ParisTech
INSTITUT DES SCIENCES ET TECHNOLOGIES
PARIS INSTITUTE OF TECHNOLOGY

AgroParisTech
INSTITUT DES SCIENCES ET INDUSTRIES DU VIVANT ET DE L'ENVIRONNEMENT
PARIS INSTITUTE OF TECHNOLOGY FOR LIFE, FOOD AND ENVIRONMENTAL SCIENCES



Ecole Doctorale ABIES
Agriculture
Alimentation
Biologie
Environnement
Santé

Doctorat ParisTech

T H È S E

pour obtenir le grade de docteur délivré par

L'Institut des Sciences et Industries du Vivant et de l'Environnement

(AgroParisTech)

Spécialité : sciences agronomiques

présentée et soutenue publiquement par

Fabien FERCHAUD

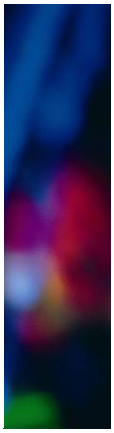
le 26 juin 2015

Etude des bilans d'eau, d'azote et de carbone dans des agrosystèmes dédiés à la production de biomasse en fonction des espèces et des pratiques culturales

Directeur de thèse : **Bruno MARY**

Jury

M. André CHANZY, Directeur de Recherche, UMR EMMAH, INRA	Rapporteur
M. Goetz RICHTER, Docteur, Sustainable Soil and Grassland Systems, Rothamsted Research	Rapporteur
M. Gilles BILLEN, Directeur de Recherche, UMR Sisyphe, CNRS	Examinateur
M. Benoît GABRIELLE, Professeur, UMR ECOSYS, AgroParisTech	Examinateur
M. Bruno MARY, Directeur de Recherche, UR AgroImpact, INRA	Encadrant



INRA SCIENCE & IMPACT

Projet FUTUROL PROCETHOL 2G

bpifrance

AgroParisTech
UR AgroImpact
INRA, Site de Laon, F-02000 Barenton-Bugny, France

Résumé

Les biocarburants de 2ème génération pourraient fournir une énergie renouvelable au secteur des transports et ainsi permettre de lutter contre le changement climatique. Toutefois, leurs bilans gaz à effet de serre, énergétiques et environnementaux seront probablement très dépendants des ressources utilisées. Les cultures lignocellulosiques candidates à la production de biocarburant 2G devront ainsi concilier forte productivité, faibles besoins en intrants et faibles impacts environnementaux. L'objectif de la thèse a été de quantifier les bilans d'eau, d'azote et de carbone à l'échelle de la parcelle, pour différentes cultures candidates et différentes pratiques culturales. Nous nous sommes appuyés sur le dispositif expérimental de long terme « Biomasse & Environnement », mis en place en 2006 à Estrées-Mons, en Picardie. Il compare deux cultures pérennes en C4 (*Miscanthus* × *giganteus* et switchgrass), deux cultures pluriannuelles fourragères en C3 (fétuque et luzerne) et deux cultures annuelles récoltées en plante entière (sorgho fibre et triticale). Il inclut deux niveaux de fertilisation et deux dates de récolte pour les cultures pérennes : récolte précoce (octobre) ou récolte tardive (février). Les mesures effectuées ont porté sur : i) la production de biomasse, ii) l'évolution des stocks d'eau du sol en continu pendant 7 ans, iii) la profondeur et la densité des systèmes racinaires, iv) le drainage et la concentration en nitrate de l'eau drainée, évalués avec le modèle STICS à partir des stocks d'eau et d'azote minéral du sol mesurés en milieu d'automne et fin d'hiver, v) les stocks de carbone organique du sol en 2006 et 2011-2012, vi) le devenir de l'engrais azoté, suivi par marquage isotopique ^{15}N de l'engrais pendant 4 ou 5 années successives.

Grâce à leur enracinement profond, les cultures pérennes et pluriannuelles ont prélevé davantage d'eau que les cultures annuelles, notamment en profondeur. Le drainage sous les cultures pluriannuelles a été plus faible que sous les cultures annuelles (64 contre 133 mm an^{-1} en moyenne sur 7 ans), malgré une production de biomasse équivalente. Il a été intermédiaire pour les cultures pérennes (56-137 mm an^{-1}) et très fortement lié à la production (elle-même fonction de l'espèce et de la fertilisation azotée). La concentration en nitrate a varié de 2 à 23 mg l^{-1}. Elle a été en général plus faible sous les cultures pérennes, sauf pour le miscanthus lors de la première année de mesure. Les stocks de carbone du sol ont augmenté fortement sous les cultures pluriannuelles (+0.93 t C ha^{-1} an^{-1}) mais n'ont pas varié significativement pour les autres cultures. Le ^{15}N retrouvé dans la biomasse récoltée a été plus faible pour les cultures pérennes, particulièrement lorsqu'elles sont récoltées tardivement, mais cela est compensé par une plus forte proportion de ^{15}N dans leurs organes souterrains et dans le sol. Le ^{15}N retrouvé dans le système sol-plante a été de 69% de l'azote apporté pour les cultures pérennes, 61% pour les cultures pluriannuelles et 56% pour les cultures annuelles, ce qui suggère que des pertes importantes ont eu lieu par volatilisation et dénitrification. Dans nos conditions pédoclimatiques, les cultures pérennes en C4 sont les plus intéressantes pour concilier forte production de biomasse, forte efficience d'utilisation de l'eau et de l'azote et faibles pertes d'azote vers l'hydrosphère et l'atmosphère. En revanche, seules les cultures pluriannuelles permettent de stocker du carbone à court terme.

Mots clés : bioénergie, cultures lignocellulosiques, expérimentation au champ, système racinaire, balance hydrique, drainage, nitrate, carbone organique du sol, ^{13}C, fertilisation azotée, ^{15}N, efficience d'utilisation de l'azote

Abstract

Title: Water, nitrogen and carbon balance of bioenergy crops: impact of crop species and cropping practices

Abstract:

Second generation biofuels could provide renewable energy to the transportation sector while mitigating climate change. However, their greenhouse gas, energy and environmental balances will probably depend on the feedstock used for their production. Bioenergy crops that could be used for second generation biofuels will have to fulfil several requirements, including high productivity, low input requirements, and low environmental impacts. The objective of this work was to assess the water, N and C balances at the plot scale for various bioenergy crops with different management. The study is based on a long term field experiment, called "Biomass & Environment", established at the INRA experimental station in Estrées-Mons, northern France. This experiment includes two perennial C4 crops (*Miscanthus* × *giganteus* and switchgrass), two semi-perennial forage C3 crops (fescue and alfalfa) and two annual C4/C3 crops (fibre sorghum and triticale). It compares two nitrogen treatments and two dates of harvest of perennial crops: early (October) or late harvest (February). Measurements have been carried out on: i) biomass production; ii) soil water stocks, monitored continuously during 7 years; iii) root depth and density; iv) drainage and nitrate concentration in drained water, assessed from soil water and mineral N content measurements (in mid-autumn and late winter) and using the STICS model; v) soil organic carbon (SOC) stocks in 2006 and 2011-2012; vi) the fate of ^{15}N-labelled fertiliser applied during 4 or 5 successive years.

Thanks to their deep rooting system, perennial and semi-perennial crops consumed more water than annual crops. The amount of drained water was lower under semi-perennial than annual crops (64 *vs.* 133 mm yr^{-1} average over 7 years), despite an equivalent biomass production. It was intermediate under perennial crops (56-137 mm yr^{-1}) and negatively correlated to biomass production, itself depending on crop species and N rate. Nitrate concentration in drained water varied between 2 and 23 mg l^{-1}. It was generally lower under perennial than other crops, except for miscanthus on the first year of measurement. SOC stocks increased markedly over time under semi-perennial crops (+0.93 t C ha^{-1} yr^{-1}), whereas no significant change occurred under perennial and annual crops. The ^{15}N recovery in the harvested biomass was lower for perennial than other crops, particularly when harvested late, but compensated by a higher ^{15}N recovery in belowground organs and soil. The overall ^{15}N recovery in the soil-plant system was 69% in perennials, 61% in semi-perennials and 56% in annual crops, suggesting that important fertiliser losses occurred through volatilisation and denitrification. In our pedo-climatic conditions, the C4 perennial crops performed best in terms of production, water and nitrogen use efficiency, and nitrogen losses towards the groundwater and the atmosphere. However, only semi-perennial crops yielded in SOC sequestration.

Keywords: bioenergy, bioenergy crops, field experiment, root system, water balance, drainage, nitrate losses, soil organic carbon, ^{13}C abundance, nitrogen fertilizer, ^{15}N, nitrogen use efficiency

Remerciements

Ce travail de thèse de l'école doctorale ABIES d'AgroParisTech a été réalisé au sein de l'unité de recherche INRA AgroImpact à Laon, dans le cadre du projet Futurol. Il a ainsi bénéficié du financement de BPI France (ex-Oséo).

Je remercie en premier lieu Bruno Mary pour avoir dirigé ce travail de thèse. Ses très larges connaissances et sa grande rigueur scientifique m'ont été très précieuses. Je le remercie pour son enthousiasme, sa confiance et son implication.

Mes remerciements vont également aux membres de mon comité de pilotage : Claude Doussan, Benoît Gabrielle et Elise Pelzer.

Merci à André Chanzy et Goetz Richter d'avoir accepté d'être les rapporteurs de cette thèse. Merci également à Gilles Billen et Benoît Gabrielle d'avoir accepté de faire partie du jury. Merci aussi à Eric Justes qui n'a finalement pas pu participer.

Cette thèse n'aurait pas pu voir le jour sans le travail des nombreuses personnes qui ont contribué à la mise en place et à la conduite du dispositif « Biomasse & Environnement ». Je remercie Ghislain Gosse pour avoir lancé les projets biomasse (notamment le projet ANR Regix) au niveau du centre INRA de Lille et permis l'installation du dispositif sur le site d'Estrées-Mons. Un remerciement particulier à tous ceux qui en ont assuré la conduite au quotidien depuis 2006 : Stéphane Cadoux, Matthieu Preudhomme, Emilie Mignot, Charlotte Journel, Guillaume Vitte et Laura Fingar. Merci également aux membres de l'unité expérimentale GCIE pour leur implication sur la conduite des cultures. Je remercie aussi les chercheurs et ingénieurs d'AgroImpact qui se sont impliqués dans le pilotage du dispositif : Nicolas Beaudoin, Hubert Boizard, Stéphane Cadoux, Jean-Marie Machet, Bruno Mary et Marion Zapater.

Une thèse basée sur l'expérimentation au champ, c'est d'abord un travail important de terrain pour les mesures et prélèvements, et au laboratoire pour le traitement des échantillons. Alors, un grand merci à tous les collègues de l'unité AgroImpact qui ont assuré la plus grosse partie des tâches expérimentales : Daniel Boitez, Frédéric Bornet, Bertrand Chauchard, Nicolas Collanges, Caroline Dominiarczyk, Charlotte Journel, Frédéric Mahu, Emilie Mignot, Anita Teixeira, Eric Venet et Guillaume Vitte. Merci à eux d'avoir effectué avec professionnalisme ce travail, qui a pu être parfois ingrat ou fastidieux (Ah, extraire les racines d'échantillons de sol à la pince à épiler…). Merci à Guillaume de m'avoir épaulé

efficacement pour l'organisation des grandes campagnes de prélèvement de 2011 et 2012. Merci également aux stagiaires qui ont passé quelques jours, semaines ou mois sur le dispositif (particulièrement à Laure Le Guen et Odile Sergent que j'ai eu le plaisir d'encadrer) et aux personnes en main d'œuvre occasionnelle. Je tiens également à remercier Manuella Catterou et Olivier Delfosse pour avoir réalisé les analyses isotopiques.

Merci à Frédéric Mahu, Frédéric Bornet, Jérôme Duval et Thomas Laemmel (stagiaire encadré par Bruno Mary) pour leur aide précieuse dans le traitement des données TDR. Merci à Jérôme (et à mon ami breton Erwan Cutullic) de m'avoir incité à m'attaquer au logiciel R, c'est effectivement un super outil ! Merci à Guillaume Vitte pour avoir assuré une bonne partie du traitement des données racinaires et d'autres. Merci à Bassem Dimassi d'avoir partagé avec moi son expérience sur les calculs et analyses statistiques concernant les stocks de carbone. Merci à Nicolas Brunet pour ses conseils en géostatistiques. Je remercie également Julie Constantin pour avoir diffusé sa procédure de « publipostage » permettant de générer des fichiers STICS à la vitesse de l'éclair.

Merci aux co-auteurs des articles pour leur aide et pour les échanges fructueux. Merci aux collègues relecteurs de l'une ou l'autre des parties de cette thèse, ainsi qu'aux *reviewers* anonymes. Je remercie aussi tous les collègues dans l'unité et au-delà avec qui j'ai pu échanger sur la thèse (notamment dans le cadre du projet Futurol) et qui m'ont permis d'avancer dans ma réflexion.

Je souhaite également remercier Valérie Dazin et Brigitte Rocourt, gestionnaires dans l'unité AgroImpact, pour leur aide toujours précieuse sur tous les aspects administratifs.

A l'heure de terminer cette longue aventure (quatre ans et demi), j'ai une pensée particulière pour mes encadrants de stage de fin d'études d'ingénieur, Claudine Thenail et Didier Le Cœur, qui ont été les premiers à me transmettre le virus de la recherche, et pour Patrick Durand, qui m'a fait confiance pour mes premiers pas à l'INRA comme ingénieur contractuel (il y a dix ans déjà !) et m'a par la suite encouragé à me présenter au concours d'ingénieur de recherche. Je remercie aussi Hubert Boizard pour son accueil en 2008 en tant que directeur de l'unité Agro-Impact et pour m'avoir permis de démarrer cette thèse fin 2010.

Je n'oublie pas bien sûr tous ceux rencontrés au cours de ces années et avec qui j'ai pu partager les indispensables moments de détente, pauses cafés (ou plutôt thé), sorties et soirées diverses : Caroline, Julien, Julie, Enguerrand, Magali, Elsa, Thomas, Marie-Laure, Loïc, Cecilia, Anne, Céline, Bénédicte et tous les autres. Merci à eux !

Merci enfin à ma famille et à mes amis pour leur soutien et leurs encouragements.

