer (DELTA V Advantage, Thermo Electron, Bremen, Germany).

The amount of N derived from the ^{15}N-labelled fertilizer in the crop organs was calculated using the equation given by Hauck and Bremner (1976), as follows:

$NC_F = T \times (p - q)/f$

Where NC_F is the weight of N derived from fertilizer (kg ha^{-1}), T the total weight of N in organ (kg ha^{-1}), p the atom% excess ^{15}N in labelled crop, q the atom% excess ^{15}N in control organs that did not receive labelled N (LN0 and EN0 treatments), f the atom excess ^{15}N in labelled fertilizer.

Statistical analysis

ANOVA (Statistica ®) was used for statiscal analysis of harvest date. Differences were compared using the Newman-Keuls test at the 5% level.

		Late harvest	Early harvest
Date	Organ	Biomass (tDM ha^{-1})	Biomass (tDM ha^{-1})
May	Stems	0,4	1,1
June	Stems	7,3	7,1
July	Stems	18,7	17,8
August	Stems	21,4	20,8
October	Stems	23,0	22,4
February	Stems	19,6	
May	Green Leaves	0,7	1,3
June	Green Leaves	3,7	3,1
July	Green Leaves	4,7	4,6
August	Green Leaves	4,1	4,3
October	Green Leaves	2,8	2,7
July	Dead Leaves	0,7	0,8
August	Dead Leaves	1,1	1,2
October	Dead Leaves	2,7	2,6
February	Dead Leaves	0,9	
February	Litter	5,2	
May	Rhizomes	13,1	11,4
June	Rhizomes	9,1	8,7
July	Rhizomes	8,8	9,5
August	Rhizomes	10,7	12,0
October	Rhizomes	17,4	15,6
February	Rhizomes	19,9	
May	Roots	1,4	1,3
June	Roots	0,8	0,8
July	Roots	0,8	0,9
August	Roots	0,7	1,0
October	Roots	1,8	1,1
February	Roots	1,5	
May	Aboveground	1,2	2,4
June	Aboveground	11,0	10,3
July	Aboveground	24,1	23,3
August	Aboveground	26,9	26,6
October	Aboveground	28,5	27,7
February	Aboveground	20,5	
May	Belowground	14,5	12,7
June	Belowground	9,9	9,5
July	Belowground	9,5	10,4
August	Belowground	11,4	13,0
October	Belowground	19,2	16,7
February	Belowground	21,4	16,2
May	Total	15,7	15,1
June	Total	20,9	19,8
July	Total	33,7	33,7
August	Total	38,3	39,6
October	Total	47,7	44,4
February	Total	42,3	

Table 1: Biomass production of *Miscanthus* x *giganteus* in the different organs of the plant, at different dates. Differences between the two harvest dates were not significant ($p > 0.05$)

3. Results

3.1 Biomass production

The aboveground biomass increased from emergence to mid-October and no significant differences were observed between treatments (Table 1). On average, the maximum aboveground biomass was 28.1 tDM.ha^{-1}. The aboveground biomass of the different organs (stems, green leaves and senescent leaves) was not significantly different between treatments at each sampling date. Stem biomass increased from emergence to mid-october and reached 22.7 tDM.ha^{-1} on average. Green leave biomass increased from emergence to mid-July and reached a mean value of 4.7 tDM.ha^{-1}. Thereafter, green leaves biomass decreased until a value of 2.7 tDM.ha^{-1} in October. Leaves senescence began between June and July, and increased until October to reach 2.7 tDM.ha^{-1}.

The belowground biomass decreased until June and July in early and late harvest treatments respectively. On average, the minimum aboveground biomass was 9.7 tDM.ha^{-1}. Thereafter, the belowground biomass increased until October and was 17.9 tDM.ha^{-1} on average. The rhizomes represented 92% of the belowground biomass and roots 8% during the entire growth cycle.

3.2 Nitrogen content and ^{15}N recovery in plants

3.2.1 Nitrogen content in aboveground organs

In May, aboveground N content was significantly higher ($p<0.01$) in E (78 kgN ha^{-1}) than in L (41 kgN ha^{-1}) treatment (Figure 1a). The same trend was observed with the ^{15}N (Figure 1a): 29.4 kgN ha^{-1} came from ^{15}N-labelled fertilizer in E treatment compared to only 1.5 kgN ha^{-1} in L treatment. Thereafter, aboveground biomass N content was significantly higher ($p<0.01$) in L treatment. The aboveground N content increased until mid-July up to 211 and

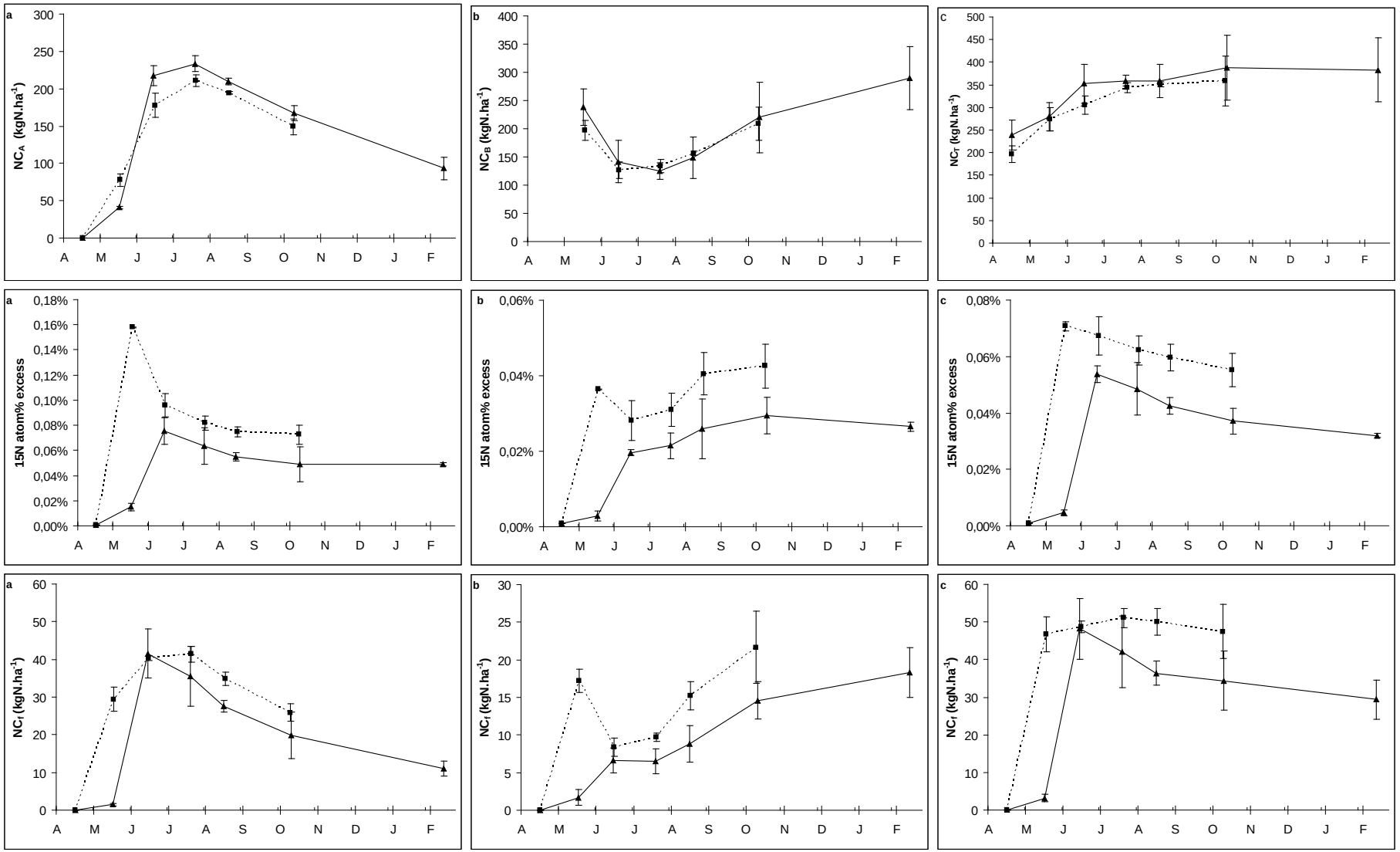


Figure 1: Time-course change in nitrogen content (NC), ^{15}N excess and N uptake from fertilization (NC_f) a) in the aboveground biomass ($_A$), left column; b) in the belowground biomass ($_B$), middle column and c) in the whole plant ($_T$), right column. Continuous lines represent late harvest treatment; dotted lines represent early harvest treatment.

234 kgN ha^{-1} in E and L treatments respectively. N uptake from ^{15}N-labelled fertilizer ended in June and no significant difference was observed between treatments. On average, 39.7 kgN ha^{-1} were coming from ^{15}N-labelled fertilizer in June and July, corresponding to 84% of whole crop ^{15}N-labelled fertilizer uptake (Table 2). During this period and until October, ^{15}N isotopic excess decreased in the aboveground biomass, notably in E treatment, suggesting N uptake from other sources with a lower ^{15}N excess than ^{15}N-labelled fertilizer (Figure 1a).

Thereafter, aboveground N content declined until harvest and was 149 and 65 kgN ha^{-1} in E and L treatments respectively. In February, total nitrogen losses due to leaf abscission were 29 kgN ha^{-1} in L treatment. At harvest, there was 25.9 and 8.6 kgN ha^{-1} of ^{15}N in the aboveground biomass in E and L treatments respectively. In L treatment, 2.5 kgN ha^{-1} of ^{15}N was found in the mulch. The partitioning of ^{15}N-labelled fertilizer in the aboveground biomass at harvest corresponded to 55 and 38% of whole crop ^{15}N-labelled fertilizer uptake in E and L treatments respectively (Table 2).