Table des matières

Résumé……1

Abstract……..2

Remerciements……3

Liste des tableaux.....……..9

Liste des figures……11

1 Contexte et problématique 13

1.1 La transition vers les énergies renouvelables : une nécessité 14

1.2 La biomasse comme source d'énergie et de carbone renouvelable 15

1.2.1 Les biocarburants de première génération 16

1.2.2 Les biocarburants de deuxième génération 20

1.3 L'évaluation des cultures candidates pour la production de biocarburants de 2ème génération 23

1.3.1 Production de biomasse et besoins en intrants 24

1.3.2 Impacts environnementaux 30

1.4 Objectifs et démarche de la thèse 33

1.4.1 Objectif général 33

1.4.2 Démarche de la thèse et questions de recherche 34

1.4.3 Dispositif d'étude 36

2 Soil water uptake and root distribution of different perennial and annual bioenergy crops 39

2.1 Introduction 41

2.2 Materials and methods 42

2.2.1 Study site and experimental design 42

2.2.2 Climatic data 43

2.2.3 Biomass production 44

2.2.4 Soil and soil water monitoring 44

2.2.5 Root mapping 47

2.2.6 Relationship between root density and proportional water capture 47

2.2.7 Statistical analysis 48

2.3 Results 48

2.3.1 Soil water content 48
2.3.2 Maximal soil water deficit 49
2.3.3 Proportional water capture 52
2.3.4 Root distribution and root intersection density 53
2.3.5 Relationship between root distribution and water uptake 57
2.4 Discussion 58
2.4.1 Soil water deficit and water capture 58
2.4.2 Root distribution 60
2.4.3 Relationship between root distribution and water capture 61
2.5 Conclusions 63
3 Drainage and nitrate leaching assessed during seven years under perennial and annual bioenergy crops 65
3.1 Introduction 67
3.2 Materials and methods 68
3.2.1 Site and experimental design 68
3.2.2 Climatic data 71
3.2.3 Crop production 72
3.2.4 Soil water content and mineral nitrogen 72
3.2.5 Nitrate concentration in subsoil 72
3.2.6 Assessment of drainage and nitrate concentration in drained water 72
3.2.7 Statistical analyses 74
3.3 Results 75
3.3.1 Soil water and mineral N contents in mid-autumn and late winter 75
3.3.2 Nitrate concentration in soil and subsoil in 2011 76
3.3.3 Ability of STICS to reproduce SWC and soil nitrate in late winter 77
3.3.4 Evolution of drainage and nitrate concentration in drained water 78
3.3.5 Comparison between obs. and sim. nitrate concentrations in the subsoil. 81
3.3.6 Mean drainage and nitrate concentration over seven years 82
3.3.7 Relationship between biomass production and drainage 83
3.4 Discussion 84
3.4.1 Water balance 84
3.4.2 N leaching and nitrate concentration 85

3.4.3 Perspectives for the development of bioenergy crops 88
4 Changes in soil carbon stocks under perennial and annual bioenergy crops 89
4.1 Introduction 91
4.2 Materials and methods 93
4.2.1 Study site and experimental design 93
4.2.2 Crop yields 95
4.2.3 Soil sampling and analysis 95
4.2.4 Crop residues and belowground biomass 96
4.2.5 Calculation of soil mass, SOC stocks and $\delta^{13}C$ 97
4.2.6 Calculation of new/old SOC stocks 100
4.2.7 Statistical analysis 100
4.3 Results 101
4.3.1 Crop yields 101
4.3.2 Crop residues and belowground biomass 101
4.3.3 Soil bulk densities 104
4.3.4 SOC concentration in the vicinity of belowground organs 104
4.3.5 Initial SOC concentrations and $\delta^{13}C$ 107
4.3.6 Changes in SOC concentrations 110
4.3.7 Changes in $\delta^{13}C$ 112
4.3.8 SOC stocks and mean weighted $\delta^{13}C$ 114
4.3.9 Changes in new and old SOC stocks 118
4.4 Discussion 121
4.4.1 Crop residues and belowground biomass 121
4.4.2 Changes in SOC concentrations and $\delta^{13}C$ 121
4.4.3 Changes in SOC stocks 122
4.4.4 Changes in new and old SOC stocks 123
4.4.5 Effect of management practices 124
5 The fate of cumulative applications of ^{15}N-labelled fertiliser in perennial and annual bioenergy crops 125
5.1 Introduction 127
5.2 Materials and methods 128
5.2.1 Site and experimental design 128

5.2.2 Sampling and analytical procedures 131
5.2.3 Calculations 133
5.2.4 Statistical analyses 134
5.3 Results 135
5.3.1 Crop production and N removal at harvest 135
5.3.2 Crop residues and living biomass 136
5.3.3 *Ndff* and ^{15}N recovery in the exported biomass 139
5.3.4 ^{15}N recovery in crop residues and living biomass 141
5.3.5 ^{15}N recovery in soil 144
5.3.6 Overall ^{15}N recovery 144
5.4 Discussion 145
5.4.1 Crop production and nitrogen removal at harvest 145
5.4.2 N content of crop residues and living biomass 146
5.4.3 ^{15}N recovery in the soil-plant system 147
5.5 Conclusions 151
6 Discussion générale, conclusions et perspectives 153
6.1 Synthèse des résultats obtenus 154
6.1.1 Bilan hydrique 154
6.1.2 Pertes de nitrate et devenir de l'azote de l'engrais 155
6.1.3 Evolution des stocks de carbone du sol 156
6.2 Discussion générale et conclusions 157
6.2.1 Intérêts et limites de la démarche expérimentale 157
6.2.2 Quelles cultures pour les biocarburants de deuxième génération ? 158
6.2.3 Quel effet des pratiques culturales sur les bilans environnementaux ? 160
6.2.4 La modélisation comme outil de généralisation 161
6.3 Perspectives 162
Références bibliographiques 165
Annexes 181

Liste des tableaux

Table 1.1. Production de biomasse (t MS ha^{-1} an^{-1}), rendement de conversion (RC, en tep t^{-1} MS) et production de biocarburants (tep ha^{-1} an^{-1}) pour des cultures conventionnelles (biocarburants 1G), des résidus de culture et des cultures lignocellulosiques (éthanol 2G). D'après Gabrielle *et al.* (2014). 27

Table 1.2. Concentrations moyennes en éléments minéraux N, P, K (g kg^{-1} MS) et quantités exportées par tep de biocarburant produit (kg tep^{-1}) pour des cultures conventionnelles, résidus de cultures et cultures lignocellulosiques. D'après Gabrielle *et al.* (2014). 29

Table 2.1. Meteorological data: P = precipitation (mm), PET = Penman potential evapotranspiration (mm) recorded at Estrées-Mons over the period 2007-2013 44

Table 2.2. Soil characteristics measured in the experimental plots 45

Table 2.3. Root intersection density (mean number of root impacts per cm^2) measured for each soil layer and crop 56

Table 2.4. Parameter and statistical criterion values obtained for each crop after fitting the model describing the relationship between root intersection density and proportional water capture. 58

Table 3.1. Treatments of the B&E long-term experiment combining rotation and fertiliser-N rate 69

Table 3.2. Meteorological data: P = precipitation (mm), PET = Penman potential evapotranspiration (mm) recorded at Estrées-Mons over seven years. 71

Table 3.3. Mean soil water, nitrate and ammonium contents over 0-150 cm measured in early November (average of 7 years from 2007 to 2013) and mid-March (average of 7 years from 2008 to 2014) 76

Table 3.4. Annual drained water (mm) calculated between 2007-08 and 2013-14. Values in brackets are standard deviations between blocks. 79

Table 3.5. Factors influencing annual drainage and nitrate concentration in drained water over seven years (2007-08 to 2013-14) 79

Table 3.6. Nitrate concentration (mg NO_3 l^{-1}) in drained water simulated between 2007-08 and 2013-14 (mean annual weighted concentration). 80

Table 3.7. Mean drainage and weighted nitrate concentration calculated over seven years in the different treatments 83

Table 4.1. Physical and chemical soil characteristics measured in 2006 for the two parts of the field trial: west (perennial crops) and east (semi-perennial and annual crops) 93

Table 4.2. Treatments of the B&E long-term experiment combining rotation and fertiliser-N rate 94

Table 4.3. Carbon content (t C ha^{-1}) in harvested biomass from 2006 to 2011. The mean harvested C content is calculated from 2006 to the last year before the soil sampling date (2010 for perennial crops, 2011 for the other crops) 102

Table 4.4. Carbon content (t C ha^{-1}) in crop residues found at soil surface or in soil layers in 2011 for perennial crops and in 2012 for semi-perennial and annual crops (mean of treatments N- and N+).. 103

Table 4.5. Carbon content (t C ha^{-1}) in roots and rhizomes of perennial crops measured in 2011 (mean of treatments N- and N+) 103

Table 4.6. Soil bulk densities (g cm^{-3}) measured in 2006, 2011 and 2012 from 0 to 40 cm depth (mean of N- and N+) 105

Table 4.7. Minimum and maximum values of belowground biomass (BG = roots + rhizomes) of miscanthus and switchgrass found in individual soil cores and the regression equation between BG and three variables: C (SOC concentration), $\delta^{13}C$ (SOC composition) and C_{New} (SOC concentration derived from the new crop) 106

Table 4.8. Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted $\delta^{13}C$ ($\delta^{13}Cw$, ‰) measured at the start of the experiment (2006) in the two parts of the field: perennial and semi-perennial/annual crops 114

Table 4.9. Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted $\delta^{13}C$ ($\delta^{13}Cw$, ‰) on ESM basis measured (a) in 2011 for perennial crops and (b) in 2012 for semi-perennial/annual crops (mean of N- and N+)..... 116

Table 4.10. Proportion of the soil organic carbon stock on an equivalent soil mass basis derived from the new crops (%) in 2011 for perennial crops and in 2012 for semi-perennial crops 120

Table 4.11. Change in new (C_{New}) and old (C_{Old}) SOC stocks (t C ha^{-1} yr^{-1}) calculated from the $\delta^{13}C$ analyses (Eq 11-13) on ESM basis between 2006 and 2011 for perennial crops or 2012 for semi-perennial crops (mean of N- and N+). 120

Table 5.1. Rotations of the B&E long term experiment..... 130

Table 5.2. Nitrogen fertilisation rates applied to the B&E long term experiment using ^{15}N-labelled UAN..... 130

Table 5.3. Mean harvested biomass (t DM ha^{-1} yr^{-1}) and nitrogen exported (kg ha^{-1} yr^{-1}) from 2007 to 2010 (perennial crops) or 2011 (other crops)..... 136

Table 5.4. Crop residues and living biomass (t DM ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops 137

Table 5.5. N content in crop residues and living biomass (kg ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops..... 138

Table 5.6. Ndff in the exported biomass (kg N ha^{-1}) during each year, N derived from fertiliser and other sources and ^{15}N recovery (%) in the exported biomass over the whole period..... 140

Table 5.7. ^{15}N recovery (%) measured in crop residues and living biomass in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops 142

Table 5.8. ^{15}N recovery (%) measured in soil layers in 2011 for perennial crops and in 2012 for semi-perennial/annual crops. Layers L1, L2, L3, L4 and L5 correspond to ca. 0-5, 5-18, 18-32, 32-38 and 38-58 cm respectively..... 143

Liste des figures

Figure 1.1. Part des énergies renouvelables (RE) dans la production totale mondiale d'énergie primaire en 2008 (IPCC, 2011). 15

Figure 1.2. Principales filières et matières premières des biocarburants de 1ère génération (source : IFPEN). 17

Figure 1.3. Emissions de GES au cours du cycle de vie des carburants fossiles et des biocarburants (FAO, 2008). 18

Figure 1.4. Principales filières et matières premières des biocarburants de 2ème génération (source : IFPEN). 20

Figure 1.5. Production moyenne annuelle de biomasse pour les différentes cultures du dispositif B&E sur la période 2007-2010. Les barres représentent les écarts types entre blocs. D'après Cadoux *et al.* (2014). 25

Figure 1.6. Relation entre le rapport coût bénéfice pour les émissions de GES (Meqt/M) et le rapport N/C de la biomasse exportée. D'après Cadoux *et al.* (2014). 28

Figure 1.7. Localisation et vue aérienne au printemps 2010 du dispositif « Biomasse & Environnement » 36

Figure 2.1. Evolution of the soil water content (SWC, 0-210 cm) over time (from July 2007 to November 2013) for each crop 49

Figure 2.2. Soil water deficit (SWD) calculated for each crop over the period 2007-2013. SWD is the mean (over 7 years) of the maximal values of SWD (0-210 cm) encountered during each year 50

Figure 2.3. Relationship between the maximal soil water deficit and the water balance (P+I-PET) observed each year for each crop. 51

Figure 2.4. Relationship between the maximal soil water deficit and the aboveground biomass production observed each year for each crop. 52

Figure 2.5. Proportional water capture (PWC) versus depth for each crop (Mis = miscanthus; Swi = switchgrass; Fes = fescue; Alf = alfalfa; Sor = sorghum; Tri = triticale). PWC is calculated at the date of the maximal soil water deficit (mean value over the period 2007-2013) 53

Figure 2.6. Spatial distribution of roots observed on the vertical trench wall (180 cm × 300 cm) for each crop in 2010 54

Figure 2.7. Relationship between proportional water capture (PWC) and root intersection density (RID) for each crop. PWC was calculated at the date of maximal soil water deficit in 2010: a) RID expressed in linear scale; b) RID expressed in logarithmic scale 58

Figure 3.1. Map representing the experimental design of the B&E long-term experiment. All plots are 12 × 30 m (360 m^2) and the whole field is 2.7 ha 70

Figure 3.2. Nitrate concentration in soil solution versus depth measured in November 2011 in N+ treatments of perennial crops (a) and other crops (b) 77

Figure 3.3. Comparison between observed and simulated values with STICS of (a) soil water content (a) and soil nitrate content (b) over 0-150 cm in mid-March for seven years (2007 to 2014). Each point represents one plot for a given year 78

Figure 3.4. Evolution of the mean nitrate concentration in drained water (mg NO_3 l^{-1}) for miscanthus E, switchgrass E, semi-perennial (mean of Fes-Alf and Alf-Fes) and annual crops (mean of Sor-Tri and Tri-Sor) 81

Figure 3.5. Comparison between observed and simulated nitrate concentrations in the subsoil (N+ treatments). The simulated concentration is the mean weighted nitrate concentration simulated over four years (2007-08 to 2010-11) using STICS model. The observed concentration was measured in November 2011 in the subsoil layer containing the amount of water drained during the four years 82

Figure 3.6. Relationship between mean water drainage and mean biomass production (average of seven years) 84

Figure 4.1. Soil layers (L) sampled in 2006 and 2011/2012 and used for soil organic carbon stocks calculations on equivalent soil mass (ESM) basis with corresponding soil depths 99

Figure 4.2. Map representing the spatial variability of soil organic carbon (SOC) concentration (g kg^{-1}) in 2006 for (a) 0-20 cm (L1-2), (b) 20-34.2 cm (L3) and (c) 34.2-40 cm (L4) obtained by ordinary kriging from the sampled points (open circles) 108

Figure 4.3. Map representing the spatial variability of $\delta^{13}C$ (‰) of soil organic carbon in 2006 for (a) 0-20 cm (L1-2), (b) 20-34.2 cm (L3) and (c) 34.2-40 cm (L4) obtained by ordinary kriging from the sampled points (open circles) 109

Figure 4.4. Profile of SOC concentration (g kg^{-1}) on ESM basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops) 111

Figure 4.5. Profile of $\delta^{13}C$ (‰) of SOC on ESM basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops) 113

Figure 4.6. Map representing the spatial variability of (a) SOC stocks (t C ha^{-1}) and (b) mean weighted $\delta^{13}C$ (‰) measured in 2006 in the old ploughed layer, obtained by ordinary kriging from the sampled points (open circles). 114

Figure 4.7. Change in (a) SOC stocks (t C ha^{-1} yr^{-1}) and (b) mean weighted $\delta^{13}C$ (‰ yr^{-1}) on ESM basis in layers L1-2 (2667 t ha^{-1}), L1-3 (4669 t ha^{-1}) and L1-4 (5553 t ha^{-1}) between 2006 and 2011 for perennial crops or 2012 for semi-perennial and annual crops 118

Figure 5.1. Relationship between the ^{15}N recovery and the apparent recovery (%) in the exported biomass of perennial crops (2007-2010) and sorghum N+ (2007-2011) 141

Figure 5.2. Overall ^{15}N recovery (%) measured for perennial (2007-2010) and semi-perennial/annual crops (2007-2011) 145

1 Contexte et problématique

1.1 La transition vers les énergies renouvelables : une nécessité

Depuis le milieu du 20ème siècle, on observe une forte augmentation des concentrations atmosphériques en dioxyde de carbone (CO_2), méthane (CH_4) et protoxyde d'azote (N_2O). La concentration en CO_2 a ainsi augmenté de 40% depuis l'époque préindustrielle pour atteindre 391 ppm en 2011 (GIEC, 2013). Cette augmentation s'explique en premier lieu par l'utilisation massive de combustibles fossiles pour répondre aux besoins énergétiques des différents secteurs économiques (industries, bâtiments, transports, etc.). Le CO_2 et les autres gaz à effet de serre (GES) induisent un « forçage radiatif », qui a déjà entraîné un réchauffement du climat « sans équivoque », d'après les données compilées par le Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC, 2013). Ce réchauffement s'est traduit par une augmentation des températures de l'atmosphère et de l'océan, une diminution de la couverture de neige et de glace et une élévation du niveau des océans. La confrontation de modèles climatiques prenant en compte les différentes sources de forçage radiatif à ces observations, montre qu'« il est *extrêmement probable* que l'influence de l'homme est la cause principale du réchauffement observé » (GIEC, 2013).

D'après les simulations climatiques, la poursuite de ces émissions de GES entraînera un réchauffement supplémentaire, augmentant ainsi la probabilité d'impacts sévères et irréversibles sur les activités humaines et les écosystèmes (IPCC, 2014)[1]. L'évolution de la température moyenne à la surface du globe pourrait être d'environ +4 °C à l'horizon 2100 par rapport à l'époque préindustrielle si l'augmentation des émissions de GES se poursuivait. Limiter le réchauffement climatique en-dessous de +2 °C par rapport à l'époque préindustrielle, et ainsi limiter les risques graves pour les activités humaines et les écosystèmes, nécessitera une réduction substantielle des émissions de GES dans les prochaines décennies.

D'autre part, les ressources fossiles, qui jouent un rôle central dans l'économie mondiale, ont une répartition géographique très hétérogène et sont, par définition, finies. Si les ressources mondiales en pétrole, gaz et surtout charbon sont encore potentiellement importantes, elles seront de plus en plus difficiles et couteuses à extraire (McGlade and Ekins, 2015). Cela constitue une incitation à rechercher des sources alternatives d'énergie. En outre, l'usage des ressources fossiles risque dans l'avenir d'être fortement limité par la question des GES. En effet, dans l'objectif de contenir le réchauffement climatique à 2°C au-dessus de la

[1] « Intergovernmental panel on climate change », équivalent anglophone de GIEC

température moyenne à l'époque préindustrielle, un tiers des réserves de pétrole, la moitié des réserves de gaz et 80% de celles de charbon devraient rester inutilisées d'ici 2050.