3.2.2 Nitrogen content in belowground organs

No significant effect of treatments was observed on the belowground N content (Figure 1 b1). N uptake by the belowground biomass from ^{15}N-labelled fertilizer began in May, notably in E treatment (Figure 1b). A part of this N was remobilized to the aboveground organs between May and June in E treatment. In June and July, 7.8 kgN ha^{-1} coming from ^{15}N-labelled fertilizer were found, on average, in the belowground organs, showing that a part of absorbed N was directly stored in the belowground organs, corresponding to 16% on average of whole crop ^{15}N-labelled fertilizer uptake (Table 2).

Thereafter, belowground nitrogen content increased until mid-October and was 209 and 290 kgN ha^{-1} in E and L treatments respectively (Figure 1b). During this period, ^{15}N isotopic

		Late harvest	Early harvest
Date	Organs	^{15}N partitioning %	
May	Aboveground	51%	63%
June	Aboveground	86%	83%
July	Aboveground	85%	81%
August	Aboveground	76%	70%
October	Aboveground	57%	55%
February	Aboveground	38%	
May	Belowground	49%	37%
June	Belowground	14%	17%
July	Belowground	15%	19%
August	Belowground	24%	30%
October	Belowground	43%	45%
February	Belowground	62%	

Table 2: ^{15}N partitioning, as percentage of total 145N-labelled fertilizer uptake by the whole crop, in the aboveground and belowground organs of the crop, at different dates, for early (autumn) and late (February) harvests.

		Late harvest	Early harvest
Date	Organs	^{15}N recovery (%)	
May	Aboveground	1,2%	24,5%
June	Aboveground	34,6%	33,6%
July	Aboveground	29,7%	34,5%
August	Aboveground	23,0%	29,1%
October	Aboveground	16,5%	21,6%
February	Aboveground + Litter	10,5%	
May	Belowground	1,4%	14,3%
June	Belowground	5,5%	7,0%
July	Belowground	5,5%	8,1%
August	Belowground	7,3%	12,7%
October	Belowground	12,2%	18,0%
February	Belowground	15,2%	
May	Total	2,6%	38,9%
June	Total	40,1%	40,6%
July	Total	35,1%	42,6%
August	Total	30,3%	41,7%
October	Total	28,7%	39,6%
February	Total	25,8%	

Table 3: ^{15}N recovery (%) in *Miscanthus* x *giganteus* in the aboveground and belowground organs of the crop, at different dates, for early (autumn) and late (February) harvests.

excess increased, suggesting nitrogen remobilization from aboveground to belowground organs (Figure 1 b2). At harvest, 22 and 18 kgN ha^{-1} of applied nitrogen was found in the belowground organs in E and L treatments respectively (Figure 1b), corresponding to 45 and 62% respectively of ^{15}N-labelled fertilizer absorbed by the whole crop (Table 2).

3.2.3 Nitrogen content in the whole crop

No significant effect of treatments was observed on the whole crop N content (Figure 1c). Total N content increased until harvest in both treatments to reach on average 371 kgN ha^{-1}. This increase occurred mainly from emergence until June and July in L and E treatments respectively. Maximum ^{15}N-labelled recovery occurred in June and July in L and E treatments respectively and was on average 49.6 kgN ha^{-1} (Figure 1c), corresponding to 41.4% on average of ^{15}N-labelled fertilizer applied to the crop (Table 3). During this period and until October, ^{15}N isotopic excess decreased in the whole crop, suggesting nitrogen uptake from other sources with a lower ^{15}N excess than ^{15}N-labelled fertilizer (Figure 1c).

3.3 Nitrogen rough fluxes within the plant

3.3.1 Rough N fluxes occurring during plant growth

The N absorbed by the crop was shared between above and belowground organs. Indeed, belowground organs absorbed and stored ^{15}N-labelled nitrogen during nitrogen accumulation in the aboveground organs. Moreover, there was simultaneity between N absorption by the crop and N remobilization within organs. We propose here to study nitrogen fluxes within the crop by using ^{15}N tracing. We have determined different N fluxes: *R* which corresponds to N remobilisation from below to aboveground organs; *M* which corresponds to N remobilisation from above to belowground organs; *Ap* which corresponds to absorbed N allocated to aboveground organs and *Ar* which corresponds to absorbed N allocated to belowground

	M (kgN ha^{-1} d^{-1})	***R*** (kgN ha^{-1} d^{-1})	***Ap*** (kgN ha^{-1} d^{-1})	***Ar*** (kgN ha^{-1} d^{-1})	***A*** (kgN ha^{-1} d^{-1})
Late harvest treatment					
April – May (21,22,23,*M*=0)	**0,00**	**0,49**	**0,83**	**0,49**	**1,32**
May – June (21,22,23,*M*=0)	**0,00**	**3,83**	**2,27**	**0,49**	**2,76**
June – July (21,22,23,*M*=0)	**0,00**	**0,50**	**-0,04**	**0,03**	**-0,01**
July – August (8,9,10,*R*=0)	**0,45**	**0,00**	**-0,39**	**0,35**	**-0,04**
August – October (9,18,19, *R*=0)	**1,15**	**0,00**	**0,39**	**0,14**	**0,53**
October – February (9,18,19, *R*=0)	**0,59**	**0,00**	**0,00**	**-0,03**	**-0,03**
Early harvest treatment					
April – May (21,22,23,*M*=0)	**0,00**	**0,94**	**1,48**	**0,94**	**2,43**
May – June (21,22,23,*M*=0)	**0,00**	**2,20**	**1,25**	**-0,21**	**1,04**
June – July (19,23,*M*=0)	**0,00**	**0,00**	**0,94**	**0,18**	**1,12**
July – August (9,18,19, *R*=0)	**1,25**	**0,00**	**0,66**	**-0,41**	**0,25**
August – October (9,18,19, *R*=0)	**0,94**	**0,00**	**0,10**	**0,03**	**0,13**

Table 4: Mean daily nitrogen fluxes per period within the plant (kgN ha^{-1} d^{-1}). *R* = N remobilisation from below to aboveground organs; *M* = N remobilisation from above to belowground organs; *Ap* = absorbed N allocated to aboveground organs; *Ar* = absorbed N allocated to belowground organs and *A* = Total nitrogen absorption (*Ar* + *Ap*). Data in parenthesis corresponded to the equations and assumptions used for N fluxes calculations.

organs. These fluxes were calculated between two successive sampling dates. We hypothesized that the daily fluxes were stable between two sampling dates and that *R* and *M* could not occur simultaneously.

3.3.2 Daily N fluxes

The detailed N fluxes between each sampling date are shown in (Table 4). From the date of N fertilizer application to the end of remobilization from below to aboveground organs (from February to June or July), *R* was slightly higher in L than in E treatment with 1.61 and 1.57 kgN ha^{-1} d^{-1} respectively but *R* was calculated on different time steps, 95 days in L treatments compared to only 61 days in E treatments.

From the date of N fertilizer application to the end of the N accumulation in aboveground organs in July, *Ap* was slightly higher in E than in L treatment with 1.23 kgN ha^{-1} d^{-1} and 1.02 kgN ha^{-1} d^{-1} respectively and *Ar* was slightly lower in E (0.30 kgN ha^{-1} d^{-1}) than in L (0.34 kgN ha^{-1} d^{-1}) treatment. Total daily N absorption by the crop (*A*) was higher in E than in L treatment with 1.53 kgN ha^{-1} d^{-1} and 1.36 kgN ha^{-1} d^{-1} respectively.

Between July and harvest, *M* was higher in E than in L treatment with 1.09 kgN ha^{-1} d^{-1} and 0.75 kgN ha^{-1} d^{-1} respectively. This was due to difference of remobilization duration between treatments, with 210 days in L treatments compared to only 82 days in E treatments. During this period, *A* was very low in both treatments with 0.19 kgN ha^{-1} d^{-1} and 0.15 kgN ha^{-1} d^{-1} in E and L treatment respectively. In L treatment, *Ap* was nil and *Ar* was 0.15 kgN ha^{-1} d^{-1}. In E treatment, *Ap* was 0.38 kgN ha^{-1} d^{-1} and *Ar* was slightly negative.

3.3.3 Cumulated N fluxes

The amount of N remobilization from below to aboveground organs was higher in L than in E treatment with 144 and 94 kgN ha^{-1} respectively (Figure 2). In July, at maximum N