Pour répondre à ces enjeux, il sera nécessaire de combiner une diminution de la demande énergétique globale et une réduction du recours aux ressources fossiles dans la consommation d'énergie des différents secteurs économiques, ainsi que de favoriser le stockage du CO_2 (en lien avec l'usage des sols). Parmi les options disponibles, les énergies renouvelables (issues de la biomasse – ou « bioénergies », hydrauliques, éoliennes, solaires, géothermiques et marines) sont appelées à jouer un rôle majeur dans cette transition vers une économie dite « décarbonée » (IPCC, 2011).

1.2 La biomasse comme source d'énergie et de carbone renouvelable

La biomasse, qui désigne l'ensemble de la matière organique d'origine animale et végétale, fût longtemps la première source d'énergie pour les populations humaines, avant le développement des énergies fossiles au cours du 20ème siècle. Aujourd'hui, la biomasse constitue la principale source d'énergie renouvelable dans le monde. En 2008, les énergies renouvelables représentaient 12.9% de la production mondiale d'énergie primaire qui s'élevait à 429 exajoules (Figure 1.1). Parmi ces énergies renouvelables, le premier contributeur était la biomasse (10.2%), dont 60% sous forme d'usages traditionnels (cuisine, chauffage) dans les pays en développement (IPCC, 2011). De même, dans l'union européenne, les énergies renouvelables représentaient en 2010 environ 10% de la consommation d'énergie primaire, soit 172.5 Mtep[2], dont 68% provenaient de la biomasse (EurObserv'ER, 2011).

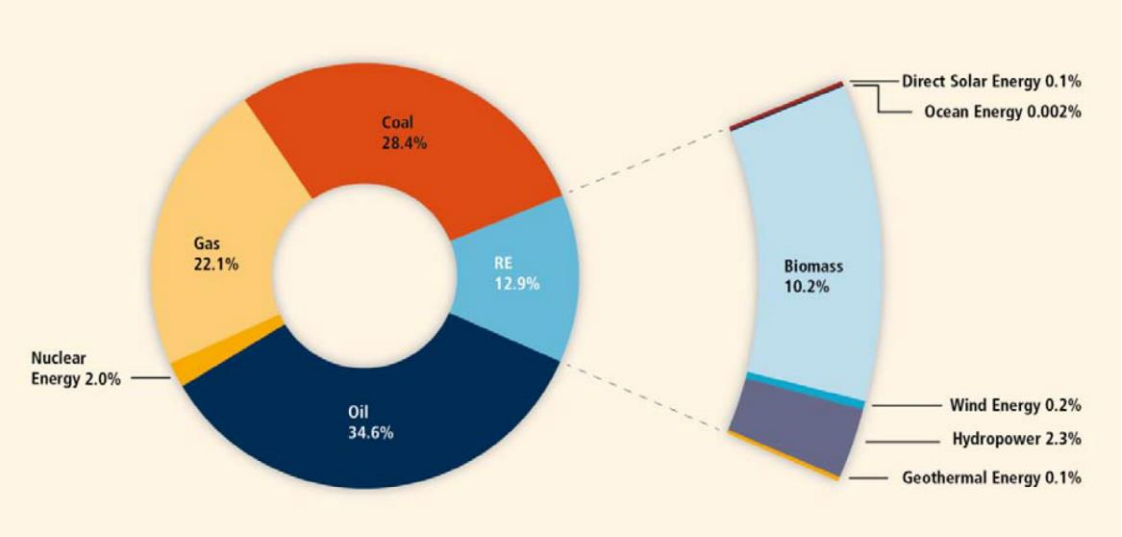


Figure 1.1. Part des énergies renouvelables (RE) dans la production totale mondiale d'énergie primaire en 2008 (IPCC, 2011).

[2] Millions de tonnes équivalent pétrole

Dans les scénarios de transition énergétique étudiés par le GIEC, la bioénergie apparaît comme l'énergie renouvelable ayant le potentiel le plus élevé à l'horizon 2050, devant le solaire et l'éolien (IPCC, 2011). De plus, la biomasse constitue une source de carbone renouvelable qui pourrait remplacer les ressources fossiles non seulement pour l'énergie, mais aussi pour la chimie et les matériaux (Ragauskas *et al.*, 2006; Cherubini, 2010).

Le secteur des transports est un des principaux secteurs économiques émetteurs de GES. Il représentait en 2010 environ 14% des émissions totales à l'échelle mondiale (IPCC, 2014). Ce secteur est extrêmement dépendant des ressources en carburants, aujourd'hui essentiellement d'origine fossile. En effet, la principale alternative existante aux carburants est l'électricité, mais la légèreté et la compacité des carburants leur confèrent encore une capacité de stockage de l'énergie bien supérieure à celle des meilleures batteries (Bessou *et al.*, 2011). Les biocarburants (*biofuels* en anglais), qui sont des « carburants constitués de dérivés industriels tels que les gaz, alcools, éthers, huiles et esters obtenus après transformation de produits d'origine végétale ou animale » (JORF n°168 du 22 juillet 2007 page 12390), constituent une alternative intéressante aux carburants fossiles qui pourrait permettre de réduire les émissions de GES. Au-delà des biocarburants (ou agrocarburants) de 1ère génération (dits aussi « conventionnels ») déjà commercialisés, des recherches sont en cours pour développer des biocarburants de 2ème génération (ou biocarburants « avancés »).

1.2.1 Les biocarburants de première génération

Les biocarburants de 1ère génération (1G) sont synthétisés à partir des organes de réserve de cultures produites traditionnellement pour l'alimentation. On distingue deux filières principales : la filière éthanol qui utilise le sucre ou l'amidon et la filière biodiésel à partir d'huiles végétales (Figure 1.2). La filière éthanol produit de l'éthanol ou de l'ETBE[3] qui sont incorporés à l'essence et la filière biodiesel de l'EMHV[4] incorporé au gazole.

[3] Ethyl Tertio Butyl Ether
[4] Esther méthylique d'huile végétale

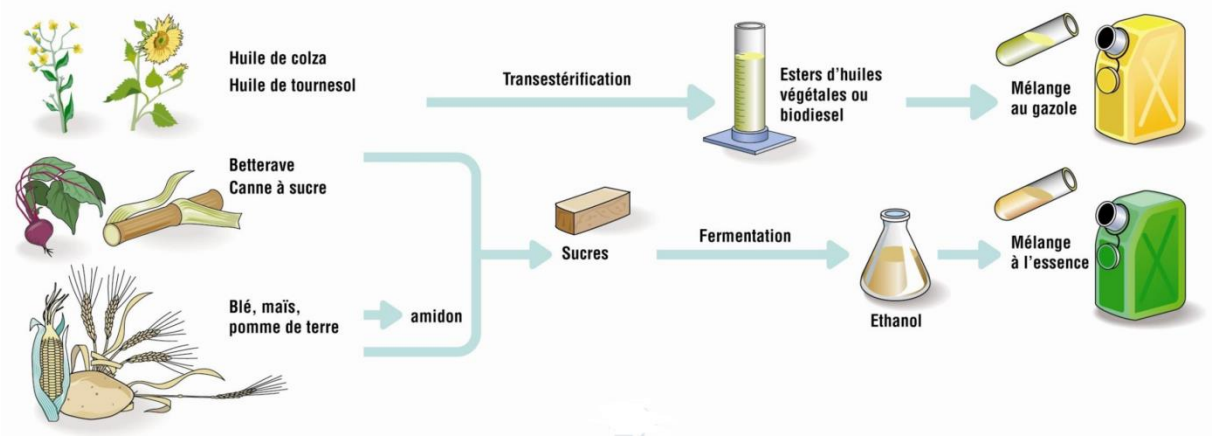


Figure 1.2. Principales filières et matières premières des biocarburants de 1ère génération (source : IFPEN).

La production mondiale de biocarburants 1G a fortement augmenté dans les années 2000, sous l'impulsion de politiques publiques ambitieuses (FAO, 2008). Ainsi, en 2009, la directive dite « énergies renouvelables » (n° 2009/28/CE) fixait pour l'Union Européenne (UE) un objectif de 10% d'énergies renouvelables dans le secteur des transports à l'horizon 2020. En 2013, la consommation de biocarburants dans l'UE était de 13.6 Mtep dont 79% sous forme de biodiesel produit en majorité à partir de colza (EurObserv'ER, 2014). Cette consommation correspond à un taux d'incorporation moyen sur une base énergétique de 4.7% dans le secteur des transports. A l'échelle mondiale, la production atteignait 100 milliards de litres en 2010 (International Energy Agency, 2011) et était largement dominée par l'éthanol avec deux principaux pays producteurs : les USA (principalement à partir de maïs) et le Brésil (principalement à partir de canne à sucre). En 2011, environ 8% de la production mondiale de céréales aurait été consommée par la filière éthanol, alors que la filière biodiesel aurait utilisée respectivement 26% et 13% de la production de colza et de soja (de Cara *et al.*, 2012).

En parallèle du développement des biocarburants 1G, une controverse a pris forme sur leur intérêt et notamment sur leur bilan environnemental. Le premier aspect de cette controverse concerne leur bilan GES. En effet, le CO_2 libéré lors de la combustion des biocarburants a été prélevé dans l'atmosphère par photosynthèse lors de l'étape de production de la biomasse. On peut donc considérer ces émissions comme nulles (carbone en « cycle court »). Cependant, si l'utilisation des biocarburants permet d'éviter les émissions de GES induites par la combustion de leur équivalent fossile, la production de la biomasse puis son transport et sa transformation produisent également des GES. Il convient donc de réaliser un bilan GES de l'ensemble de la filière, en comparaison avec la filière fossile (Figure 1.3). Ce bilan utilise la méthode de l'Analyse de Cycle de Vie (ACV).

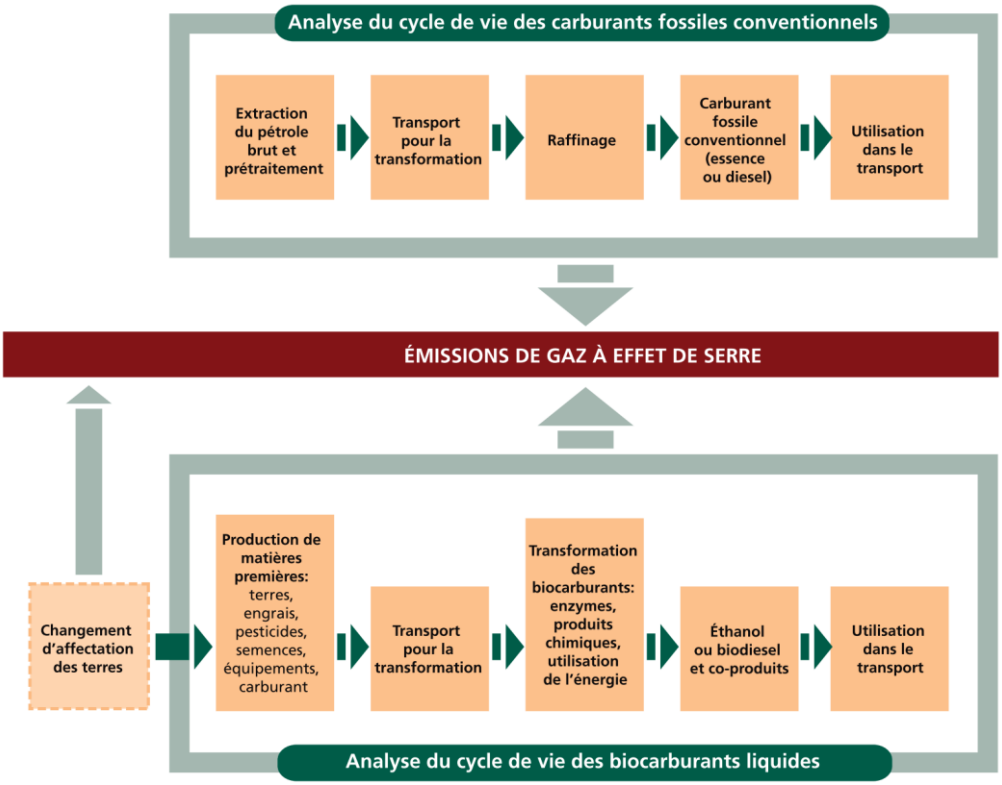


Figure 1.3. Emissions de GES au cours du cycle de vie des carburants fossiles et des biocarburants (FAO, 2008).

Si la plupart des études concluent à des émissions de GES plus faibles pour les biocarburants que pour leurs équivalents fossiles (IPCC, 2011), l'amplitude de cette réduction est très variable selon les filières et les ressources utilisées, ainsi que selon les hypothèses et méthodes de calcul retenues (Whitaker *et al.*, 2010; Bessou *et al.*, 2011). Elle peut être faible, pour des filières pourtant quantitativement importantes comme l'éthanol de maïs. Ainsi, Hill *et al.* (2006) estimaient que le gain de GES permis par la consommation d'éthanol de maïs aux USA n'était que de 12% par rapport à l'essence. Dans certains cas de changement d'affectation des sols (CAS), par exemple lorsque des forêts ou des prairies permanentes sont converties en terres agricoles pour produire des biocarburants, le bilan GES devient même négatif par rapport aux filières fossiles (Fargione *et al.*, 2008). Cela s'explique par les émissions de CO_2 liées au déstockage de carbone (initialement stocké dans le sol ou la végétation) provoqué par ces CAS. En France, les travaux de l'ADEME (2010) évaluaient la réduction des émissions de GES à 59% pour le biodiésel de colza et à 66% pour l'éthanol de betterave (en l'absence de CAS).

Les principales incertitudes sur ces bilans sont liées à la phase agricole de production de la biomasse. Elles concernent en particulier les émissions de N_2O et les émissions liées au

CAS direct ou indirect[5]. En effet, les émissions de N_2O, GES dont le pouvoir de réchauffement global est 296 fois supérieur à celui du CO_2, sont généralement calculées à partir des quantités d'intrants azotés, mais le facteur d'émission à utiliser fait encore débat (Crutzen *et al.*, 2008; Davidson, 2009; Reay *et al.*, 2012). Concernant les CAS, si le CAS direct peut être considéré comme nul en Europe du fait des contraintes réglementaires (ADEME, 2010), le CAS indirect reste difficile à quantifier (de Cara *et al.*, 2012) et n'est le plus souvent pas intégré dans les évaluations. Il pourrait cependant, dans certaines situations, conduire à des émissions de GES supérieures aux carburants fossiles (Searchinger *et al.*, 2008). La méta-analyse conduite par de Cara *et al.* (2012) montre que les émissions de GES liées aux CAS varient fortement en fonction des cultures, du type de biocarburant et des régions considérées : le facteur CAS serait plus faible pour l'éthanol 1G que pour le biodiesel et dans le cas de l'éthanol, pour la betterave que pour le maïs ou le blé (notamment du fait des différences de rendements).

L'autre aspect de cette controverse liée aux biocarburants 1G est lié au fait que ceux-ci sont produits à partir de grandes cultures conventionnelles, avec des itinéraires de production intensifs. Ces biocarburants contribuent donc aux impacts environnementaux de l'agriculture tels que l'eutrophisation des milieux aquatiques[6]. Ils pourraient ainsi avoir, en dehors des émissions de GES, un coût environnemental plus élevé que celui des carburants fossiles (Scharlemann and Laurance, 2008). De plus, leur production induit une demande supplémentaire en biens agricoles par rapport à l'alimentation animale et humaine et pourrait donc contribuer à l'augmentation des prix de ces biens (FAO, 2008). Cet effet sur les marchés, associé à une pression croissante sur les terres agricoles, font craindre le développement d'une concurrence entre énergie et alimentation, dans un contexte de croissance de la population mondiale et donc d'augmentation de la demande alimentaire (Karp and Richter, 2011).

La réglementation européenne a en partie intégré ces différentes limites. Tout d'abord, des critères de durabilité pour les biocarburants ont été adoptés dans la directive n° 2009/30/CE sur la qualité des carburants :

[5] On distingue deux types de CAS. Le CAS direct a lieu lorsque l'implantation d'une culture sur une terre pour produire des biocarburants modifie l'usage de cette terre. Le CAS indirect a lieu quand une demande supplémentaire en biens agricoles pour produire des biocarburants induit par les mécanismes de marché des changements d'affectation sur d'autres terres, afin de produire des biens non destinés à la production de biocarburant.

[6] Détérioration des écosystèmes aquatiques liée à l'accumulation de nutriments.

1) réduction des émissions GES d'au moins 50% dès 2017 puis 60% à partir de 2018 par rapport aux filières fossiles (sans prise en compte du CAS indirect) ;
2) pas de production sur des terres de grande valeur en termes de diversité biologique ou présentant un important stock de carbone (prairies, forêts, tourbières) ;
3) respect des exigences environnementales de la politique agricole commune.

D'autre part, le parlement européen a récemment limité la part des biocarburants 1G à 7% de la consommation d'énergie dans le secteur des transports. De plus, les producteurs devront reporter à la Commission Européenne les émissions estimées de GES liées au CAS indirect (European Parliament, 2015). Le débat sur le bienfondé des politiques publiques traitant des biocarburants reste cependant assez vif.

1.2.2 Les biocarburants de deuxième génération

Les biocarburants de 2ème génération (2G) ne se basent plus sur les molécules de réserve (sucres et huiles) mais sur la lignocellulose, le constituant principal de la paroi des cellules végétales composé de lignines, de cellulose et d'hémicelluloses. Ils permettent donc d'augmenter considérablement la gamme des ressources mobilisables. Les procédés de transformation en cours de développement se divisent en deux types : la voie thermochimique qui passe par une gazéification de la biomasse et aboutit à la production d'hydrocarbures de synthèse et la voie biochimique, qui permet la production d'éthanol (Figure 1.4).

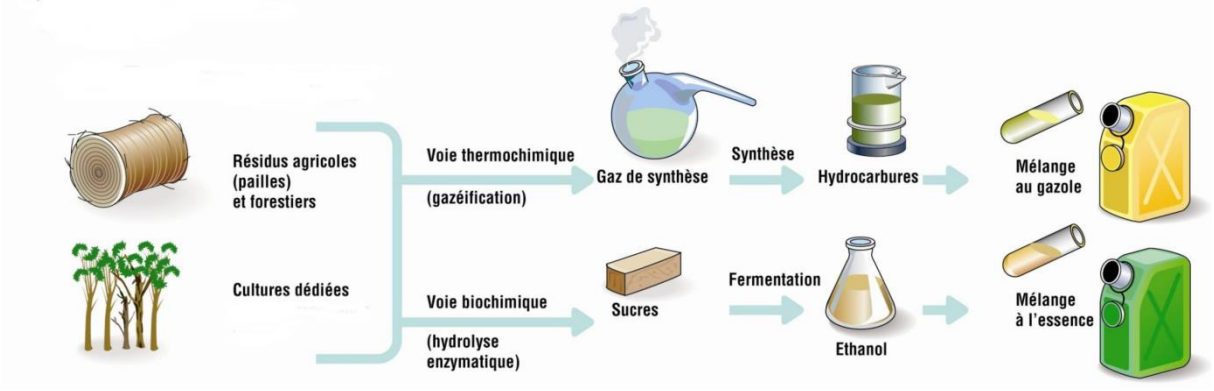


Figure 1.4. Principales filières et matières premières des biocarburants de 2ème génération (source : IFPEN).

La voie biochimique reprend le principe de la production d'éthanol par fermentation, utilisé dans la première génération, mais les sucres sont issus de l'hydrolyse enzymatique de la cellulose et des hémicelluloses. C'est cette voie qu'utilise le procédé développé dans le

cadre du projet Futurol[7]. Si les procédés de transformation sont globalement connus, les travaux actuels visent à les rendre économiquement et énergétiquement viables et à les transférer à l'échelle industrielle (Bessou *et al.*, 2011). Une des difficultés majeures est liée à la nature de la lignocellulose : il s'agit d'un substrat complexe qui présente une forte récalcitrance à l'hydrolyse enzymatique. En effet, les lignines sont reliées par des liaisons covalentes à la cellulose et aux hémicelluloses, ce qui rend difficile la pénétration des enzymes au sein de la structure lignocellulosique. Il est donc nécessaire d'appliquer un prétraitement physicochimique à la biomasse pour rendre la cellulose et les hémicelluloses accessibles aux enzymes (Bessou *et al.*, 2011). Un autre défi sur lequel portent aussi les recherches actuelles consiste à pouvoir fermenter les sucres en C5 (à 5 atomes de carbone) issus de l'hydrolyse des hémicelluloses. La lignine est, elle, brûlée pour fournir l'énergie nécessaire au procédé.

Les ressources lignocellulosiques exploitables par les procédés de 2ème génération sont de différents types. On peut distinguer en particulier deux grandes catégories :

1) les résidus lignocellulosiques d'origine agricole (ex. : pailles de céréales, rafles de maïs), sylvicole (rémanents forestiers), industrielle ou urbaine (ex. : résidus de bois, palettes ou cagettes) ;
2) les cultures lignocellulosiques[8] qui peuvent être des plantes annuelles (ex. : sorgho), pluriannuelles (ex. : fétuque, luzerne), des plantes pérennes herbacées (ex. : miscanthus, switchgrass), ou des plantes pérennes ligneuses (ex. : peuplier, saule) cultivées en taillis à courte ou très courte rotation (TCR, TtCR).

Les résidus lignocellulosiques ont l'avantage, en tant que coproduits (de la production alimentaire, du bois matériaux, etc.) de constituer un gisement immédiatement disponible sans impacter l'utilisation des terres. Ainsi, la production annuelle de résidus agricoles serait au niveau de l'UE de 258 millions de tonnes (Scarlat *et al.*, 2010) et de près de 4 milliards de tonnes à l'échelle mondiale (Lal, 2008), ce qui représente un gisement important, même en tenant compte de la part déjà utilisée pour l'élevage (environ 28 millions de tonnes à l'échelle européenne d'après Scarlat *et al.* (2010)). Par contre, l'exportation systématique de ces résidus entraînerait une diminution des stocks de carbone du sol et pourrait donc avoir des

[7] Futurol est un projet de recherche et développement qui vise à mettre au point et valider un procédé de production d'éthanol par voie biologique à partir de lignocellulose. Différents modules s'intéressent aux étapes du procédé et forment l'axe principal du projet. Celui-ci s'appuie notamment sur une installation pilote, inaugurée en 2011 sur le site de Pomacle-Bazancourt (Marne). L'autre axe du projet est consacré aux ressources candidates, à travers un module « ressources ». Le travail de thèse s'inscrit dans le cadre de ce module financé par BPI France (ex-Oséo).

[8] Dans les chapitres en anglais, ces cultures lignocellulosiques seront appelées *bioenergy crops*.

conséquences négatives à long terme sur la fertilité de ces sols (Saffih-Hdadi and Mary, 2008; Powlson *et al.*, 2011). Ainsi, dans le cas du département de l'Aisne, sur les 660 000 tonnes de pailles potentiellement disponibles chaque année, seul environ un tiers (soit 220 000 tonnes) pourrait être exporté durablement, à condition de cibler les parcelles les moins sensibles (Mary *et al.*, 2009). Etant donnée la taille envisagée des unités de production d'éthanol (entre 50 et 200 millions de litres d'éthanol par an, soit environ 130 000 à 520 000 tonnes de biomasse en considérant un rendement massique de 30%) et les contraintes sur le rayon de collecte, ces résidus lignocellulosiques ne seront probablement pas suffisants. Des cultures dédiées pourraient compléter l'approvisionnement, tout en apportant éventuellement d'autres services.

Les cultures lignocellulosiques sont des cultures pouvant être récoltées en plante entière et offrant *a priori* un bon compromis entre productivité et besoins en intrants. Parmi les cultures candidates, on trouve des cultures traditionnellement utilisées en alimentation animale comme le triticale ou la luzerne et des cultures « dédiées », souvent pérennes comme le miscanthus ou les TCR. Ces cultures dédiées ont généralement été d'abord développées dans l'objectif d'une utilisation en combustion (production de chaleur et/ou d'électricité) ou pour l'industrie papetière, mais elles pourraient être des candidates intéressantes pour la production de biocarburants 2G (Somerville *et al.*, 2010; Don *et al.*, 2011).

Parmi les cultures dédiées, les plantes pérennes herbacées en C4, comme le miscanthus (*Miscanthus* × *giganteus*) et le switchgrass (*Panicum virgatum*) suscitent un vif intérêt (Lewandowski *et al.*, 2003; Heaton *et al.*, 2010; Monti *et al.*, 2012; van der Weijde *et al.*, 2013). Il s'agit de plantes à rhizome, récoltées annuellement (parfois deux fois par an pour le switchgrass), mais dont la durée de production après la première implantation peut être d'une vingtaine d'année. Le rhizome (et les racines associées) jouent un rôle d'organe de réserve : une partie des éléments minéraux et du carbone contenus dans la biomasse aérienne est mise en réserve à l'automne puis remobilisée au printemps lors du début de croissance des nouvelles parties aériennes (Garten *et al.*, 2010; Strullu *et al.*, 2011). Ces cultures peuvent être récoltées en fin d'hiver, avec de faibles teneurs en éléments minéraux et un taux de matière sèche élevé, en utilisant du matériel agricole classique. D'autre part, les TCR et TtCR correspondent à des plantations à forte densité d'arbres à croissance rapide, avec des récoltes tous les 5 à 10 ans (TCR) voire tous les 2-3 ans (TtCR). Des programmes de sélection variétale sont en cours sur plusieurs espèces comme le peuplier et le saule afin d'améliorer la productivité de ces systèmes (Kauter *et al.*, 2003; Karp and Shield, 2008). Ceux-ci ont

l'avantage de demander peu d'intrants et peu d'opérations culturales. Ils nécessitent par contre du matériel spécifique pour l'implantation et surtout la récolte.

En plus de ces cultures pérennes, des cultures pluriannuelles traditionnellement fourragères pourraient aussi être utilisées pour la production de biocarburants 2G (Sanderson and Adler, 2008). En effet, ces cultures sont relativement productives, notamment grâce à une longue saison de croissance, et sont déjà bien maîtrisées par les agriculteurs (implantation, récolte, etc.). Elles ont également l'avantage de pouvoir être implantées et détruites facilement et ainsi d'entrer en rotation avec des cultures alimentaires. Elles nécessitent par contre plusieurs récoltes par an. Parmi ces cultures, la luzerne (*Medicago sativa*) a une importante capacité de fixation symbiotique de l'azote atmosphérique et produit des protéines de qualité pour l'alimentation animale. Avec des procédés de fractionnement adaptés, elle pourrait être produite pour cette double finalité protéines/énergie (Sanderson and Adler, 2008).

Enfin, des cultures annuelles récoltées en plante entière pourraient aussi être utilisées. Ces cultures auraient l'avantage d'être facilement insérables dans les rotations existantes. Parmi les cultures candidates, on peut citer le triticale (× *Triticosecale* Wittmack), une céréale rustique avec une forte production de paille, récoltée en vert ou à maturité, (Lewandowski and Schmidt, 2006; Davis-Knight and Weightman, 2007), et le sorgho (*Sorghum bicolor* (L.) Moench) dont certains types variétaux (dits « sorgho fibre » ou « sorgho biomasse ») à floraison très tardive ont été sélectionnés pour la production de biomasse (Olson *et al.*, 2012; Zegada-Lizarazu *et al.*, 2013).

1.3 L'évaluation des cultures candidates pour la production de biocarburants de 2ème génération

Le contexte développé précédemment montre que le développement des biocarburants devrait se faire avec une triple contrainte (Tilman *et al.*, 2009; Karp and Richter, 2011) : la sécurité alimentaire, la sécurité énergétique et le respect de l'environnement (en particulier la limitation des GES). La diversité des ressources mobilisables constitue dans ce cadre un atout majeur des biocarburants 2G. Cependant, les cultures qui seraient mobilisées pour leur production (en complément des résidus lignocellulosiques) doivent répondre à plusieurs exigences :

1) une production élevée de biocarburant à l'hectare afin de fournir une quantité significative d'énergie tout en limitant la pression sur les terres et donc la concurrence avec la production alimentaire ou les autres usages du sol ;

2) de faibles besoins en intrants et en particulier en fertilisation azotée pour limiter les émissions de GES direct et indirect et ainsi permettre une réduction substantielle des GES par rapport aux filières fossiles ;
3) de faibles impacts environnementaux à l'échelle locale (impacts sur l'eau, l'air, les sols) voire des impacts positifs comme le stockage de carbone dans les sols.

Les différentes cultures candidates doivent donc être comparées sur ces trois critères afin d'une part, de renseigner les ACV et permettre ainsi de préciser l'évaluation environnementale des filières de biocarburant 2G, et d'autre part, de permettre le choix des cultures les plus adaptées pour optimiser le bilan environnemental des biocarburants 2G.

1.3.1 Production de biomasse et besoins en intrants

Si de nombreuses publications fournissent des mesures de rendement pour une culture donnée, peu d'études ont permis de comparer différentes espèces candidates dans les mêmes conditions. Le dispositif « Biomasse & Environnement » (B&E), qui constitue le support principal de cette thèse et sera décrit plus en détails par la suite, permet la comparaison de 6 espèces différentes. Implanté en 2006 à Estrées-Mons (Somme), il regroupe des cultures pérennes (miscanthus, switchgrass), pluriannuelles (fétuque élevée, luzerne) et annuelles (sorgho fibre et triticale). De plus, les cultures pérennes sont conduites soit en récolte précoce (P) au mois d'octobre, soit en récolte tardive (T) en février. Deux niveaux de fertilisation azotée sont également appliqués (N- et N+), avec des doses dépendantes de l'espèce. Les résultats des quatre premières années sur la production et les exportations d'éléments minéraux ont été précédemment publiés (Cadoux *et al.*, 2014). Dans les conditions du dispositif B&E (sol limoneux profond avec une forte réserve utile, climat tempéré), les cultures pérennes miscanthus et switchgrass sont les plus productives, particulièrement en récolte précoce (Figure 1.5).

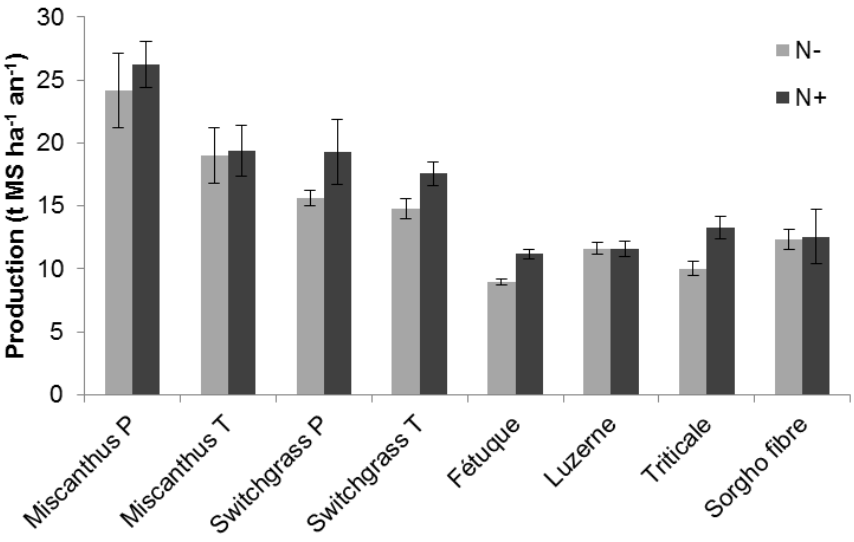


Figure 1.5. Production moyenne annuelle de biomasse pour les différentes cultures du dispositif B&E sur la période 2007-2010. Les barres représentent les écarts types entre blocs. D'après Cadoux *et al.* (2014).

Dans un article de synthèse (Gabrielle *et al.*, 2014), ces données ont été comparées aux valeurs trouvées dans la littérature pour les différentes espèces, aux mesures effectuées sur un réseau de 10 sites en France regroupant les mêmes espèces (Cadoux *et al.*, 2010) et aux rendements grain et paille de cultures conventionnelles (Table 1.1).