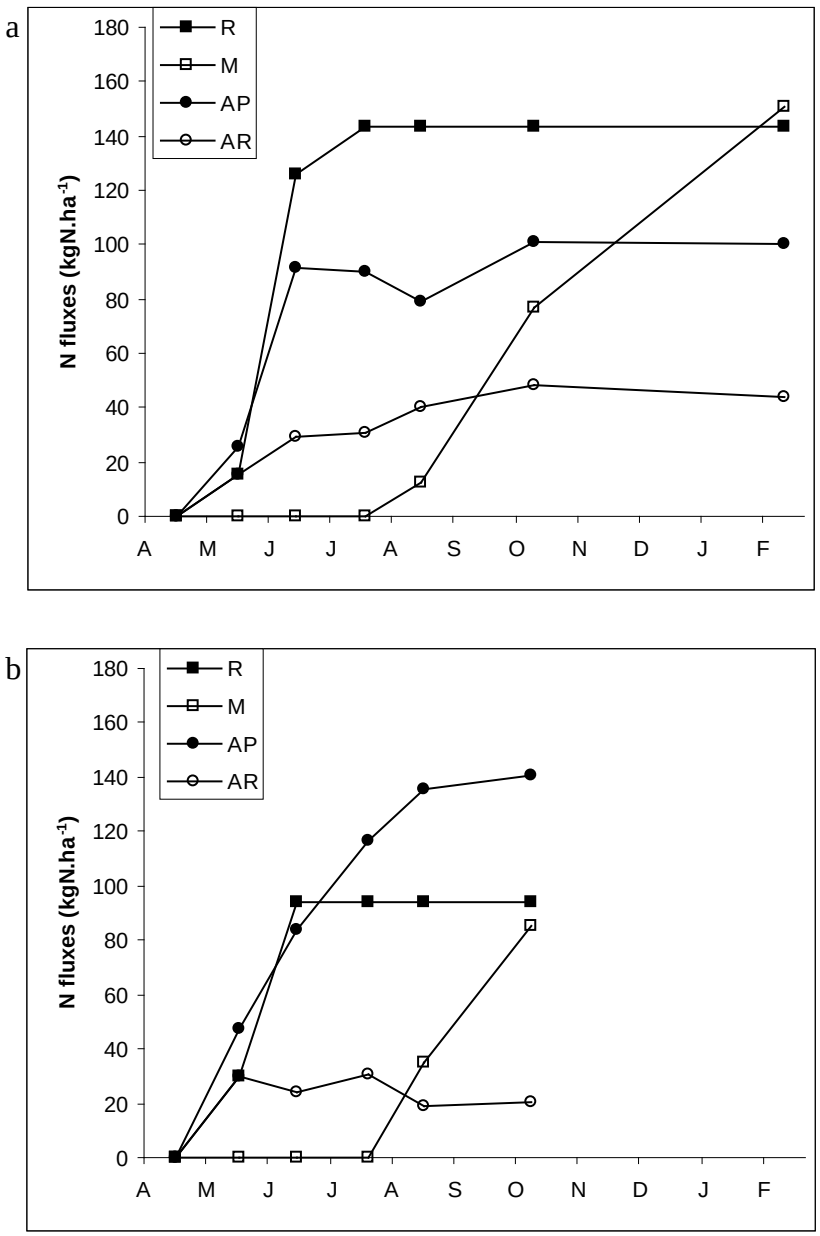


Figure 2: Cumulated nitrogen fluxes (kgN ha^{-1}) within the plant during one growing season a) in L treatment and b) in E treatment. *R* = N remobilisation from below to aboveground organs; *M* = N remobilisation from above to belowground organs; *Ap* = absorbed N allocated to aboveground organs and *Ar* = absorbed N allocated to belowground organs.

accumulation in aboveground biomass, *Ap* was lower in L than in E treatment with 90 and 117 kgN ha^{-1} respectively (Figure 2). *Ar* did not differ between treatments and was around 30 kgN ha^{-1}. Hence, *A* was higher in E than in L treatment with 147 and 121 kgN ha^{-1} respectively.

Between July and October, *M* was similar between treatments with 72 and 77 kgN ha^{-1} in E and L treatments respectively (Figure 2). In L treatments, *M* continued until harvest in February with supplementary 74 kgN ha^{-1}. Hence, in L treatment, total *M* was twice of that observed in E treatment, with 149 kgN ha^{-1}. During this period, *Ap* was low in L treatment with 11 kgN ha^{-1} and higher in E treatment with 24 kgN ha^{-1}. *Ar* was slightly negative in E treatment with 9 kgN ha^{-1} N losses. In L treatment, *Ar* was low with 13 kgN ha^{-1}. Hence, *A* was higher in L than in E treatment with 24 and 14 kgN ha^{-1} respectively. Total N absorption by the crop during the vegetative period was higher in E than in L treatment with 161 and 145 kgN ha^{-1} respectively.

3.3.4 N cycling

In L treatments, the amount of N remobilized from below to aboveground organs in spring and the one from above to belowground biomass during aboveground organs senescence was close with 143 and 151 kgN ha^{-1} (Figure 2). This underlined that the crop was able to recover the same amount of N at the end of the vegetative growth than the amount of N remobilized in spring. Hence, N absorption by the belowground organs (*Ar*) during vegetation period could have allowed the crop to accumulate supplementary N reserves for subsequent years of growth. In L treatment, 50% of nitrogen uptake by the aboveground organs came from N remobilisation of belowground organs and the other 50% from external N uptake (fertilizer, mineralization from soil or N fixation).

The amount of N remobilized from below to aboveground organs influenced allocation of absorbed N in the crop. Indeed, E treatments tended to compensate the lowest N remobilization from below to aboveground organs by a higher allocation of absorbed N to the aboveground organs with, on average, 79% of absorbed N allocated to the aboveground organs in E treatment compared to 69% in L treatment.

3.3.5 ^{15}N recovery uncertainties

We could not calculate total N balance due to the lack of the part contained in the soil organic matter. The decline of ^{15}N-labelled fertilizer recovery observed in L treatments could be due to stems and leaves senescence which occurred between June and August and could have trapped a part of labelled fertilizer but was not measured. Similarly, we did not assess root mortality which can have led to ^{15}N losses. However, this decline was not observed in E treatments. We hypothesized that N-labelled losses observed in L treatments could be due to i) N storage in deeper roots than those collected in the 0-25 cm depth soil during stems senescence, or ii) N exudation by roots. In E treatment, N in senescent stems and leaves could have been re-allocated to growing stems due to the lower N content in the aboveground organs.

4. Discussion

4.1 Biomass production and N content at harvest

Our crops had higher aboveground and belowground biomass dry matter production and higher N stocks in rhizomes than those studied by Christian *et al.* (2006). Surprisingly, the highest aboveground biomass dry matter production observed in our experiment (21.5 vs. 14 t ha^{-1}) was not associated with higher N content compared to those reported by Christian *et al.* (2006) (61.6 *vs.* 90.2 kgN ha^{-1}). This could be due to greater N remobilization from above to belowground organs in our experimental conditions. Hence, it seemed that N remobilisation varied between sites. Further studies are needed to determine the effect of environmental factors like photoperiod or temperatures and phenological stage of the crop on N remobilization from above to belowground organs.

4.2 Recovery of ^{15}N-labelled fertilizer

At harvest in February, ^{15}N-labelled fertilizer recovery in the whole crop (Table 2) was 30% lower in our experiment than in the study of Christian *et al.* (2006) who reported that 55.5% of ^{15}N-labelled fertilizer was recovered in the whole crop. In our experiment, ^{15}N-labelled fertilizer recovery in the whole crop in L treatment decreased with time, with 41.4% in June and July on average and 25.8% at harvest. Differences of ^{15}N-labelled fertilizer recovery in the whole crop could be due to an earlier N application in our experiment, which could have led to greater N losses by volatilization, denitrification or to immobilization in soil. Recous and Machet (1999) have shown in winter wheat that, as residence time of N fertilizer in the soil increases, more of the N fertilizer is subjected to other processes (gaseous loses, immobilization) to the detriment of uptake by the crop. In addition, ^{15}N-labelled fertilizer recovery in winter wheat was found as positively correlated with its DM growth rate (Limaux *et al.*, 1999). Unfortunately, data on N immobilization in soil were not available and

so we cannot precisely quantify the global ^{15}N balance. Moreover, the higher N application rate to the crop in our study (120 kgN ha^{-1}) compared to the 60 kgN ha^{-1} in the study of Christian *et al.* (2006) could also explain the differences of ^{15}N-labelled fertilizer recovery in the whole crop. Christian *et al.* (2006) reported that 21% of ^{15}N was removed with aboveground biomass at harvest, higher than the 8.5% observed in our L treatment and comparable to the 22% observed in our E treatment (Table 2). As observed by Christian *et al.* (2006), we showed that, in February, the greater proportion of ^{15}N was found in the belowground organs, notably in rhizome when the crop was harvested in February. In case of early harvest in October, the reverse statement was observed with a greater proportion of N-labelled found in the aboveground organs.

4.3 N fluxes within the crop

4.3.1 N absorption

The earlier emergence of the crop and the lower N remobilization observed in E treatment compared to L treatment (Strullu *et al.*, submitted) could explain the earlier and greater 15N absorption observed in May in E treatment. Louhalia *et al*. (1999) showed that N uptake by *Lolium perenne* was regulated by the amount of N reserves available before regrowth, the higher were the N reserves and the lower was the crop N uptake. It appeared that the same phenomenon could occur in *M. giganteus.*

We observed that a part of absorbed N was directly stored in belowground organs as suggested by the study of Strullu *et al.* (submitted), who reported that around 60% of absorbed N was allocated to the aboveground biomass. However, we observed that the crop was able to compensate low N remobilization by changing N allocation between above and belowground organs. This phenomenon was not observed by Strullu *et al.* (submitted) when studying net apparent N fluxes, because nitrogen absorption by belowground organs during N

remobilization from below to aboveground organs could not be distinguished with this methodological approach.

Finally, the lower N absorption by the crop observed between July and October compared to those observed between the date of N fertilizer application and July was probably due to the dry climate observed during summer 2009 which could have limited crop N demand as well as soil N mineralization and N absorption.

4.3.2 N remobilisation

Perennial plants have different strategies of N remobilisation and N uptake from soil during regrowth (Millard and Grelet, 2010). In some species, N uptake from soil is delayed from 20 to 30 days after the beginning of N remobilisation (Millard *et al.,* 2006; Grassi *et al.,* 2002). In few species, N uptake from soil begins at the end of remobilisation process in spring (Millard *et al.,* 2001). In other perennial species like *Jugulans nigra* x *regia* (Frak *et al.,* 2002), *Betula pendula* and *Pinus sylvestris* (Millard *et al.*, 2001), N uptake from soil and N remobilisation occurred concomitantly. We showed that in *Miscanthus* x *giganteus*, N remobilisation and N absorption occurred simultaneously or were slightly delayed.

In L treatment, N remobilization from above to belowground organs represented 65% of maximum aboveground biomass N content, while it represented only 41% in E treatment. These values confirm the apparent N fluxes reported by Strullu *et al.* (submitted). Strullu *et al.* (submitted) determined that there was a linear relationship between N remobilisation from below to aboveground organs and initial belowground N stocks, with a slope of 0.79 and a part of non available N for remobilisation of 39 kgN ha^{-1} (R^2 = 0.84). This relationship was determined by net apparent N fluxes. The proportion of remobilized N from below to aboveground organs calculated in the current study followed the same relationship, meaning that rough fluxes are close to net ones. Hence the belowground organs N absorption observed

in our study during N remobilisation could have either i) been too low to change significantly the relationship or ii) not interacted with N remobilization.