Concernant les cultures lignocellulosiques, les données de la littérature montrent une grande variabilité des rendements pour une espèce donnée, probablement à cause de la diversité des conditions pédoclimatiques et des pratiques. Les valeurs médianes des données de la littérature ne peuvent pas être utilisées directement pour classer les cultures entre elles, du fait des interactions entre cultures et conditions pédoclimatiques (Laurent *et al.*, 2015). Par exemple, les données sur sorgho fibre proviennent majoritairement du sud de l'Europe alors que les expérimentations sur saule sont plutôt conduites en Europe du nord. On retrouve également une forte variabilité au sein du réseau Regix (10 sites), avec des valeurs médianes peu différentes entre espèces : le classement des espèces change en fonction du site. Les rendements théoriques de conversion en éthanol 2G, calculés à partir de l'équation proposée par Cadoux *et al.* (2014) et des teneurs moyennes en cellulose, hémicelluloses et lignines trouvées dans la littérature (Gabrielle *et al.*, 2014), sont par contre plus élevés pour les cultures pérennes (du fait de teneurs en cellulose plus importantes) et pour le triticale (en considérant que les grains sont traités par procédé 1G) que pour les autres espèces. La luzerne a un rendement de conversion en éthanol particulièrement faible à cause de sa teneur importante en protéines (qui pourraient cependant être extraites et valorisées par ailleurs). A

l'échelle des 10 sites du réseau Regix, miscanthus, switchgrass et triticale ont ainsi les rendements éthanol les plus élevés.

Concernant les cultures conventionnelles, le colza apparaît comme l'espèce la moins productive et la betterave la plus productive quelle que soit l'échelle considérée. La production de biocarburant obtenue avec la betterave est également plus élevée que celle permise par le colza, malgré un rendement de conversion plus faible pour l'éthanol que pour le biodiesel. Les rendements paille sont globalement équivalents aux rendements grain mais la production d'éthanol est réduite à cause de rendements de conversion plus faibles en 2G.

On peut comparer les rendements en éthanol 2G obtenus sur le dispositif B&E à ceux des cultures conventionnelles dans la Somme. Si les cultures pérennes miscanthus et switchgrass permettent un gain de production important par rapport au colza, la betterave reste la deuxième espèce la plus productive après miscanthus en coupe précoce.

Table 1.1. Production de biomasse (t MS ha^{-1} an^{-1}), rendement de conversion (RC, en tep t^{-1} MS) et production de biocarburants (tep ha^{-1} an^{-1}) pour des cultures conventionnelles (biocarburants 1G), des résidus de culture et des cultures lignocellulosiques (éthanol 2G). D'après Gabrielle *et al.* (2014).

	Production de biomasse (t MS ha^{-1} an^{-1})			RC (tep t^{-1} MS)	Production de biocarburant (tep ha^{-1} an^{-1})		
Cultures conventionnelles (biocarburants 1G) : *rendements moyens réels*							
	UE-27	France	Somme		UE-27	France	Somme
Blé	4.2	6.1	7.3	0.22	0.9	1.4	1.6
Maïs	5.7	7.4	7.9	0.27	1.6	2.0	2.2
Colza	2.7	2.9	3.3	0.40	1.1	1.2	1.3
Betterave	12.3	15.9	16.0	0.25	3.0	3.9	3.9
Résidus de cultures (éthanol 2G) : *rendements moyens estimés*							
	UE-27	France	Somme		UE-27	France	Somme
Blé	4.6	5.8	6.5	0.16	0.7	0.9	1.0
Maïs	5.9	7.2	7.6	0.15	0.9	1.1	1.1
Colza	4.4	4.6	4.9	0.15	0.6	0.7	0.7
Cultures lignocellulosiques (éthanol 2G) : *rendements en parcelles expérimentales*							
	Littérature*	Regix*	B&E		Littérature*	Regix*	B&E
Saule TCR	9 (5-11)	-	-	0.16	1.5 (0.7-1.8)	-	-
Peuplier TCR	6 (2-10)	-	-	0.15	0.9 (0.4-1.5)	-	-
Miscanthus P#	29 (14-60)	-	27	0.16	4.7 (2.3-9.7)	-	4.3
Miscanthus T#	15 (5-43)	15 (3-23)	19	0.16	2.4 (0.8-6.9)	2.4 (0.4-3.7)	3.1
Switchgrass P#	12 (1-22)	-	19	0.15	1.8 (0.2-3.3)	-	2.9
Switchgrass T#	-	14 (5-19)	16	0.15	-	2.2 (0.7-3.0)	2.5
Fétuque	9 (4-14)	11 (3-23)	10	0.12	1.1 (0.5-1.7)	1.3 (0.3-2.8)	1.2
Luzerne	11 (1-17)	14 (3-16)	12	0.09	1.0 (0.1-1.5)	1.2 (0.2-1.4)	1.0
Triticale	13 (5-16)	13 (3-19)	12	0.18	2.3 (0.9-2.9)	2.3 (0.6-3.3)	2.2
Sorgho fibre	26 (16-43)	14 (5-23)	13	0.13	3.5 (2.1-5.7)	1.9 (0.7-3.1)	1.8

* médiane (min-max)

Miscanthus et switchgrass : P = récolte précoce (septembre-novembre) et T = récolte tardive (janvier-avril)

Les rendements grains des cultures conventionnelles sont issus de la moyenne des rendements 2000-2009 d'après Eurostat pour l'UE-27 et Agreste pour la France et la Somme.

Les biomasses de résidus sont calculées à partir des rapports grains/pailles issus de Scarlat *et al.* (2010).

Les rendements des cultures lignocellulosiques proviennent de :

- *Littérature* : revues bibliographiques et compilation d'études individuelles ; voir Gabrielle *et al.* (2014) ;
- *Regix* : Réseau expérimental du projet Regix (10 sites en France), années 2007-2008 (Cadoux *et al.*, 2010) ;
- *B&E* : site expérimental « Biomasse & Environnement » (Somme), 2007-2010 (Cadoux *et al.*, 2014).

La production de biocarburant est obtenue en multipliant la production de biomasse par un rendement de conversion (RC) réel (biocarburants 1G) ou théorique (éthanol 2G) ; voir Gabrielle *et al.* (2014).

Les données du dispositif B&E ont également permis de quantifier les exportations en éléments minéraux : azote (N), phosphore (P) et potassium (K). Ces exportations sont variables entre espèces et les cultures pérennes sont caractérisées par de faibles teneurs en N et P, particulièrement en récolte tardive (Cadoux *et al.*, 2014). A partir de ces données expérimentales, des niveaux de fertilisation « optimisés » (permettant une balance nulle entre apports et exportations) ont été calculés pour chaque espèce. Ces situations ont été utilisées pour quantifier le rapport entre les émissions de GES liées à la fertilisation NPK (*Meqt*) et le gain de GES obtenu par le remplacement du carburant fossile (*M*) : plus ce rapport coût/bénéfice (*Meqt*/*M*) est faible, plus la culture permettra *a priori* d'obtenir un bilan GES intéressant (démarche adaptée de Crutzen *et al.* (2008)). La teneur en N de la biomasse exportée, exprimée par rapport au carbone (C), apparaît fortement reliée à ce rapport, sauf pour la luzerne dont l'azote provient majoritairement de la fixation symbiotique (Figure 1.6). La faible teneur en azote des cultures pérennes et la capacité de fixation symbiotique de la luzerne apparaissent donc comme des atouts majeurs de ces cultures vis-à-vis du bilan de GES.

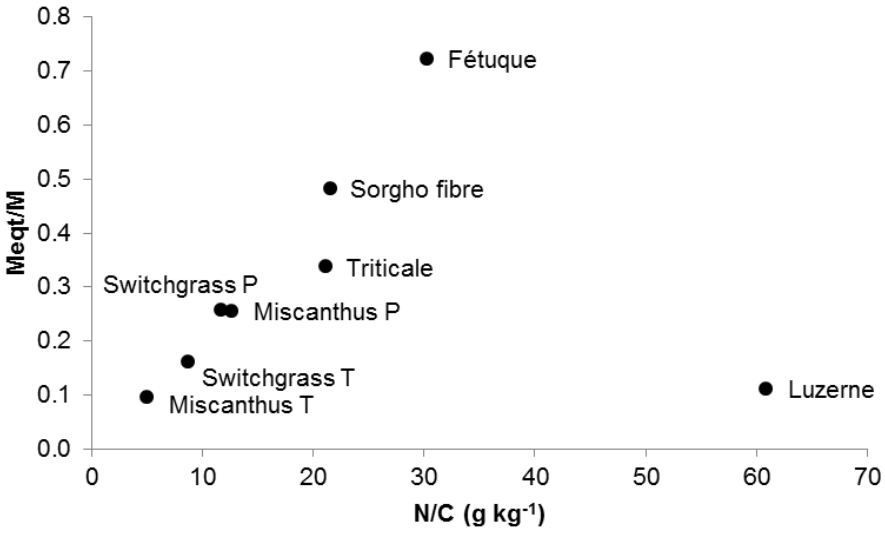


Figure 1.6. Relation entre le rapport coût bénéfice pour les émissions de GES (Meqt/M) et le rapport N/C de la biomasse exportée. D'après Cadoux *et al.* (2014).

Ces différences de teneurs en éléments minéraux entre espèces se retrouvent dans les données de la littérature, malgré la variabilité liée aux pratiques culturales et à la disponibilité en éléments dans les sols (Gabrielle *et al.*, 2014). Les cultures pérennes, les TCR ainsi que les résidus de cultures ont généralement de faibles teneurs en N et P à la récolte, notamment par rapport aux grains des cultures conventionnelles (Table 1.2).

Table 1.2. Concentrations moyennes en éléments minéraux N, P, K (g kg^{-1} MS) et quantités exportées par tep de biocarburant produit (kg tep^{-1}) pour des cultures conventionnelles, résidus de cultures et cultures lignocellulosiques. D'après Gabrielle *et al.* (2014).

	Concentration en éléments minéraux (g kg^{-1} MS)			Exportation d'éléments minéraux par tep de biocarburant produit (kg tep^{-1})		
	N*	P*	K*	N	P	K
Cultures conventionnelles						
Blé	20.3 (2.6)	2.7	4.6 (0.4)	91	12	21
Maïs	12.9 (1.0)	2.9 (0.8)	5.9 (3.4)	47	11	22
Colza	31.8 (1.6)	6.0	7.8	79	15	19
Betterave	7.9 (2.0)	1.2	7.9	32	5	32
Résidus de cultures						
Blé	6.0 (0.9)	0.7 (0.3)	13.5 (3.0)	37	4	83
Maïs	6.2 (1.2)	1.0 (0.4)	13.9 (5.3)	42	7	96
Colza	6.3 (1.1)	0.8	13.7	42	6	93
Cultures lignocellulosiques						
Saule TCR	4.8 (0.9)	0.8 (0.3)	2.1 (0.7)	30	5	13
Peuplier TCR	5.2 (1.4)	0.8 (0.4)	3.3 (0.7)	34	5	22
Miscanthus P$^{\#}$	5.3 (0.5)	0.6 (0.2)	7.3 (1.8)	33	4	45
Miscanthus T$^{\#}$	3.3 (0.9)	0.4 (0.0)	5.0 (1.2)	21	2	31
Switchgrass P$^{\#}$	6.9 (2.1)	1.0 (0.1)	7.5 (1.9)	45	7	49
Switchgrass T$^{\#}$	4.4 (1.4)	0.7 (0.2)	3.2 (1.6)	29	4	21
Fétuque	15.5 (3.7)	2.4 (0.3)	19.9 (3.4)	129	20	165
Luzerne	27.2 (2.5)	2.6 (0.2)	21.4 (3.6)	311	29	245
Triticale	10.3 (1.2)	2.0	8.8 (1.2)	58	11	50
Sorgho fibre	9.2 (0.1)	1.8	12.3	70	14	93

* moyenne (écart type)
$^{\#}$ Miscanthus et switchgrass : P = récolte précoce (septembre-novembre) et T = récolte tardive (janvier-avril)
Synthèse de données bibliographiques. Voir Gabrielle *et al.* (2014) pour les références.
Les écarts types ont été calculés quand il y avait au moins 3 références disponibles.

En conclusion sur cette partie, il semble que la productivité des différentes espèces varie fortement avec les conditions pédoclimatique et les pratiques culturales. Le choix des espèces adaptées devra donc être effectué en fonction des conditions de production. Dans des conditions favorables comme celles rencontrées sur le dispositif B&E, les cultures pérennes pourraient permettre une production d'éthanol 2G à l'hectare bien plus élevée que la production de biodiesel obtenue à partir de colza. Les niveaux de production restent cependant équivalents à ceux obtenus avec la betterave. Un intérêt majeur des cultures pérennes comme le miscanthus ou le switchgrass et des TCR est leur faible teneur en éléments

minéraux (et en particulier en azote) à la récolte, ce qui limite les besoins en fertilisation. Cela permet d'espérer des bilans GES plus intéressants que ceux obtenus avec les biocarburants 1G comme le biodiesel de colza ou l'éthanol de maïs.

1.3.2 Impacts environnementaux

Les impacts environnementaux se produisant à l'échelle de la parcelle agricole peuvent affecter fortement le bilan environnemental des filières de biocarburants. Parmi ceux-ci, les impacts relatifs aux cycles de l'eau, de l'azote et de carbone sont de première importance. Nous allons par la suite exposer les enjeux liés à ces différents cycles et introduire de manière synthétique l'état de l'art concernant les différents types de culture lignocellulosiques. Cet état de l'art sera présenté de manière plus détaillée dans les différents chapitres.

1.3.2.1 Impacts liés à l'eau

Le bilan hydrique à l'échelle de la parcelle détermine la quantité d'eau retournant vers l'atmosphère par évapotranspiration (ETR) et celle restituée au milieu par le drainage et éventuellement le ruissellement. Ce bilan est donc sous l'influence de l'occupation des sols et, au niveau des terres agricoles, des cultures dont les demandes en eau peuvent varier, notamment du fait des différences de durée et de position dans le temps de la période de croissance (Amigues *et al.*, 2006). A l'échelle du paysage agricole, la consommation en eau des cultures est donc une composante importante du cycle hydrologique.

Plusieurs auteurs ont suggéré que les cultures pérennes comme le miscanthus ou le switchgrass pourraient consommer davantage d'eau que les cultures annuelles, à cause d'une production plus élevée, d'une période de croissance plus longue et d'un enracinement plus profond (Powlson *et al.*, 2005; Rowe *et al.*, 2009; Heaton *et al.*, 2010). Cela pourrait également être le cas pour les cultures pluriannuelles et les TCR. Il est important de pouvoir quantifier ces différences car le déploiement de ces cultures sur des surfaces conséquentes à l'échelle d'un territoire pourrait alors impacter l'alimentation en eau des aquifères. Peu d'études ont pour l'instant tenté ces comparaisons (Hickman *et al.*, 2010; McIsaac *et al.*, 2010; Pugesgaard *et al.*, 2014; Yimam *et al.*, 2014; Abraha *et al.*, 2015) et les résultats sont parfois contradictoires, notamment dans le cas du switchgrass. De plus, si des capacités d'enracinement profond ont été reportées pour le miscanthus, le switchgrass ou la luzerne (Dardanelli *et al.*, 1997; Neukirchen *et al.*, 1999; Ma *et al.*, 2000; Riche and Christian, 2001), l'effet de cet enracinement sur l'utilisation de l'eau du sol est mal connu.

1.3.2.2 Impacts liés à l'azote

Depuis le milieu du 20ème siècle, le recours massif aux engrais azotés de synthèse a largement contribué à l'accroissement des rendements et de la production agricole mondiale (Tilman *et al.*, 2002). Cette entrée massive d'azote réactif sur les surfaces agricoles a profondément modifié le cycle global de l'azote (Galloway *et al.*, 2008), avec des conséquences environnementales importantes (Galloway *et al.*, 2008; Sutton *et al.*, 2011). Ainsi, sur les 11 millions de tonnes de N apportées par les engrais sur les terres agricoles de l'UE, environ la moitié serait perdue dans l'environnement (Sutton *et al.*, 2011). On peut distinguer trois principales pollutions azotées issues de l'agriculture : les pertes de nitrate vers les eaux, les émissions de N_2O et les émissions d'ammoniac (NH_3). Les teneurs en nitrate dans les rivières, lacs, aquifères et zones côtières sont élevées dans beaucoup de régions européennes (Grizzetti *et al.*, 2011). Cet enrichissement en azote des écosystèmes aquatiques est à l'origine de phénomènes d'eutrophisation comme les blooms algaux, avec des impacts négatifs sur la biodiversité, et participe aussi aux émissions indirectes de N_2O via la dénitrification (Butterbach-Bahl *et al.*, 2011). Les émissions directes et indirectes de N_2O contribuent à augmenter le changement climatique et les émissions de NH_3 ont des conséquences importantes sur les écosystèmes terrestres (via l'augmentation des dépositions d'azote) et la santé humaine (via leur effet sur la qualité de l'air) (Sutton *et al.*, 2011).