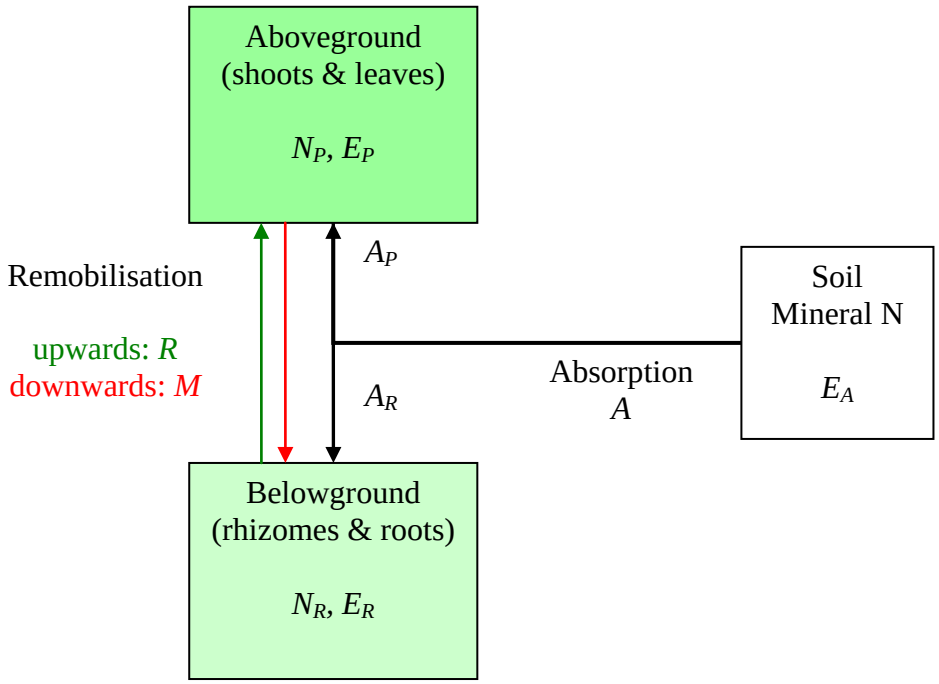
5. Conclusion

This study underlines that N fertilizer uptake by *M. giganteus*, for an application of 120 kgN.ha^{-1} in Mid-April, was low, with maximum ^{15}N recovery of 41% in the whole crop, corresponding to only 33% of total external N uptake by the crop and to 13% of the whole crop N content. The main part of nitrogen uptaken by the aboveground organs comes from N remobilisation from below to aboveground organs and from N mineralization in soil. The proportion of N coming from external uptake and its partitioning within above and belowground organs was dependent on belowground biomass N stocks before regrowth. Finally, it appeared that N uptake by the belowground organs during vegetation period allowed to the crop to accumulate N reserves for subsequent years of growth. *M. giganteus* is able to keep almost close its internal N cycle between years. This pattern could result from a crop species adaptation to frequent defoliation in its original biotope. Indeed, the high efficiency of N remobilisation from above to belowground organs allowed to the crop to recover the amount of N remobilized from below to aboveground organs during regrowth in spring. Further studies are needed in order to have a better understanding of the environmental effects like photoperiod, temperature or water stress on N remobilisation from above to belowground organs. Study on the effect of time and rate of N fertilizer applied to *M. giganteus* is needed in order to increase N recovery by the crop.

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Appendix
Calculation of N fluxes in the crop using ^{15}N tracing



Let be:

N_P the amount of N in the aboveground plant parts (kg/ha)
N_R the amount of N in the belowground plant parts (kg/ha)
E_P the ^{15}N isotopic excess of aboveground plant parts (atom%)
E_R the ^{15}N isotopic excess of belowground plant parts (atom%)
E_A the ^{15}N isotopic excess of the N absorbed by the plant (atom%)

The calculation is based on the differential ^{15}N enrichment which appears in aboveground and belowground organs after the addition of a ^{15}N labelled fertilizer. The basic hypotheses of our compartmental analysis are the following:

- ^{15}N mixes uniformly when entering in any compartment,
- each N flux (R or M, A, A_R, A_P) is positive and constant during each time interval of measurement,
- remobilisation occurs either upwards (R) or downwards (M) but not simultaneously in both directions.

The mass conservation equations are:

a) for total N ($^{14}N+^{15}N$)

$$\frac{dN_P}{dt} = A_P + R - M \qquad (1)$$

$$\frac{dN_R}{dt} = A_R - R + M \qquad (2)$$

b) for ^{15}N in excess (symbol *)

$$\frac{dN_P^*}{dt} = A_P \cdot E_A + R \cdot E_R - M \cdot E_P \quad (3)$$

$$\frac{dN_R^*}{dt} = A_R \cdot E_A - R \cdot E_R + M \cdot E_P \quad (4)$$

Equations (1,3) and (2,4) can be combined to provide the following isotope dilution equations:

$$N_P \cdot \frac{dE_P}{dt} = R \cdot (E_R - E_P) + A_P \cdot (E_A - E_P) \quad (5)$$

$$N_R \cdot \frac{dE_R}{dt} = M \cdot (E_P - E_R) + A_R \cdot (E_A - E_R) \quad (6)$$

1) Downwards remobilisation ($M > 0$; $R = 0$)

1a) General case

Equation (5) can be written:

$$N_P \cdot \frac{dE_P}{dt} = A_P \cdot (E_A - E_P) \quad (7)$$

This equation can be integrated approximately in order to obtain the mean flux A_P during the time interval Δt:

$$A_P \approx \frac{1}{\Delta t} \cdot \frac{\overline{N}_P \cdot \Delta E_P}{\overline{E}_A - \overline{E}_P} \quad (8)$$

where symbols $\overline{N}_P$, $\overline{E}_A$, $\overline{E}_P$ represent the mean value of the variables N_P, E_A, E_P during the time interval Δt. Then M can be calculated using equation (1):

$$M = A_P - \frac{\Delta N_P}{\Delta t} \quad (9)$$

In equation (8), the mean isotopic excess of absorbed N ($\overline{E}_A$) during the time interval Δt can be assessed as follows. Integrating and combining (1) and (2) yields:

$$\frac{\Delta N_P + \Delta N_R}{\Delta t} = A_P + A_R \quad (10)$$

Integrating and combining (3) and (4) yields:

$$\frac{\Delta N_P^* + \Delta N_R^*}{\Delta t} \approx \left(A_P + A_R\right) \cdot \overline{E}_A \quad (11)$$

so that

$$\overline{E}_A \approx \frac{\Delta N_P^* + \Delta N_R^*}{\Delta N_P + \Delta N_R} \quad (12)$$

1b) Particular case

The ^{15}N labelled fertilizer has almost entirely disappeared from the soil mineral N pool, so that $E_A \approx 0$

Equation (1) becomes:

$$\frac{dN_P}{dt} = A_P - M \quad (13)$$

and can be integrated:

$$N_P = N_{P0} - \left(M - A_P\right) t \quad (14)$$

Equation (5) becomes:

$$N_P \cdot \frac{dE_P}{dt} = -A_P \cdot E_P \quad (15)$$

so that

$$\frac{dE_P}{E_P} = \frac{-A_P}{N_{P0} - (M - A_P)t} dt \quad (16)$$

Integration yields:

$$E_P = E_{P0} \left[1 - \frac{M - A_P}{N_{P0}} t \right]^{\frac{A_P}{M - A_P}} \quad (17)$$

The flux A_P over the time interval $[t_0, t_1]$ is:

$$A_P = -\frac{\Delta N_P}{\Delta t} \cdot \frac{\ln\left(\frac{E_{P1}}{E_{P0}}\right)}{\ln\left(\frac{N_{P1}}{N_{P0}}\right)} \quad (18)$$

Then M is calculated using equation (9) and A_R using equation (2):

$$A_R = \frac{\Delta N_R}{\Delta t} - M \quad (19)$$

2) Upwards remobilisation ($R > 0$; $M = 0$)

2a) General case

Equation (6) can be written:

$$N_R \cdot \frac{dE_R}{dt} = A_R \cdot (E_A - E_R) \quad (20)$$

This equation can be integrated approximately in order to obtain the mean flux A_R during the time interval Δt:

$$A_R \approx \frac{1}{\Delta t} \cdot \frac{\overline{N}_R \cdot \Delta E_R}{\overline{E}_A - \overline{E}_R} \quad (21)$$

Then R can be calculated using equation (2)

$$R = A_R - \frac{\Delta N_R}{\Delta t} \quad (22)$$

and A_P using equation (1)

$$A_P = \frac{\Delta N_P}{\Delta t} - R \quad (23)$$

2b) Particular case

The ^{15}N labelled fertilizer is added at a small rate and long before the experiment (e.g. the year before). In this case the soil mineral N pool remains almost unlabelled: $E_A \approx 0$.

Equation (2) can be written:

$$\frac{dN_R}{dt} = A_R - R \quad (24)$$

It can be integrated into:

$$N_R = N_{R0} - (R - A_R)t \quad (25)$$

Equation (6) becomes:

$$N_R \cdot \frac{dE_R}{dt} = -A_R \cdot E_R \tag{26}$$

Using equation (25), equation (26) can be integrated into:

$$E_R = E_{R0}\left[1 - \frac{R - A_R}{N_{R0}}\,t\right]^{\frac{A_R}{R-A_R}} \tag{27}$$

The flux A_R during the time interval $[t_0, t_1]$ is:

$$A_R = -\frac{\Delta N_R}{\Delta t} \cdot \frac{\ln\left(\frac{E_{R1}}{E_{R0}}\right)}{\ln\left(\frac{N_{R1}}{N_{R0}}\right)} \tag{28}$$

with

$$N_{R0} = \frac{N_{R1}^{*} + N_{P1}^{*}}{E_{R0}} \tag{29}$$

Finally R is calculated using equation (22).