Si l'azote émis sous forme de N_2O, soit directement depuis la parcelle cultivée ou indirectement via la cascade de l'azote, ne représente que quelques pourcents de l'azote apporté, ces émissions ont un impact important sur le bilan GES. Les émissions directes de N_2O sont très variables dans le temps et l'espace et dépendent, au-delà de la seule fertilisation azotée, de nombreux facteurs biotiques et abiotiques (Bessou *et al.*, 2011). Aussi, l'impact des cultures et pratiques culturales sur les émissions de N_2O est une question de recherche complexe qui ne sera pas abordée spécifiquement dans ce travail de thèse.

Les pertes d'azote par lixiviation dépendent de la disponibilité en azote minéral dans le sol et du drainage. En climat tempéré, la plus grande partie des pertes par lixiviation s'opère pendant la période hivernale car l'essentiel du drainage a lieu à cette période. De nombreux facteurs peuvent jouer sur ces pertes avec en particulier le type de culture et la rotation, la présence de cultures intermédiaires et la fertilisation azotée (Beaudoin *et al.*, 2005; Constantin *et al.*, 2010). Les conditions pédoclimatiques jouent également un rôle important (Beaudoin *et al.*, 2005). L'impact des cultures lignocellulosiques sur les pertes de nitrate par lixiviation a encore fait l'objet de peu de travaux. De plus, le faible nombre d'espèces étudiées conjointement et la variabilité des conditions pédoclimatiques entres études rend difficile les

comparaisons d'espèces. Quelques travaux ont cependant montré que les cultures pérennes, les TCR et les cultures pluriannuelles pourraient permettre de réduire les pertes par lixiviation par rapport à des grandes cultures annuelles (McIsaac *et al.*, 2010; Dimitriou *et al.*, 2012; Lesur *et al.*, 2014; Pugesgaard *et al.*, 2014). Par contre, pendant l'année ou les deux années suivant l'implantation des cultures pérennes et des TCR, des pertes et concentrations en nitrate plus élevées que par la suite ont souvent été observées (Christian and Riche, 1998; Mortensen *et al.*, 1998; Lesur *et al.*, 2014).

Enfin, étant donné le coût énergétique et en termes de GES de la fabrication des engrais azotés, ainsi que les conséquences environnementales des pertes d'azote sous forme de nitrate, de N_2O et de NH_3, il paraît important de s'intéresser à l'efficience de la fertilisation azotée. Cette efficience peut être définie comme la proportion de l'azote apportée effectivement absorbée par la plante. Si une efficience « apparente » peut être mesurée en comparant la quantité d'azote présente dans la plante à la récolte à celle mesurée dans un témoin non fertilisé, la mesure de l'efficience réelle nécessite l'utilisation d'un marquage isotopique ^{15}N de l'engrais. L'utilisation du marquage ^{15}N permet également de suivre le devenir de l'engrais dans les différents compartiments du système sol-plante et de quantifier les pertes vers l'hydrosphère et l'atmosphère par défaut de bilan (Gardner and Drinkwater, 2009). Cette technique a été appliquée au miscanthus (Christian *et al.*, 2006) et au switchgrass (Pedroso *et al.*, 2014), mais aucune étude n'a comparé différents types de cultures lignocellulosiques dans les mêmes conditions.

1.3.2.3 Impacts liés au carbone

A l'échelle globale, le carbone organique du sol représente un pool de C de 1550 Gt, supérieur aux pools atmosphérique (760 Gt) et biotique (560 Gt) (Lal, 2004). Compte-tenu de ces quantités, une variation même modeste des stocks de C dans les sols peut avoir un impact important sur les niveaux de CO_2 atmosphérique et le climat. Or, ces stocks varient de manière importante en fonction de l'occupation des sols : ils sont en France en moyenne de 43 t C ha^{-1} (sur 30 cm) pour les terres cultivées et d'environ 70 t C ha^{-1} pour les prairies permanentes et les forêts (Arrouays *et al.*, 2001). Au cours des 200 dernières années, les sols auraient perdu globalement entre 55 et 78 Gt de C du fait des changements d'utilisation des terres, participant ainsi à l'augmentation des concentrations en CO_2 atmosphérique (Lal, 2004).

A l'échelle de la parcelle, le stock de C organique du sol est la résultante de deux flux opposés : d'un côté, l'humification du C provenant des résidus végétaux (aériens et

souterrains) et éventuellement des apports extérieurs de matières organiques, de l'autre la minéralisation du C du sol sous forme de CO_2. Des changements d'occupation du sol, de type de culture ou de pratiques culturales peuvent, en modifiant ces flux, occasionner un stockage ou un déstockage de carbone, jusqu'à atteindre un nouvel équilibre (Bessou *et al.*, 2011). Les émissions ou séquestrations de CO_2 résultant de ces variations de stocks doivent être comptabilisées dans le bilan GES de la culture. Dans le cas des biocarburants, elles peuvent avoir un impact important sur le bilan GES global de la filière. En plus du bilan GES, l'évolution des stocks de C a également un effet sur les propriétés du sol et peut ainsi impacter sa fertilité (Lal, 2004).

L'impact des cultures énergétiques sur les stocks de carbone du sol dépendra donc non seulement du type de culture et des pratiques, mais aussi de l'occupation du sol précédente. La conversion de forêts ou prairies en cultures annuelles conduit à un déstockage de carbone important (Fargione *et al.*, 2008). Sur des terres arables, l'exportation systématique des résidus de culture, en diminuant les apports de C au sol, provoque généralement une diminution des stocks de C (Saffih-Hdadi and Mary, 2008; Powlson *et al.*, 2011). On peut donc attendre le même effet de l'introduction de cultures annuelles récoltées en plante entière. Au contraire, l'introduction de cultures pluriannuelles de type fourragères (fétuque, luzerne, etc.) pourrait conduire à une augmentation des stocks de C, comme cela a déjà été observé après conversion de terres arables en prairies (Post and Kwon, 2000; Conant *et al.*, 2001; Soussana *et al.*, 2004). Les cultures pérennes comme miscanthus ou switchgrass et les TCR sont également souvent considérées comme des cultures pouvant permettre de stocker du C dans les sols (Powlson *et al.*, 2005; Somerville *et al.*, 2010; Karp and Richter, 2011). Les résultats disponibles dans la littérature sont cependant très variables (Anderson-Teixeira *et al.*, 2009; Don *et al.*, 2011; Poeplau and Don, 2014). Cette variabilité est probablement en partie due aux difficultés méthodologiques liées à la mesure des variations de stocks de C du sol. De plus, très peu d'études ont comparé différentes espèces dans les mêmes conditions ou analysé l'impact de pratiques culturales telles que la fertilisation azotée ou le mode de récolte des cultures pérennes.

1.4 Objectifs et démarche de la thèse

1.4.1 Objectif général

Dans les paragraphes précédents, nous avons vu que la phase agricole joue un rôle central dans le bilan environnemental des biocarburants. Ainsi, les cultures lignocellulosiques qui seraient utilisées pour la production de biocarburant 2G doivent pouvoir réponde à une

triple exigence : forte productivité, faibles besoins en intrants et faibles impacts environnementaux. Si des connaissances sont déjà disponibles sur les deux premiers points, les données accessibles sur les impacts environnementaux de ces cultures sont encore peu nombreuses et les évaluations souvent incertaines. Il y a donc un enjeu fort à apporter des éléments de comparaison sur les impacts environnementaux des différentes espèces candidates.

Les cultures candidates présentent une diversité de fonctionnement pour de nombreux aspects : pérennité (pérennes, pluriannuelles, annuelles), capacité ou non de fixation symbiotique de l'azote atmosphérique, phénologie, type de métabolisme pour la photosynthèse (C3, C4), etc. Nous faisons l'hypothèse que cette diversité induit une variabilité de leurs fonctions puits-sources au sein du système sol-plante (puits d'eau et d'azote minéral, source de carbone et d'azote organique) et que ces cultures peuvent donc avoir des conséquences différentes sur les cycles de l'eau de l'azote et du carbone.

Une deuxième hypothèse est que certaines pratiques culturales peuvent aussi fortement modifier ces cycles :

1) La présence d'organes souterrains de réserve chez les cultures pérennes comme le miscanthus et le switchgrass conduit à l'existence d'un cycle annuel de C et N interne à la plante. Ce cycle peut être en partie tronqué par une récolte précoce en automne, qui peut permettre une production de biomasse plus élevée mais qui engendre aussi des exportations d'azote plus importantes et une diminution des réserves azotées de la plante.
2) La fertilisation azotée peut avoir à la fois un effet sur la production des cultures (et donc sur les exportations et restitutions de C et N) et sur la disponibilité en azote dans le milieu (et donc potentiellement sur les différentes pertes).

Dans ce cadre, l'objectif général de la thèse est de quantifier les bilans d'eau, d'azote et de carbone pour différentes cultures candidates et en faisant varier deux pratiques culturales : le mode de récolte (pour les cultures pérennes) et la fertilisation azotée.

1.4.2 Démarche de la thèse et questions de recherche

L'état de l'art sur les impacts environnementaux des cultures lignocellulosiques montre, d'une part, qu'il existe une hétérogénéité importante des connaissances selon les espèces et les impacts, et d'autre part, qu'il est difficile d'établir des comparaisons à partir de différentes études traitant chacune d'une seule espèce. Nous avons donc choisi de comparer différents types d'espèces dans les mêmes conditions pédoclimatiques et pour plusieurs

impacts. De plus, l'étude des cycles eau-C-N à l'échelle de la parcelle cultivée nécessite une approche à long terme, soit pour intégrer l'effet de la variabilité climatique interannuelle (cas de processus très variables comme le drainage), soit pour pouvoir quantifier les processus lents comme les évolutions de stocks de C. Pour répondre à ces besoins, le travail de thèse s'est appuyé sur un dispositif expérimental de long terme permettant la comparaison de différents types d'espèces candidates : le dispositif « Biomasse & Environnement » (B&E). Ce dispositif permet en effet de comparer les principaux types de cultures lignocellulosiques décrits précédemment, sauf les TCR ou TtCR.

A la suite de ce premier chapitre, le manuscrit de thèse est divisé en quatre parties qui abordent différentes questions de recherche en utilisant les données expérimentales acquises sur le dispositif B&E. Le dernier chapitre consistera en une discussion générale.

Dans le deuxième chapitre, nous analysons les capacités de prélèvement d'eau des différentes espèces, avec les questions suivantes :

- *Comment évoluent les stocks d'eau du sol au cours de l'année ?*
- *Quelle est la capacité des espèces étudiées à prélever l'eau du sol à différentes profondeurs ?*
- *Quelle est la densité et la profondeur d'enracinement de ces espèces ?*
- *Y a-t-il une relation entre le prélèvement d'eau et les caractéristiques du système racinaire ?*

Le troisième chapitre complète le précédent sur le bilan hydrique et aborde les pertes d'azote par lixiviation :

- *Quel est l'impact des espèces et pratiques sur le drainage ?*
- *Quelles sont les concentrations en nitrate dans l'eau drainée et les quantités d'azote lixivié ?*

Le quatrième chapitre traite des questions liées au carbone :

- *Quel est l'impact des espèces et pratiques culturales sur l'évolution des stocks de carbone organique du sol ?*
- *S'il y a des évolutions contrastées, sont-elles liées à des différences sur les entrées de carbone ou sur la minéralisation ?*

Enfin, le cinquième chapitre s'intéresse au devenir de l'azote de l'engrais :

- *Quelle est l'efficience d'utilisation de l'azote des différents systèmes étudiés ?*
- *Comment se répartit l'azote de l'engrais dans les différents compartiments du système sol-plante (exportations, organes de réserve, résidus, sol) ?*
- *Quel est le niveau des pertes ?*

1.4.3 Dispositif d'étude

Le dispositif B&E a été implanté en 2006 à Estrées-Mons dans la Somme, sur le domaine expérimental de l'INRA (Figure 1.7). Le sol est un sol brun lessivé (Haplic Luvisol d'après IUSS Working Group WRB (2006)) sur matériau limoneux recouvrant la craie, qui apparaît entre trois et quatre mètres de profondeur. C'est un sol typique des plateaux du Santerre en Picardie, très fertile et propice aux grandes cultures. Le climat est de type océanique dégradé (Joly *et al.*, 2010) avec une température moyenne annuelle de 10.6 °C et une pluviométrie annuelle de 699 mm (moyenne 2006-2014).

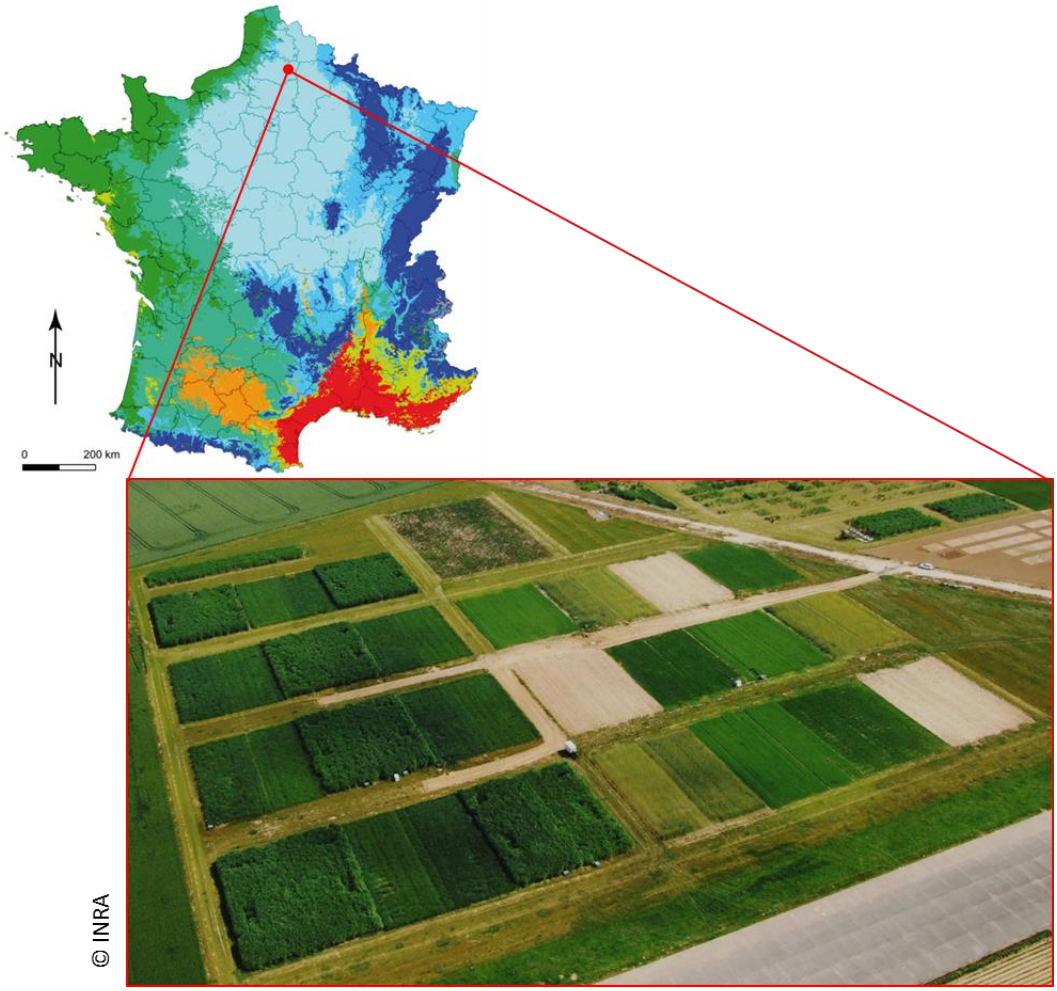


Figure 1.7. Localisation et vue aérienne au printemps 2010 du dispositif « Biomasse & Environnement ». La carte représente les principales zones climatiques françaises (Joly *et al.*, 2010).

Ce dispositif a été mis en place pour étudier la production de biomasse et les impacts environnementaux de différents types de cultures lignocellulosiques. Il est piloté par l'unité AgroImpact et conduit en interaction entre AgroImpact et l'unité expérimentale GCIE. Il regroupe deux cultures pérennes en C4 (miscanthus et switchgrass), deux cultures pluriannuelles de type fourragères en C3 (fétuque et luzerne) et deux cultures annuelles (sorgho fibre et triticale). Les cultures pérennes sont conduites soit en récolte précoce

(octobre), soit en récolte tardive (février). Les cultures pluriannuelles sont implantées pour deux à trois ans et les deux espèces sont en rotation. Les deux espèces annuelles sont également en rotation. Ces différentes modalités sont croisées avec deux niveaux de fertilisation azotée (N- et N+), les doses d'azote pour chaque modalité étant fixées par espèce. Le dispositif B&E n'a donc pas vocation à étudier des systèmes de culture optimisés et directement transférables dans la pratique mais plutôt des agrosystèmes contrastés, avec trois grands types (cultures pérennes, pluriannuelles et annuelles).