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Chapitre 4

Discussion des résultats et éléments de modélisation du fonctionnement pluriannuel de la culture

1. Introduction

La mise en culture de *Miscanthus* x *giganteus* à des fins de production de biocarburant nécessite d'en évaluer les impacts à la fois énergétiques, économiques et environnementaux. A terme, il faudra optimiser sa conduite en termes de densité et condition d'implantation, rythme de coupe, fertilisation azotée, irrigation en fonction du pédoclimat et du mode de valorisation. Ces travaux d'évaluation d'impacts et d'optimisation multicritères doivent prendre également en compte la dimension temporelle inhérente à la phase d'installation de la culture et à sa pérennité (15-20 ans) ainsi que la dimension spatiale, dans la répartition de la culture dans le territoire (Karp et al., 2010). La modélisation déterministe fonctionnelle est un outil privilégié pour assurer l'intégration temporelle et spatiale des relations du système sol-culture-atmosphère (Corwin et Wagenet, 1996 ; Beaudoin et al, 2008).

Nous allons faire la synthèse des avancées des chapitres précédents concernant la définition d'un module mise en réserve de l'azote et son rôle dans la nutrition azoté (fonction de stress) en vue de la modélisation du fonctionnement de la culture à moyen et long terme.

2. Variabilité de la biomasse des organes souterrains et conséquences

La forte variabilité de la biomasse des organes souterrains observée notamment dans le Chapitre 1 peut s'expliquer par différents facteurs :

- La variabilité du développement des plantes au niveau intra-parcellaire.
- La prise en compte volontaire des différences de développement entre les différents blocs servant de répétitions.
- La réalisation de prélèvements destructifs et donc effectués sur des plantes différentes à chaque date.

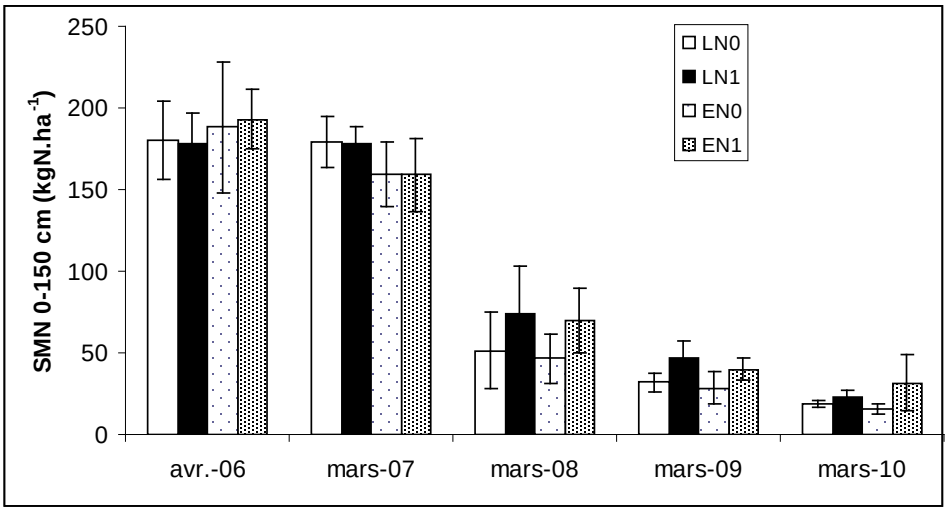


Figure 1: Evolution de l'azote minéral du sol dans les 0-150 cm depuis l'implantation de la culture de *Miscanthus* x *giganteus* en avril 2006. LN0 = coupe tardive sans fertilisation ; LN1 = coupe tardive avec 120 kgN.ha^{-1}.an^{-1} ; EN0 = coupe précoce sans fertilisation ; LN1 = coupe précoce avec 120 kgN.ha^{-1}.an^{-1}.

La teneur en azote des organes souterrains, quant à elle, était plus stable (Chapitre 1). La forte variabilité de la biomasse associée à la faible variabilité de la teneur en azote aboutit à une variabilité importante des réserves en azote des organes souterrains. Cette variabilité a un impact sur les calculs des flux d'azote apparents ou net déterminés dans les Chapitre 1 et 3 de la thèse. En effet les intervalles de confiance associés à ces mesures sont importants. Cependant, nous avons obtenu des relations significatives malgré cette forte variabilité de la biomasse des organes souterrains, grâce à la mise en oeuvre d'un protocole rigoureux.

La prise en compte partielle du système racinaire (0-25cm de sol) et le rôle des racines comme organes de réserve, a pu conduire à une légère sous estimation des flux de remobilisation au printemps.

Les organes souterrains, rhizome et racines, naissent et meurent au cours du temps. Le renouvellement des rhizomes était faible jusqu'en 4ème année de culture, avec une mortalité moyenne de 4%. Le renouvellement des racines quant à lui n'a pas été étudié, ni dans la thèse ni dans la littérature. Plus de recherches sont nécessaires afin de quantifier le taux de renouvellement des organes souterrains sur le long terme.

3. Dynamique de l'azote à moyen et long terme dans une culture de *M. giganteus*

En première année de culture, l'absorption d'azote par la plante est très faible à cause d'un enracinement faible de la culture (0.16 t.ha^{-1} de racines dans les 0-25cm de sol ; O. Postaire, communication personnelle) et d'une production de biomasse aérienne faible (4 à 8 tMS.ha^{-1} ; O. Postaire, communication personnelle). En 2ème année, la forte diminution de l'azote minéral présent dans les 0-150cm de sol (Figure 1) suggère une absorption d'azote importante par la plante et donc un développement important du système racinaire. En effet, les pertes d'azote par lixiviation en fin de deuxième année sont relativement faibles, entre 10 et 12 kgN.ha^{-1} (couplage de mesures de reliquats avec la modélisation du transfert des solutés

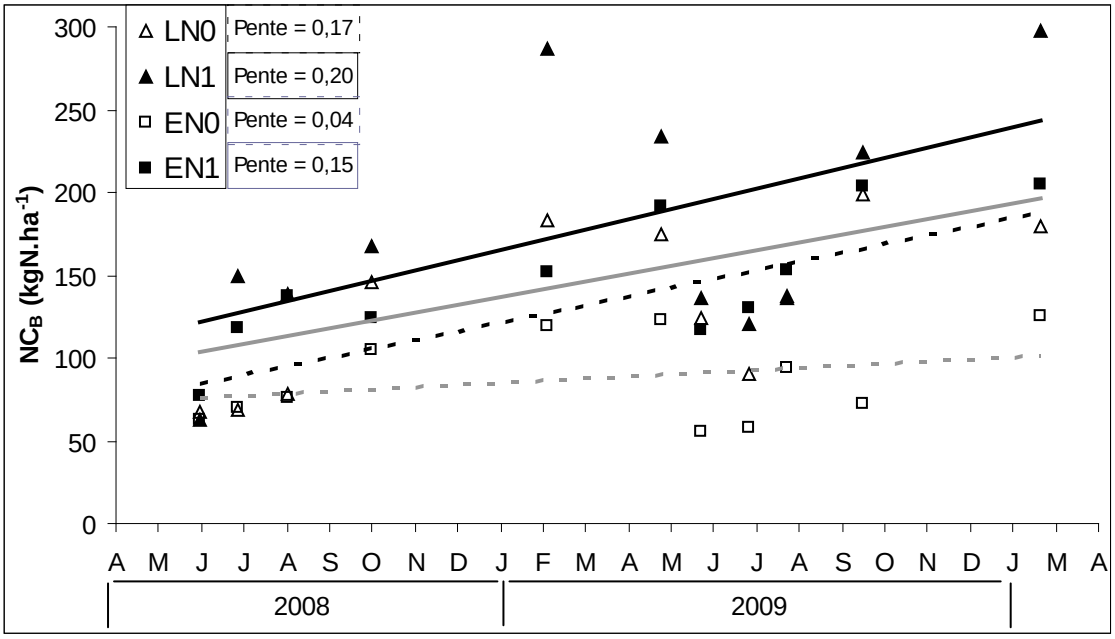


Figure 2: Evolution des réserves en azote contenues dans les organes de réserve (NC_B) de *M. giganteus* au cours des deux années d'expérimentation. LN0 = coupe tardive sans fertilisation ; LN1 = coupe tardive avec 120 $kgN.ha^{-1}.an^{-1}$; EN0 = coupe précoce sans fertilisation ; LN1 = coupe précoce avec 120 $kgN.ha^{-1}.an^{-1}$.

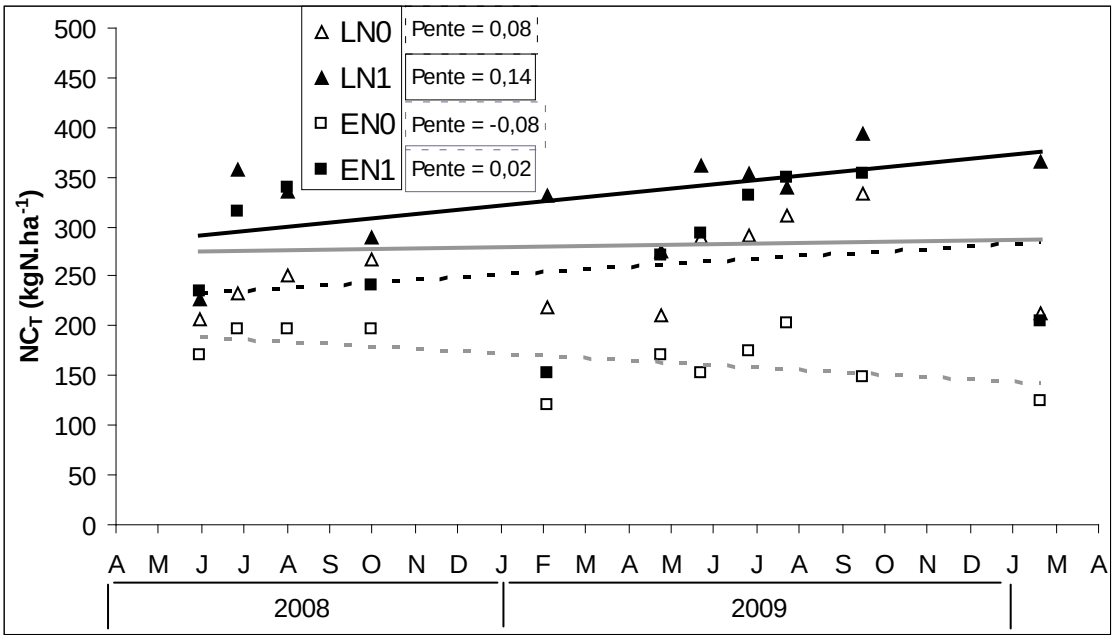


Figure 3: Evolution de la quantité d'azote dans les plantes entières au cours des deux années d'expérimentation. LN0 = coupe tardive sans fertilisation ; LN1 = coupe tardive avec 120 $kgN.ha^{-1}.an^{-1}$; EN0 = coupe précoce sans fertilisation ; LN1 = coupe précoce avec 120 $kgN.ha^{-1}.an^{-1}$.

pendant l'hiver, pour les coupes précoces, Ferchaud *et al.* 2010). Au bout de trois ans de culture, les reliquats azotés du sol sont faibles, mais ce n'est qu'en début de cinquième année qu'ils atteignent une valeur qui pourrait être considérée comme minimale (Figure 1). Il faut signaler ici qu'une culture de *M. giganteus* a un système racinaire dense en surface (1.3 $t.ha^{-1}$ dans les 0-25 premiers centimètres de sol en $3^{ème}$ et $4^{ème}$ année de culture) et qui descend profondément dans le sol (jusqu'à 2.5m), ce qui permet à *M. giganteus* un accès aisé aux ressources hydriques et azotées du sol quelle que soit leur localisation (Neukirchen *et al.*, 1999), une fois la culture implantée.

Les plantes récoltées en coupe tardive accumulent de l'azote au fur et à mesure des saisons de croissance dans leurs organes de réserve (Figure 2), grâce à une efficience de remobilisation élevée de l'azote et à une mise en réserve de l'azote dans les organes souterrains qui se prolonge en fin de croissance et pendant l'hiver. Nos résultats montrent que la fertilisation permet une accumulation d'azote plus importante dans les organes de réserve. Les pentes des droites de régression linéaires sont de 62, 73, 15 et 55 $kgN.ha^{-1}.an^{-1}$ pour les traitements LN0, LN1, EN0, EN1 respectivement. Les quantités accumulées se différencient donc entre traitements, seules les 3 pentes les plus fortes étant significativement différentes de zéro. Dans nos essais, les plantes récoltées en coupe précoce et ne recevant pas de fertilisation azotée n'accumulent pas ou peu d'azote dans leurs organes de réserve.

Les différences entre traitements de l'évolution de l'accumulation d'azote dans la culture sont aussi marquées mais tendent davantage vers l'équilibre avec des pentes nulles. Les pentes des droites de régression linéaires sont de 29, 51, -29 et 7 $kgN.ha^{-1}.an^{-1}$ pour les traitements LN0, LN1, EN0, EN1 respectivement. La quantité d'azote total dans la plante a tendance à diminuer au cours du temps dans les plantes récoltées en coupe précoce et ne recevant pas de fertilisation azotée (Figure 3). Les plantes récoltées en coupe tardive, quant à elles, accumulent de l'azote.

Une coupe précoce sans apport de fertilisant conduira à un appauvrissement progressif du sol en terme de taux d'azote organique et donc de disponibilité en N minéral, mais également à un épuisement progressif des réserves en azote contenues dans les organes souterrains. En coupe précoce, la fertilisation azotée permet de "maintenir" le système avec une quantité d'azote total dans la plante stable au cours du temps. Cependant, cette fertilisation azotée ne semble pas nécessaire en coupe tardive voire néfaste pour le bilan environnemental de la culture compte tenu des pertes d'azote qu'elle engendre sous forme gazeuse ou immobilisation dans le sol (chapitre 3).

La production de *M. giganteus* atteint généralement un état stationnaire au bout de 3-5 ans (Christian *et al.,* 2008) qui n'est pas encore observé ici. Il est probable que le système racinaire atteindra alors un état d'équilibre. Les rhizomes ont un rôle majeur dans le recyclage interne de l'azote dans la culture, et la dynamique ultérieure de l'accumulation d'azote dans la culture. La simulation des bilans d'azote à long terme, nécessite donc de prendre en compte les organes souterrains et de renseigner leur dépendance vis-à-vis des différentes sources d'azote externe (azote minéral du sol, fertilisation). Sur un plan pratique, l'accompagnement de leur évolution pluriannuelle nécessite de mieux connaître la dynamique à long du stockage d'N du rhizome et son taux de renouvellement à l'équilibre.

4. Module de mise en réserve et de remobilisation

4.1 Phase de remobilisation au printemps

Comme observé chez d'autres espèces pérennes comme les arbres (Millard *et al.*, 2010) ou la luzerne (Ourry *et al.,* 1994), nous avons montré que la quantité d'azote remobilisé au printemps chez *M. giganteus* dépend des réserves accumulées dans les organes souterrains lors de l'année précédente. Ce stock permet une accumulation d'azote plus rapide dans les

organes aériens de la plante mais également moins aléatoire que l'absorption de l'azote minéral du sol.

D'après les résultats du Chapitre 1 confirmés dans le Chapitre 3, la quantité d'azote remobilisé entre les organes souterrains et aériens (QN remob) peut être déterminée de la façon suivante :

QN remob = 0.79 * QNR – 39.34

Avec QNR ($kgN.ha^{-1}$) qui correspond aux réserves en azote des organes souterrains à la récolte lors du cycle de culture précédent.

Il est important de noter ici que cette équation ne peut pas s'appliquer à des cultures en $1^{ère}$ année à cause de la valeur de l'ordonnée à l'origine. Les organes souterrains sont alors très peu développés et ne contiennent que peu de réserves azotées (environ 2 $kgN.ha^{-1}$ à l'implantation ; O. Postaire, communication personnelle). Des recherches sont nécessaires sur la phase d'implantation de la culture afin de déterminer la relation qui pourrait s'appliquer lors de cette phase. Une modélisation non linéaire prenant en compte à la fois la phase d'installation du peuplement et la phase de production maximale sera plus générique.

4.2 Phase de mise en réserve de l'azote

La quantité d'azote mis en réserve dans les rhizomes dépend de l'azote accumulé dans la partie aérienne pendant la saison de croissance. L'azote mis en réserve dans les organes souterrains de la plante, et dans une moindre mesure la chute des feuilles pendant l'hiver, permettent de limiter les exportations d'azote du système sol-plante. Une coupe précoce avant la fin de la sénescence de la culture ne permet pas le retour des feuilles au sol et supprime une part importante de la translocation d'azote. La quantité d'azote remobilisé entre les organes aériens et souterrains (QN remob) dans le Chapitre 1 et confirmée dans le Chapitre 3 partie de la thèse, peut être déterminée de la façon suivante:

Pour les coupes tardives : QN remob = 0.71 * QNA – 13.34

Avec QNA ($kgN.ha^{-1}$) qui correspond à la quantité maximale d'azote accumulé dans les organes aériens.

Cette relation ne s'applique pas aux coupes précoces étant donné que la translocation est stoppée par la récolte. Elle ne s'applique pas non plus aux données de la bibliographie pour des coupes tardives (*Cf* discussion Chapitre 1). Nous formulons donc l'hypothèse que les facteurs environnementaux et/ou phénologiques peuvent affecter la mise en réserve de l'azote. Nous n'avons pas pu déterminer ces facteurs qui déclenchent la mise en réserve de l'azote afin de prendre en compte l'effet de la date de coupe et du climat. Différentes hypothèses peuvent être avancées vis-à-vis de ce qui a été observé dans d'autres plantes pérennes comme les arbres (Millard and Grelet, 2010) ou la luzerne (Teixeira *et al.*, 2008) :

1) Effet de la photopériode qui varie principalement en fonction des espèces (Millard and Grelet, 2010)

2) Effet de la température et donc du stade de développement atteint par la culture (Teixeira *et al.*, 2008 ; Millard and Grelet, 2010)

5. Diagnostic de nutrition azoté

5.1 Indicateur de nutrition

L'indice de nutrition azotée est un indicateur souvent utilisé pour connaître le statut de nutrition azotée d'une culture (Gastal et Lemaire, 2002). Son calcul nécessite la connaissance des paramètres de la courbe de dilution critique de l'azote (qui définit, de façon dynamique, la concentration minimale d'azote dans la plante pour une croissance maximale), pour *M. giganteus.* Pour un niveau de production donné, le rapport entre la concentration d'azote observée dans la plante et sa concentration critique est définit comme l'indice de nutrition azotée (INN). La relation entre l'accumulation d'azote dans la biomasse aérienne est robuste

dans des environnements différents mais varie entre espèces, même au sein d'un même groupe métabolique (Lemaire *et al.*, 2007).

Nous avons vu que les organes souterrains de réserve permettaient la fourniture d'une partie importante de l'azote accumulé dans les organes aériens. L'effet de la fertilisation azotée va donc dépendre de l'état des réserves en azote des organes souterrains. Nous avons estimé un seuil situé entre 150 et 187 $kgN.ha^{-1}$ en dessous duquel une réponse significative de la culture à la fertilisation azotée est probable. La réalisation d'une courbe de dilution critique de l'azote devra donc se faire de préférence dans une culture qui aura été récoltée de manière précoce afin de limiter les réserves en azote des organes souterrains.

5.2 Fonction de stress « azote » sur la mise en place de l'indice foliaire

Nous avons déterminé que la mise en place de l'indice foliaire dépend de l'accumulation d'azote dans les organes aériens. L'intérêt de cette relation est également de permettre la simulation de la baisse de l'indice foliaire pendant la phase de remobilisation de l'azote des parties aériennes vers les organes souterrains. Cette relation pourrait donc être utilisée pour simuler l'évolution de l'indice foliaire d'une culture de *M. giganteus*, à la fois pendant la période de sa mise en place et pendant sa période de sénescence. Toutefois cette relation dépend de l'efficience d'utilisation de l'azote pour la formation de l'indice foliaire (NUE-LAI). La NUE-LAI dépend à la fois des réserves remobilisées depuis les organes souterrains et de l'azote absorbé venant du sol, et en conséquence, du niveau de nutrition azotée. D'un autre côté, la relation entre l'indice foliaire et l'azote accumulé dans la biomasse aérienne est relativement robuste pour une espèce donnée, mais varie en fonction de l'environnement (Lemaire *et al.*, 2007). Ces approches sont donc une simplification d'une réalité plus complexe.