2 Soil water uptake and root distribution of different perennial and annual bioenergy crops

Ce chapitre correspond à un article publié dans *Plant and Soil* :

Ferchaud, F., Vitte, G., Bornet, F., Strullu, L., Mary, B., 2015. Soil water uptake and root distribution of different perennial and annual bioenergy crops. *Plant and Soil* 388, 307-322.

List of authors

Fabien Ferchaud[1], Guillaume Vitte[1], Frédéric Bornet[1], Loïc Strullu[1] and Bruno Mary[1]

[1] INRA, UR1158 AgroImpact, site de Laon, F-02000 Barenton-Bugny, France

Abstract

Bioenergy crops are expected to provide biomass as a replacement for fossil resources, but their impact on the water cycle is still under question. This study aimed at both quantifying the ability of bioenergy crops to use soil water and analysing the relationship between their root systems and soil water uptake. Water content was monitored continuously for seven years (2007-2013) under perennial (*Miscanthus* × *giganteus* and *Panicum virgatum*), semi-perennial (*Festuca arundinacea* and *Medicago sativa*) and annual (*Sorghum bicolor* and × *Triticosecale*) bioenergy crops. Root distribution was characterized in 2010 down to 3 meters depth. Soil water deficit (SWD) was calculated as the difference between field capacity and actual water content. Maximal SWD (0-210 cm) during the growing season was higher for semi-perennials, despite a lower biomass production than perennials. Water capture in deep soil layers was greater under perennials and semi-perennials than under annual crops. A curvilinear asymptotic relationship was found between water capture and root density and described by a model the parameters of which varied between crops, indicating a variable soil water capture for a given root density. This study provides quantitative information required to simulate the impact of bioenergy crops on drainage and aquifer loading.

Keywords

Bioenergy, energy crops, soil water, root system, miscanthus, switchgrass

2.1 Introduction

In response to the challenges of climate change and depletion of fossil resources, biomass is expected to contribute significantly to the energy transition by providing renewable carbon for bioenergy, biomaterials and biochemicals (Ragauskas *et al.*, 2006; IPCC, 2011). Among biomass resources, dedicated bioenergy crops have large technical potential and will probably be a major player in the increase of bioenergy production (Chum *et al.*, 2011; Bentsen and Felby, 2012). The wide range of conversion technologies leads to a large diversity of candidate crops: short rotation coppices, perennial crops, semi-perennial forage crops and annual crops (Lewandowski *et al.*, 2003; Karp and Shield, 2008; Sanderson and Adler, 2008; Zegada-Lizarazu and Monti, 2011; van der Weijde *et al.*, 2013).

Perennial C4 rhizomatous crops like *Miscanthus* × *giganteus* (hereafter referred to as miscanthus) or *Panicum virgatum* (hereafter referred to as switchgrass) are considered as promising energy crops because of their high biomass production, low nutrient requirements and low greenhouse gas emissions (Somerville *et al.*, 2010; Don *et al.*, 2011; Cadoux *et al.*, 2014). However, large deployment of these crops could modify their regional environment. One particular concern is the effect on the water cycle through modifications in evapotranspiration (McIsaac *et al.*, 2010; Vanloocke *et al.*, 2010). Several authors have suggested that perennial bioenergy crops consume more water than annual crops, because of their higher biomass production, longer growing period and deeper root system (Powlson *et al.*, 2005; Rowe *et al.*, 2009; Heaton *et al.*, 2010). Using soil moisture measurements over four growing seasons in central Illinois, McIsaac *et al.* (2010) estimated that miscanthus had higher evapotranspiration than switchgrass and maize-soybean rotation. Hickman *et al.* (2010) found similar results at the same site during one growing season by using a micrometeorological method. Using a model-based approach, Le *et al.* (2011) predicted an average 58% and 36% increase of total seasonal evapotranspiration for miscanthus and switchgrass respectively, compared to maize in the Midwest United States. This higher water consumption during crop growth will reduce the amount of water drained during winter. Vanloocke *et al.* (2010) predicted with a dynamic global vegetation model a decrease in drainage ranging from 50 to 250 mm yr^{-1} with miscanthus cultivation instead of current land cover for the Midwest United States. This decrease of drainage is likely to impact aquifers in case of large-scale land conversions.

Among factors influencing crop water use, morphology and distribution of roots within the soil profile are of prime importance because they define the amount of water that can be supplied to the crop (Jackson *et al.*, 2000). Deep rooting (> 2 m) has been reported by

several authors for miscanthus and switchgrass (Neukirchen *et al.*, 1999; Ma *et al.*, 2000; Riche and Christian, 2001). This extensive root system may allow these crops to maintain their growth in case of drought period but is also likely to lead to a greater soil water deficit (SWD) than annual crops at the end of the growing season (Riche and Christian, 2001). To our knowledge, only one study has compared soil water uptake and root distribution of different bioenergy crops (Monti and Zatta, 2009). However this study was restricted to one growing season and the soil sampling depth was only 120 cm. There is a need to compare a wide range of candidate bioenergy crops over multiple seasons to take into account climate variability and in a deep soil to maximize the differences in root distribution between crops.

We hypothesized that perennial C4 crops use deep soil water resources because of their extensive root system and high biomass production, leading to a higher SWD than with other crops. The aim of this study was (1) to quantify soil water utilization for perennial, semi-perennial and annual bioenergy crops using a long term and continuous monitoring of soil water, and (2) to study the relationship between the root system of the crops and soil water uptake.

2.2 Materials and methods

2.2.1 Study site and experimental design

This study is based on an ongoing long-term experiment carried out by INRA at the experimental station of Estrées-Mons, northern France (49.872°N, 3.013°E), on a Haplic Luvisol (IUSS Working Group WRB, 2006). The experiment was initiated in 2006 and six crops were compared, representing a wide range of bioenergy crop types: two perennial C4 crops, two semi-perennial forage crops and two annual crops. The chosen crops were miscanthus (*Miscanthus×giganteus* Greef & Deuter ex Hodkinson & Renvoize), switchgrass (*Panicum virgatum* cv. Kanlow), fescue (*Festuca arundinacea* cv. Dulcia from 2006 to 2008, Noria from 2009 to 2010 and Bariane after 2010), alfalfa (*Medicago sativa* cv. Alpha from 2006 to 2008, Orca from 2009 to 2010 and Salsa after 2010), fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (× *Triticosecale* Wittmack cv. Triskell from 2006 to 2008, Amarillo from 2009 to 2011 and Tarzan after 2011). The annual crops were grown in rotation (triticale grown after sorghum and *vice-versa*) as well as the semi-perennial crops (alfalfa grown after fescue and *vice-versa*) and all crops were present each year. A catch crop was sown every year in August or early September between triticale and sorghum (rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2013). The perennial crops were established in 2006 and the semi-perennial crops were sown

in 2006 (first rotation), 2009 (second rotation) and 2011 (third rotation). Two harvest dates were compared for miscanthus and switchgrass: an early harvest in October and a late harvest in February. The experiment also included two nitrogen treatments for each crop except alfalfa, with a plot size of 360 m^2 and three replicates per treatment. Details about crop management and experimental treatments are given by Cadoux *et al.* (2014). In this study, we selected experimental treatments maximizing plant growth and thus water consumption: the late harvest for miscanthus and switchgrass and the highest nitrogen treatment for all crops. During the period 2007-2013, the mean N fertilization rates for the selected treatments were 120 kg ha^{-1} yr^{-1} for miscanthus, switchgrass, sorghum and triticale, 170 kg ha^{-1} yr^{-1} for fescue and 0 for alfalfa. The experiment did not receive irrigation, except in May 2011 for fescue, alfalfa, sorghum and triticale (58 mm in total) to facilitate crop establishment during a drought period.

2.2.2 Climatic data

Climatic data were obtained from an automatic weather station situated on the experimental site. Over the period 2007-2013, mean temperature was 10.6 °C, annual rainfall (P) and Penman potential evapotranspiration (PET) were 686 and 714 mm respectively and annual global radiation was 4154 MJ m^{-2}. The water balance (P-PET) during the growing season displayed a rather large variability between years (Table 2.1). We considered March 1 as the beginning of the growing season because it corresponds approximately to the time when winter crops like triticale start growing again and PET begins running higher than 1 mm d^{-1}. We considered November 1 as the end of the growing season because all annual and semi-perennial crops have been harvested and perennials are close to total senescence. The wettest year was 2008 and 2009 was the driest, with only 116 mm of precipitation from June to September. Springs 2010 and 2011 were drier than the seven-year average.

Table 2.1. Meteorological data: P = precipitation (mm), PET = Penman potential evapotranspiration (mm) recorded at Estrées-Mons over the period 2007-2013

Year	March-June			July-October			March-October		
	P	PET	P-PET	P	PET	P-PET	P	PET	P-PET
2007	290	364	-73	216	327	-110	507	690	-184
2008	281	350	-69	317	318	-1	598	668	-70
2009	200	341	-142	145	432	-288	344	773	-429
2010	136	374	-239	270	346	-76	406	720	-315
2011	108	328	-219	243	294	-52	351	622	-271
2012	282	294	-13	219	346	-127	501	640	-139
2013	202	293	-91	321	345	-24	523	637	-114
Mean	214	335	-121	247	344	-97	461	679	-217

2.2.3 Biomass production

The aboveground biomass at harvest was estimated for each crop. At each harvest date, plants were harvested manually and weighed. Miscanthus and switchgrass were harvested in February or early March. Fescue and alfalfa were harvested in two or three cuttings depending on the years, with the last cut in October. Sorghum was harvested in late September and triticale in late July or early August. Details about sampling methodologies are given by Cadoux *et al.* (2014). The dry matter content was determined after drying representative subsamples at 65°C for 96 h. The biomass production was expressed in tons of dry matter per hectare and per year for all crops.

2.2.4 Soil and soil water monitoring

2.2.4.1 Measurements

We used water content reflectometers (Campbell Scientific CS616) to monitor the soil moisture profile continuously from July 2007 to November 2013. Probes were installed in May 2007 in six plots (one plot per crop), inserted horizontally into the soil at 15, 45, 75, 105, 135, 165 and 195 cm depth (three replicates at 15 cm depth and two replicates at the other depths). Temperature sensors (Campbell Scientific 107) were also placed at 15 and 195 cm depth. Data were recorded at an hourly time step using CR1000 Campbell Scientific data loggers. Probes placed at 15 cm depth were removed for soil tillage and reinserted as soon as possible.

Soil cores taken down to 210 cm from the six plots in 2006 were analysed to determine soil characteristics which were very homogeneous in the six plots (Table 2.2). Bulk density was measured at each CS616 depth in May 2007 using steel cylinders of 98 cm^3 (5 cm

diameter, three replicates) and measurements were repeated for the upper depth (15 cm) in 2010 and 2011 or 2012 with six replicates. Gravimetric water content was measured in the three blocks twice a year (in mid-March and early November) from 2007 to 2013. At each date of measurement, soil cores were collected down to a depth of 150 cm with a driller 18 mm in diameter. The cores were divided into five layers (0-30, 30-60, 60-90, 90-120 and 120-150 cm). In each soil layer, one soil sample was formed by mixing five soil cores for each plot. In the instrumented plots, additional measurements were made during summer 2009 and from November 2011 to November 2013 down to 210 cm, with three individual cores per plot divided into seven layers. Only gravimetric measurements made in the instrumented plots were used to calibrate CS616 probes.

Table 2.2. Soil characteristics measured in the experimental plots. Values in brackets are standard deviations.

	Soil layers (cm)						
	0-30	30-60	60-90	90-120	120-150	150-180	180-210
Clay (g kg^{-1})	169 (25)	227 (33)	267 (22)	243 (11)	222 (12)	190 (13)	234 (21)
Fine silt (g kg^{-1})	320 (17)	305 (21)	283 (44)	277 (20)	275 (13)	272 (29)	305 (10)
Coarse silt (g kg^{-1})	459 (16)	418 (19)	410 (22)	440 (16)	467 (23)	489 (28)	404 (5)
Fine sand (g kg^{-1})	38 (9)	43 (18)	37 (21)	36 (15)	35 (13)	46 (13)	52 (15)
Coarse sand (g kg^{-1})	13 (3)	6 (2)	2 (1)	2 (1)	1 (1)	1 (1)	3 (1)
Organic C [a] (g kg^{-1})	9.8 (0.3)	6.0 (0.3)	3.0 (0.2)	2.3 (0.2)	2.1 (0.3)	1.7 (0.3)	2.6 (0.7)
pH in water	7.7 (0.2)	7.8 (0.2)	7.8 (0.1)	8.0 (0.1)	8.0 (0)	8.2 (0.1)	8.2 (0.1)
$CaCO_3$ (g kg^{-1})	2 (1)	2 (2)	1 (1)	2 (2)	1 (1)	1 (2)	3 (4)
WFC [b] (g kg^{-1})	243 (3)	221 (3)	219 (3)	217 (4)	222 (2)	228 (6)	238 (3)
WWP [c] (g kg^{-1})	90 (12)	102 (18)	107 (11)	104 (9)	95 (13)	89 (6)	104 (5)
Bulk density (g cm^{-3})	1.47 (0.05)	1.55 (0.02)	1.57 (0.03)	1.58 (0.01)	1.55 (0.03)	1.49 (0.03)	1.51 (0.03)

[a] Anne Method (AFNOR X 31-109)
[b] Water content at field capacity (median of field measurements made in March over the period 2007-2013), corresponding to *ca.* -20 kPa water potential
[c] Water content at permanent wilting point (measured with Richard's pressure plates at -1.5 MPa water potential)

2.2.4.2 Data processing and calculations

Data from CS616 probes need proper correction and calibration in order to obtain accurate soil moisture measurements (Rudiger et al. 2010). First of all, data were regularly collected in a database managed with PostgreSQL and eventual outliers were eliminated. Secondly, the soil temperature was simulated at each CS616 depth using a script developed with R software (R Core Team, 2014). We used Fourier's law to simulate heat conduction transfer through the soil profile. Soil temperature at 15 and 195 cm depth were taken as boundary conditions and the initial temperature along the soil profile was determined by

linear interpolation between the two depths. Depth and time increments as well as thermal diffusivity (*alpha*) were optimized using two other plots of the same experiment with supplementary soil temperature measurements at 75 and 135 cm depth. The optimized value for *alpha* was 24 cm^2 h^{-1} and the root mean square error (RMSE) was 0.2°C over a period of 434 days. Thirdly, period measurements from the CS616 probes were corrected for measured or simulated soil temperature, using the temperature correction equation provided by Rudiger *et al.* (2010) with the slope coefficient for silt loam. The fourth step consisted in deriving a relationship between corrected period measurements and volumetric soil water contents, obtained from gravimetric water contents and bulk density measurements. A covariance analysis was applied with R software for the two or three replicates of each plot and depth in order to choose (with a 95% confidence level) between an individual calibration with a specific linear regression equation for each replicate, a common calibration or an individual calibration of the intercept with a common slope. The mean coefficient of determination was 0.86 for the 90 probes (n=17) and the mean RMSE was 0.016 cm^3 cm^{-3}. Finally, all corrected period measurements were converted into volumetric and gravimetric water content and the two or three replicates were averaged. Missing data were filled in by linear interpolation and data were aggregated to obtain daily measurements. Standard deviation between replicates was, on average over the period 2007-2013, 0.019 cm^3 cm^{-3} for the first layer (3 replicates) and 0.007 cm^3 cm^{-3} for the other layers (2 replicates).

The soil water content (SWC, in mm) was calculated in each 30 cm soil layer and summed up over the monitored soil profile (0-210 cm). For each crop, SWC calculated over the three replicated plots with gravimetric measurements were compared to SWC calculated in the single instrumented plot using CS616 probes. We found a good, unbiased relationship between the two estimates (y = 1.005 x; R^2=0.94; n=65), which indicated that SWC assessed with CS616 probes were representative of the whole field. The soil water deficit (SWD, in mm) was defined as the difference between SWC at field capacity and the measured SWC (Beale et al. 1999) for each soil layer or over the monitored soil profile. For each soil layer, the proportional water capture (PWC, in %) was calculated as the fraction of potentially available water that had been captured by plant roots (Monti and Zatta 2009):

$$PWC = \frac{SWC_{FC} - SWC}{SWC_{FC} - SWC_{WP}} \cdot 100 \quad (1)$$

where SWC_{FC} is the water content at field capacity (in mm) and SWC_{WP} the water content at permanent wilting point (in mm). SWC_{FC} was calculated as the median of the gravimetric

measurements made in winter (March) over the period 2007-2013. SWC_{WP} was measured with Richard's pressure plates at -1.5 MPa water potential.