Nous proposons donc d'utiliser une relation INN/NUE-LAI, qui sera à déterminer quand la courbe de dilution critique sera établie, pour expliquer la variabilité du NUE-LAI et permettre la simulation de l'évolution de l'indice foliaire.

5.3 Fonction de stress N sur la production de biomasse par la culture

Nous avons montré au cours de la thèse que l'efficience d'utilisation des rayonnements (RUE) par *Miscanthus* x *giganteus* est dépendante des réserves azotées accumulées lors de l'année de culture précédente. La relation déterminée dans le Chapitre 2 est la suivante :

RUE = 0.013 * QNR + 1.06

L'utilisation de cette relation permettra de prendre en compte l'état du développement des organes souterrains de réserve de la culture, et donc l'effet temporel de l'évolution des réserves azotées, sur la production de biomasse aérienne par celle-ci.

La relation ci-dessus est établie pour la phase de remobilisation de printemps et d'été. Elle peut être perturbée par l'alimentation hydrique, tel que cela a été démontré pour la luzerne (Durand *et al.*, 1989). A l'échelle de l'année, la variation de la RUE aérienne en fonction de la saison a été mise en évidence pour de nombreuses cultures : Andrade *et al.* (1993) pour maïs Brown *et al.* (2005) pour luzerne, Justes *et al.* (2000) pour colza. Cette variation est lié à la conjugaison du rôle de la température sur la RUE total (Brown *et al.*, 2005) et la variabilité de l'allocation, qui est liée aux température et la photopériode chez la luzerne (Brown *et al.*, 2005 ; Teixeira *et al.*, 2007). Il pourrait donc être intéressant de considérer la stabilité de la relation en fonction de ces variables environnementales sur un jeu de données plus ample.

5.4 Conclusion sur le modèle conceptuel

Nous présentons dans la figure 4 les formalismes et paramètres qui seront utilisables pour la modélisation sur le long terme d'une culture de *M. giganteus*. Nous avons pu renseigner la

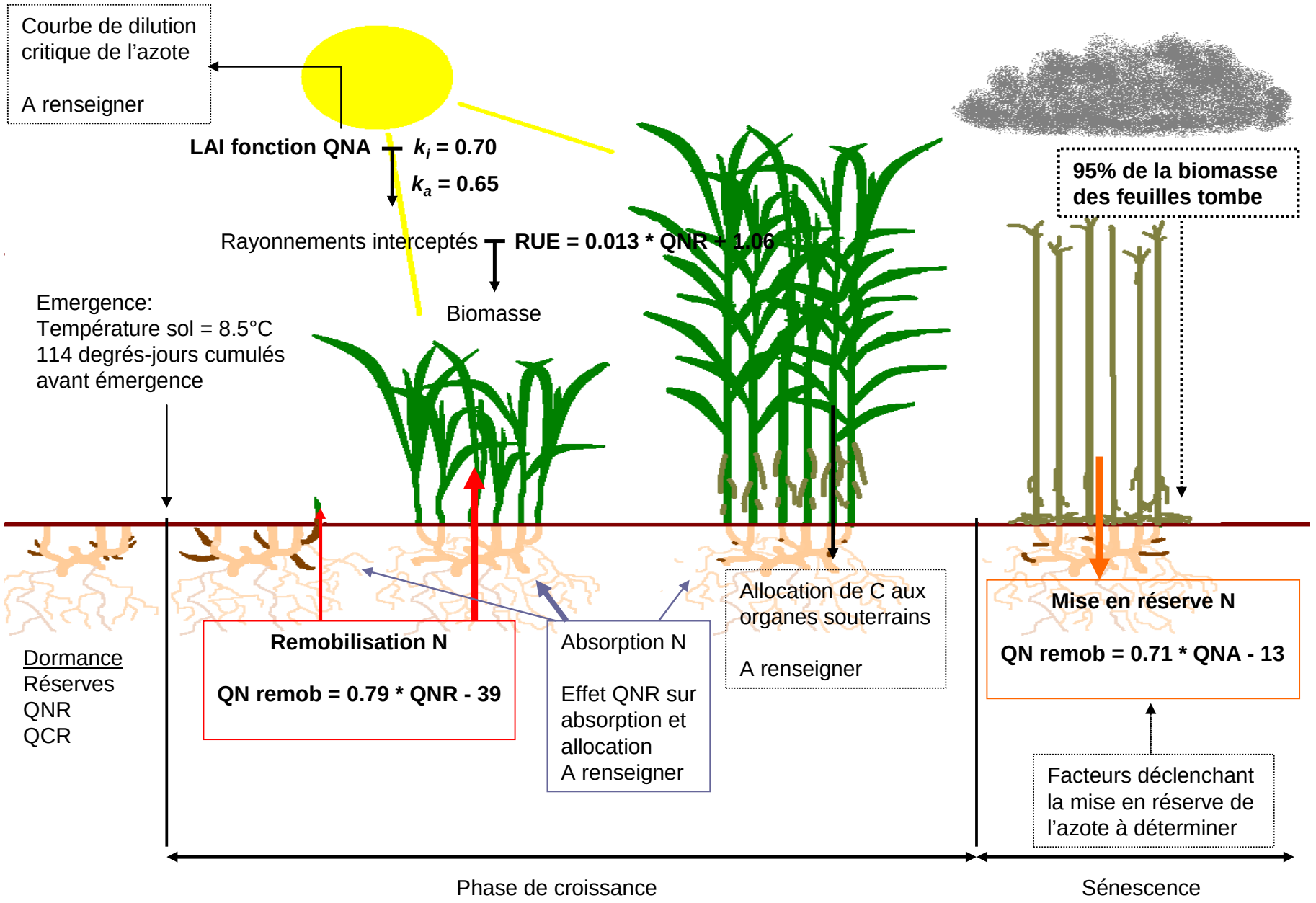


Figure 4: Schéma du fonctionnement d'une culture de *M. giganteus* à partir des résultats de la thèse

phase de remobilisation de l'azote au printemps en déterminant une relation entre la quantité d'azote remobilisé et les réserves en azote contenues dans les organes souterrains. Nous avons également déterminé une relation permettant de faire varier la RUE en fonction des réserves en azote disponibles avant le redémarrage de la culture. La mise en réserve potentielle de l'azote depuis les organes aériens a également été déterminée, mais il faut encore déterminer les facteurs environnementaux et/ou phénologiques déclenchant et influençant ces flux. L'allocation du carbone et de l'azote dans la plante sera également à renseigner dans le futur afin de pouvoir modéliser la phase d'implantation de la culture.

Conclusion et perspectives

Conclusions

Scientifiques

Ce travail de thèse nous a permis de mieux comprendre la physiologie d'une culture de *M. giganteus*, en particulier les relations entre la croissance de la plante et l'azote. Les quantités d'azote contenues dans les organes de réserve sont élevées et peuvent être remobilisées au printemps pour la croissance de la plante. Ces réserves jouent donc un rôle primordial dans la nutrition azotée de la plante en permettant une fourniture d'azote, supplémentaire à celle de l'azote fournit par le sol. Nous avons montré que l'azote accumulé dans les organes de réserve lors de l'année de culture précédente avait un rôle direct sur la production de biomasse par la culture l'année suivante *via* son effet sur l'efficience d'utilisation des rayonnements par la plante. Les organes de réserves, l'allocation d'une partie de l'azote absorbé vers ces organes et l'efficience de mise an réserve de l'azote élevée, permettent à la plante un recyclage et une gestion de l'azote sur le long terme. Les connaissances acquises sur les organes souterrains nous ont permis de déterminer des formalismes qui permettent de prédire les flux de remobilisation et de mise en réserve potentiels de l'azote dans la culture.

Nous n'avons pas étudié les flux de mise en réserve du carbone dans le premier chapitre de la thèse, mais les données expérimentales acquises au cours de la thèse donnent la possibilité de faire ces calculs.

L'efficience d'utilisation élevée de *M. giganteus* liée a son appareil photosynthétique en C4 et à son efficience de remobilisation et de mise en réserve de l'azote lui donnent un avantage indéniable par rapport aux cultures utilisées pour la production de biocarburants 1ère génération, car ces dernières sont entièrement dépendantes de l'azote minéral disponible dans le sol et de la fertilisation.

Finalisées

M. giganteus est capable de produire une biomasse aérienne importante tout en ayant des besoins en azote très faibles voire nuls ainsi qu'en limitant les exportations d'azote du système sol-plante quand il est récolté après la sénescence des organes aériens. Le rapport biomasse sur exportation d'azote qui est beaucoup plus favorable pour une culture de *M. giganteus* (20 $tMS.ha^{-1}$ de biomasse aérienne / 34 $kgN.ha^{-1}$ pour une coupe tardive sans fertilisation) que pour les cultures annuelles. Les références utilisées dans le logiciel de fertilisation AZOFERT ® pour des plantes annuelles sont pour le maïs (9.4 $tMS.ha^{-1}$ de grains / 187 $kgN.ha^{-1}$), la betterave (16 $tMS.ha^{-1}$ de pivot / 160 $kgN.ha^{-1}$), le colza (3.6 $tMS.ha^{-1}$ de grains / 108 $kgN.ha^{-1}$), ou le blé (7.7 $tMS.ha^{-1}$ de grains / 150 $kgN.ha^{-1}$ ou 15.3 $tMS.ha^{-1}$ plante entière/ 190 $kgN.ha^{-1}$) (Jean-Marie Machet ; communication personnelle).

En ce qui concerne les itinéraires culturaux étudiés lors de la thèse, nous avons observé que lorsque la plante est récoltée tardivement, l'apport d'azote par fertilisation permet seulement une consommation de luxe de l'azote. Cependant, dans des situations avec faibles fournitures d'azote par le sol, la fertilisation azotée pourrait s'avérer nécessaire pour permettre à la plante de constituer ses réserves en azote.

Une coupe précoce, même si elle permet d'avoir une production de biomasse supérieure par rapport à une coupe tardive, rend l'apport d'azote par fertilisation indispensable afin de maintenir la quantité de réserves en azote des organes souterrains et donc la production de biomasse par la culture. Un tel itinéraire cultural appliqué en routine à *M. giganteus* nécessitera donc des apports réguliers d'azote et pourrait donc impacter négativement le bilan environnemental et énergétique de cette culture dédiée.

Perspectives

Impacts des phases d'installation et de destruction de la culture

Nous avons obtenu des données concernant une culture commençant à être en pleine production. Une bonne implantation de la culture est nécessaire compte tenu de l'impact du développement des organes souterrains et de leurs réserves en azote sur la production de biomasse par la culture. En effet, celle-ci sera synonyme d'une arrivée en phase de production de biomasse maximale par la culture plus rapide. Plus de recherches sont donc nécessaires sur cette phase cruciale pour la production de biomasse future par la plante. La couverture des besoins en eau par irrigation en cas de sécheresse et une bonne maîtrise des adventices afin de limiter la concurrence pour les ressources hydriques et minérales sont donc indispensables. Parallèlement, les conditions d'entrée en phase de dégénérescence du peuplement nécessitent d'être investiguées. Les enjeux scientifiques consistent à en connaître les déterminismes, les enjeux finalisés concernent aussi bien la production (en terme de date d'occurrence) que les impacts environnementaux (devenir de l'azote et du carbone stocké).

Rôle des réserves en carbone

Il n'y a aucune donnée dans la littérature sur les flux de mise en réserve du carbone chez *M. giganteus*. Cependant, il y a nécessité de préciser si les réserves de carbone ont un rôle actif dans les remobilisations et/ou la pérennité de la culture. On peut aussi noter que les stress hydrique et azoté peuvent influencer directement le flux de carbone assimilé mais aussi la répartition des assimilats carbonés dans la plante.

Rôle du stress hydrique et d'autres carences sur les réserves pérennes

L'impact de l'alimentation hydrique sur la production aérienne à court terme du *M giganteus* a déjà été étudié et modélisé. Cependant, le facteur hydrique est connu pour affecter la

partition des assimilats carbonés dans d'autres cultures, notamment en augmentant l'allocation vers les organes souterrains de réserve. Plus de recherches sont donc nécessaires afin de déterminer les règles de partage des assimilats carbonés dans la plante en fonction de l'âge de la culture, des stress hydrique et azoté et du climat. Les conséquences en terme de mise en réserve du carbone et de l'azote et leur arrière effet sur la production doivent être encore être étudiés.

L'étude réalisée sur l'azote devra être complétée par une analyse des réserves et des flux d'autres éléments minéraux indispensables à la croissance de la plante tels que le potassium, le phosphate ou encore le magnésium. Cette étude pourra également passer par l'étude des courbes de dilution de ces éléments dans la matière sèche aérienne.

Modélisation des interactions sol-culture et sol-climat

Des données complémentaires seront nécessaires au paramétrage d'un modèle sol-plante permettant de simuler la production de biomasse et les impacts environnementaux liés à l'azote, au carbone et à l'eau.

Il sera nécessaire de déterminer la courbe de dilution critique de l'azote pour *M. giganteus* en prenant en compte les organes de réserve. Il faudra également déterminer le rôle de ces organes dans la régulation de la partition des assimilats carbonés et azotés afin de pouvoir renseigner la phase d'implantation de la culture.

La thèse permet d'attirer l'attention à porter aux spécificités de Miscanthus dans la description de certaines interactions entre culture et sol ou culture et climat : les propriétés de stockage de l'eau sur une très grande profondeur (2.5m), le bilan de carbone lié à la mortalité des rhizomes et la rhizodéposition, la réception des précipitations liée à la densité de la canopée, les bilans hydrique et thermique liés à la présence du mulch, la dynamique de minéralisation de l'azote liée au bilan d'azote à long terme en sol non labouré .

Optimisation multicritères des systèmes de culture à vocation énergétique

L'efficience d'utilisation élevée de *M. giganteus* et son efficience de remobilisation et de mise en réserve de l'azote lui donnent un avantage indéniable par rapport à des cultures annuelles. En effet, cela devrait permettre à *M. giganteus* d'avoir un meilleur rendement énergétique pour un même apport de fertilisation azoté, et de limiter les impacts environnementaux négatifs associés à la fertilisation azotée par rapport à des cultures annuelles. Cependant, il faudra intégrer les autres impacts, en particulier sur les aquifères, la production alimentaire ou la biodiversité dans une optimisation multicritères.

Les plantes pérennes ont donc un réel avantage comparé aux plantes annuelles pour une production de biomasse à des fins énergétiques qui soit durable, à condition de leur permettre de finir la mise en réserve des nutriments absorbés par les organes aériens pendant la sénescence de ces derniers. Il convient donc de bien intégrer la dimension temporelle dans les optimisations. L'efficience de remobilisation et de mise en réserve des éléments minéraux et la production de biomasse par hectare sont des caractères primordiaux pour la sélection de plantes pérennes dédiées à la production de bioénergies.

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Abstract

Using dedicated plants in order to produce bioenergy is often presented as one of the solutions in order to limit global warming and to contribute to replacing fossil energy. The use of biofuels will be acceptable only if it allows reducing negative impacts of agriculture on a global (GHG emissions) and a local (nitrate leaching, water consumption) scale. Energy crops have to answer to these requirements in allying high yields per hectare, in order to limit food and non food productions competition. We decided to quantify the role of *Miscanthus* x *giganteus* storage organs in nitrogen nutrition of the crop and to analyse internal nitrogen cycle in the crop during this Phd work, with the aim of modelling these processes on the long term. The experimental approach was done on a three year experiment, crossing two nitrogen fertilisation rates and two harvest dates.

In a first part, we show that aboveground biomass production and nitrogen content in belowground organs were different between treatments. Belowground biomass nitrogen stocks play a key role on nitrogen and carbon accumulation in aboveground organs during plant growth during the subsequent year of growth. We determined strong linear relationships between nitrogen fluxes and nitrogen accumulated in source organs during nitrogen remobilisation in spring and autumn.

In a second part, we show that differences in aboveground biomass production were mainly due to differences of belowground biomass nitrogen stocks before regrowth due to their effect on radiation use efficiency (RUE) of the crop.

In a third part, we underline the low nitrogen fertiliser recovery in the crop thanks to ^{15}N tracing. We also show that rhizomes absorbed nitrogen during nitrogen remobilisation in spring. *M. giganteus* is able to maintain a conservative nitrogen cycle: the amount of remobilised nitrogen in autumn and spring are in the same order of magnitude.

Finally, we draw some conclusions on knowledge and questions on the role of nitrogen remobilisation in nitrogen nutrition of *M. giganteus*. This plays a key role, *via* spring and autumn remobilisation processes that have direct impacts on biomass production by the crop. Taking into account belowground biomass nitrogen stocks is essential in order to succeed in an operational model which allows us to simulate the biomass production by the crop and the nitrogen balance on the long term.

Key words: bioenergy, nitrogen, rhizomes, remobilisation, harvest date, fertilisation.

Résumé

L'utilisation de plantes dédiées pour la production d'énergie est souvent présentée comme l'une des solutions pour limiter le réchauffement climatique et contribuer au remplacement des énergies fossiles. La production de biocarburants sera durable si elle contribue à réduire les impacts négatifs de l'agriculture au niveau global (émissions de GES), mais aussi local (lessivage des nitrates, consommation en eau). Les cultures énergétiques doivent satisfaire ces exigences tout en alliant un rendement élevé à l'hectare, afin de limiter la concurrence entre productions alimentaires et non alimentaires. Nous avons orienté la thèse vers la quantification du rôle des organes de réserve de *Miscanthus* x *giganteus* dans la nutrition azotée de la plante et l'analyse du cycle interne de l'azote dans la culture, en vue d'une modélisation fonctionnelle de ces processus à long terme. L'approche expérimentale s'appuie sur un essai de longue durée de 3 ans, croisant 2 doses d'azote et 2 dates de coupe.

Dans une première partie, nous montrons que les traitements ont différencié la production de biomasse aérienne et la teneur en azote des rhizomes. Les réserves souterraines jouent sur l'accumulation d'azote et de carbone dans les parties aériennes au cours de la croissance le printemps suivant. Il existe des relations linéaires étroites entre les flux d'azote et l'état des organes source d'azote lors des phases de remobilisation de l'azote au printemps et à l'automne.

Dans une deuxième partie, nous montrons que ce sont principalement les réserves en azote des parties souterraines avant le redémarrage de la culture qui expliquent les différences de production de biomasse en affectant l'efficience de bioconversion des rayonnements (RUE).

Dans une troisième partie, nous mettons en évidence la faible proportion de recouvrement dans la plante de l'azote apporté par la fertilisation, grâce à un traçage au ^{15}N de l'azote apporté. Nous montrons également que le rhizome absorbe en même temps qu'il remobilise. *M. giganteus* est capable de maintenir un cycle de l'azote conservatif : la quantité d'azote remobilisée à l'automne est du même ordre de grandeur que l'azote remobilisé au printemps.

Enfin, nous concluons sur les acquis et questions sur le rôle de la mise en réserve de l'azote dans la nutrition azotée de *M. giganteus*. Ce rôle est primordial, *via* les processus de remobilisation et de mise en réserve de l'azote. Il a un impact direct sur la production de biomasse par la culture. Prendre en compte les réserves azotées des organes souterrains est indispensable pour aboutir à une modélisation opérationnelle qui permette de simuler la production de biomasse par la culture et les bilans d'azote sur le long terme.

Mots clefs : bioénergies, azote, rhizomes, remobilisation, date de coupe, fertilisation.