2.2.5 Root mapping

We collected data on root distribution during the year 2010 for each plot in which soil water was monitored using a modified trench profile method (Tardieu, 1988). First of all, a trench 300 cm deep was dug into the plot. The observed vertical profile (180 cm wide, 300 cm deep) which was perpendicular to the crop row was then prepared. After the working surface had been smoothened, roots were made visible by removing approximately 1 cm of soil with a knife. Next, roots were mapped on three adjacent 60 × 300 cm grids with cells of 1.9 × 1.9 cm. Since root counting was a very time-intensive operation, the number of root impacts in each cell was measured only on 20% of the cells for each 60 cm wide grid (the seven cells at the right of the grid), and the presence or absence of root impact was noted for the other cells. The distribution of roots was recorded on 11 and 25 June 2010 for miscanthus and switchgrass respectively (4-year-old crops), on 14 and 21 September 2010 for fescue and alfalfa respectively (1.5-year-old crops), on September 9, 2010 for sorghum (at the beginning of anthesis) and on July 13, 2010 for triticale (ten days before physiological maturity).

2.2.6 Relationship between root density and proportional water capture

In studies dealing with roots and water uptake, the root distribution is often described using the root length density (RLD), *i.e.* the total root length per unit of soil volume (Gregory, 2006). Experimental measurement of RLD by extracting soil cores or soil monoliths can be extremely labour-intensive. Mapping and counting root impacts on a vertical soil profile has the advantage of being easier to do in the field but provides no direct measurement of RLD. However, the root intersection density (RID), *i.e.* the mean number of root impacts per cm^2 (Chopart *et al.*, 2008), can be calculated from such measurements and linear relationships between RID and RLD have been found for various crops (Chopart and Siband, 1999; Chopart *et al.*, 2008; Dusserre *et al.*, 2009). We therefore assumed that RID could be used as an indicator of RLD. Indeed, we also determined RLD for miscanthus and switchgrass (Ferchaud *et al.*, 2012) and verified the linear relationship between RLD and RID for these two crops. RID was calculated in each 60 × 300 cm grid for each layer of 30 cm thickness.

The relationship between RID and water capture was studied in 2010 using PWC calculated at the date of the maximal soil water deficit (over 0-210 cm). The relationship was described for each crop with a model derived from King *et al.* (2003):

$$PWC = (a - y_0) \cdot (1 - e^{-k.RID}) + y_0 \quad (2)$$

where k is a "resource capture coefficient" which summarizes details of water uptake physiology and soil water transport. A higher k value leads to a faster increase in water extraction when root density increases. Compared to the original model of King *et al.* (2003), we added two parameters: a which is the highest PWC achievable by the crop (a = 100% in the original model) and y_0 which is the PWC obtained in free root soil layers due to possible water capillary rise (y_0 = 0 in the original model). A common value for all crops was chosen for y_0. The parameter optimization for a and k was realised with the Excel solver using the GRG nonlinear method. The minimized criterion was the RMSE.

2.2.7 Statistical analysis

All statistical analyses were performed with R (R Core Team, 2014). The effect of the crop on highest and lowest SWC of each year was evaluated by one-factor analysis of variance (ANOVA), using the different probes as replicates. We used a two-factor ANOVA without replication with crop and year as factors in order to test the crop effect on mean SWD for each soil layer and for 0-210 cm. For PWC, we included the soil layer as third factor. For RID, the effect of the crop was evaluated in each layer by one-factor ANOVA, using the three adjacent grids as replicates. Differences between crops were evaluated with Tukey's HSD (honest significant difference) test for all variables. The assumptions of ANOVA were checked by visual examination of the residuals against predicted values and using Shapiro-Wilk and Levene's tests. If necessary, we used square root transformation or arcsine square root transformation to satisfy these assumptions.

2.3 Results

2.3.1 Soil water content

SWC over the soil profile (0-210 cm, measured with CS616 probes) fluctuated over the seven-year period with a regular pattern for all crops (Figure 2.1). It was at its highest level in winter (above 700 mm), decreased every year during spring and summer and rose in autumn. SWC in winter was rather stable between years and crops. It was close to the estimated SWC at field capacity (mean = 730 mm) and peaked from time to time at approximately 750 mm. Differences in maximum SWC between crops were only significant three years out of six. In 2009-2010 and 2010-2011, SWC did not reach field capacity for several crops (miscanthus and switchgrass in 2009-2010, miscanthus, switchgrass, fescue and alfalfa in 2010-2011). Indeed, in these cases, autumn and winter precipitations were not sufficient for the deeper layers to reach field capacity. The timing of SWC decrease depended

on the crops, with an earlier decline for triticale, fescue and alfalfa (except in 2009 and 2011 when these two crops were newly sown) than for the other crops. The lowest SWC over a year were observed on average over the period 2007-2013 in mid-October for miscanthus, in mid-September for switchgrass, fescue, alfalfa and sorghum and in early July for triticale. Minimal SWC were more variable between years and crops than maximal SWC. Differences in minimal SWC between crops were significant every year, with a range of variation between crops higher than 100 mm five years out of seven. The lowest SWC over the period 2007-2013 were observed in 2009 for all crops except for triticale, for which the lowest SWC was observed in 2013. These values (433 mm for alfalfa, 451 mm for fescue, 495 mm for switchgrass, 507 mm for sorghum, 526 mm for miscanthus and 528 mm for triticale) were significantly higher than the estimated SWC at permanent wilting point (mean = 318 mm). The timing of SWC increase after this minimal point was more stable between crops than the timing of SWC decrease in spring, but generally faster for annual crops than for the other crops.

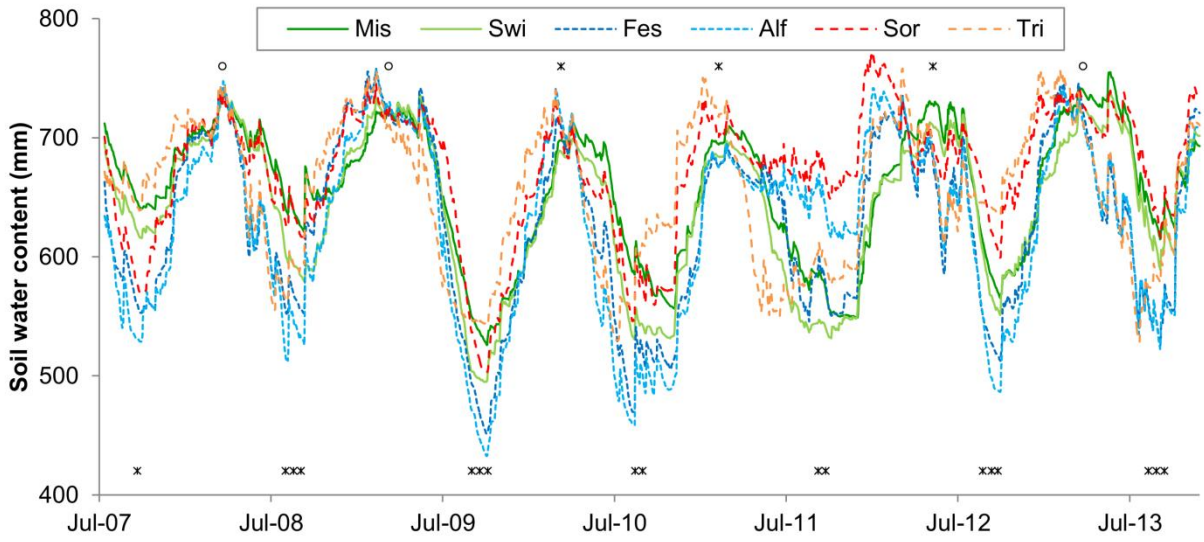


Figure 2.1. Evolution of the soil water content (SWC, 0-210 cm) over time (from July 2007 to November 2013) for each crop (Mis = miscanthus; Swi = switchgrass; Fes = fescue; Alf = alfalfa; Sor = sorghum; Tri = triticale). For fescue, alfalfa, sorghum and triticale, changes between plots were made the first day of March. Asterisks indicate significant differences between crops for the highest and lowest SWC of each year (° = NS; * = p<0.05; ** = p<0.01; *** = p<0.001).

2.3.2 Maximal soil water deficit

In order to quantify soil water utilization by the crops, we calculated the maximal SWD each year over the soil profile (0-210 cm), corresponding to the minimal SWC for the year. Maximal SWD ranged from 61 mm for triticale in 2007 to 294 mm for alfalfa in 2009. For each crop, the variability of the maximal SWD between years was large, with standard deviations of 41 to 59 mm. Nevertheless, alfalfa had the largest maximal SWD six years out

of seven and fescue always had the second largest maximal SWD. The ranking of other crops was more variable between years. On an average, over the period 2007-2013, the differences between crops were significant (Figure 2.2). Alfalfa had a higher maximal SWD (218 mm) than the other crops except fescue. Maximal SWD was lowest for sorghum (142 mm) and intermediate for triticale, miscanthus and switchgrass (156, 157 and 171 mm respectively).

At maximal SWD, SWD calculated in each soil layer was higher in the topsoil (Figure 2.2). On an average, 47% of the total SWD was located in the upper two layers (0-60 cm), 38% in the three intermediate layers (60-150 cm) and only 15% in the two deeper layers (150-210 cm). However, this distribution was crop-dependent: annual crops had a higher proportion of the total SWD in the 0-60 cm layer (56%) and a lower proportion in the 150-210 cm layer (11%) than the other crops. SWD in the 150-210 cm layer was 16 mm for sorghum and triticale, significantly lower than alfalfa (46 mm). SWD in the first layer (0-30 cm) was significantly lower for miscanthus and switchgrass (44 and 35 mm respectively) than for fescue, alfalfa and triticale (66, 56 and 56 mm respectively).

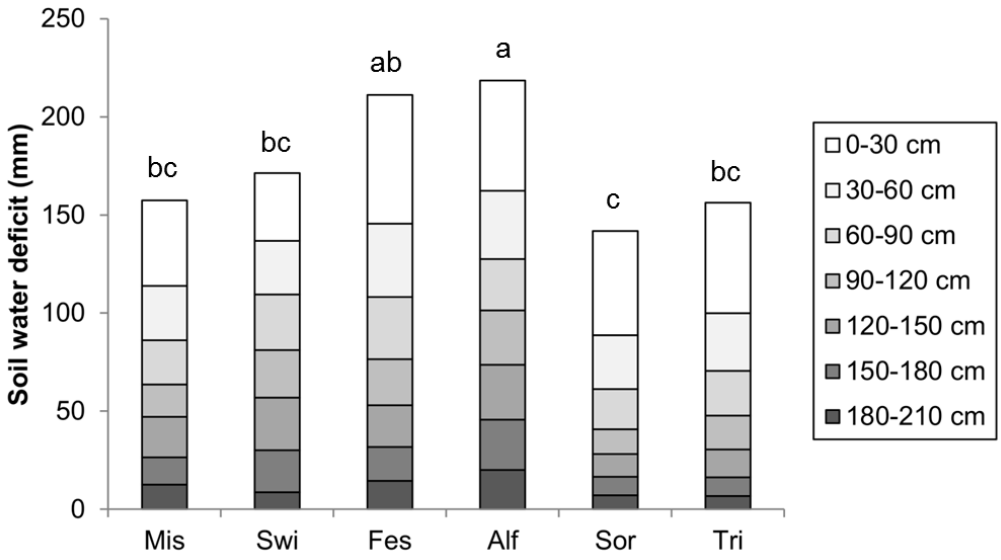


Figure 2.2. Soil water deficit (SWD) calculated for each crop over the period 2007-2013. SWD is the mean (over 7 years) of the maximal values of SWD (0-210 cm) encountered during each year. Different letters indicate significant differences ($p<0.05$) between crops.

We examined the influence of climate conditions on maximal SWD. Using data of precipitation (P), irrigation (I) and potential evapotranspiration (PET), the water balance (P+I-PET) was calculated each year and for each crop from March 1 to the date of maximal SWD. The water balance was -238 mm on an average and was very similar for most crops (-256 ± 5 mm), except for triticale (-148 mm). It was negatively correlated with maximal SWD ($r = -0.63$; $p < 0.001$; Figure 2.3).

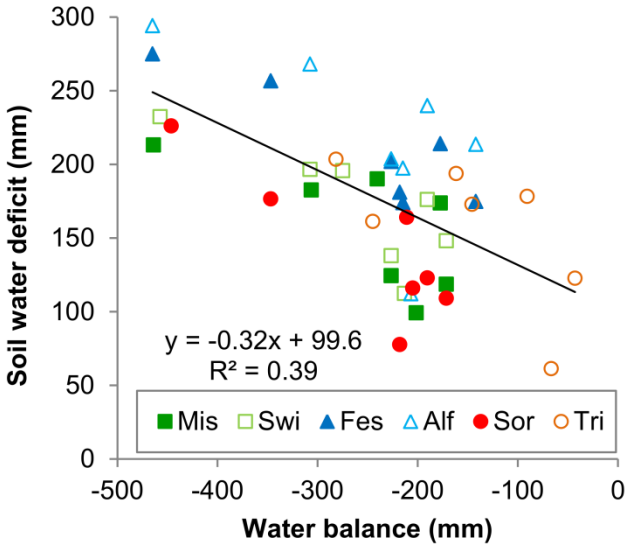


Figure 2.3. Relationship between the maximal soil water deficit and the water balance (P+I-PET) observed each year for each crop.

The influence of aboveground biomass production on maximal SWD was also investigated. No significant correlation was found between biomass production and maximal SWD (Figure 2.4). This was true not only when all crops were grouped together but also for each crop independently, despite large differences in biomass production between crops and years for some crops. Miscanthus, switchgrass and triticale had a rather stable biomass production over the period 2007-2013 but fescue, alfalfa and sorghum displayed a higher variability with low biomass production (< 10 t ha^{-1}) some years. Surprisingly, the highest maximal SWD (-294 and -275 mm for alfalfa and fescue respectively) was observed for low biomass production (3.2 and 6.4 t ha^{-1}) during the year 2009, which was the driest year and when fescue and alfalfa were newly sown. The biomass production of catch crops grown between triticale and sorghum was not taken into account because it was very low (0.5 ± 0.2 t DM ha^{-1} on average over the period 2007-2013).

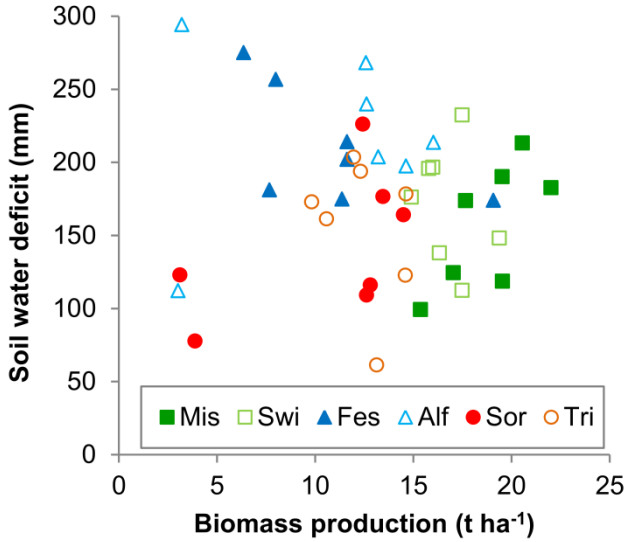


Figure 2.4. Relationship between the maximal soil water deficit and the aboveground biomass production observed each year for each crop.

2.3.3 Proportional water capture

PWC was calculated for each year and each soil layer at the date of the maximal SWD. Crops, soil depth and their interaction significantly affected PWC. On average, PWC decreased with depth from 74% in the 0-30 cm layer to 19% in the 180-210 cm layer, and never reached 100% below 30 cm. However, differences between crops were significant for all soil layers (Figure 2.5). For the first soil layer (0-30 cm), PWC was significantly lower for miscanthus and switchgrass than for other crops. The highest PWC observed in the 0-30 cm layer over the period 2007-2013 was only 76% and 57% for miscanthus and switchgrass respectively, compared to 100% for the other crops. The differences between crops were smaller in the 30-60 and 60-90 cm layers, with a higher PWC for fescue and alfalfa. PWC was smaller for sorghum and triticale below 90 cm. Alfalfa had the highest PWC (42% and 33% on average) in the 150-180 and 180-210 cm layers respectively. It was significantly higher than for sorghum and triticale (15% and 16% in the 150-180 cm layer; 12% and 11% in the 180-210 cm layer, respectively). The variability of PWC between years was smaller in deeper than in upper layers for annual crops, but not for other crops.

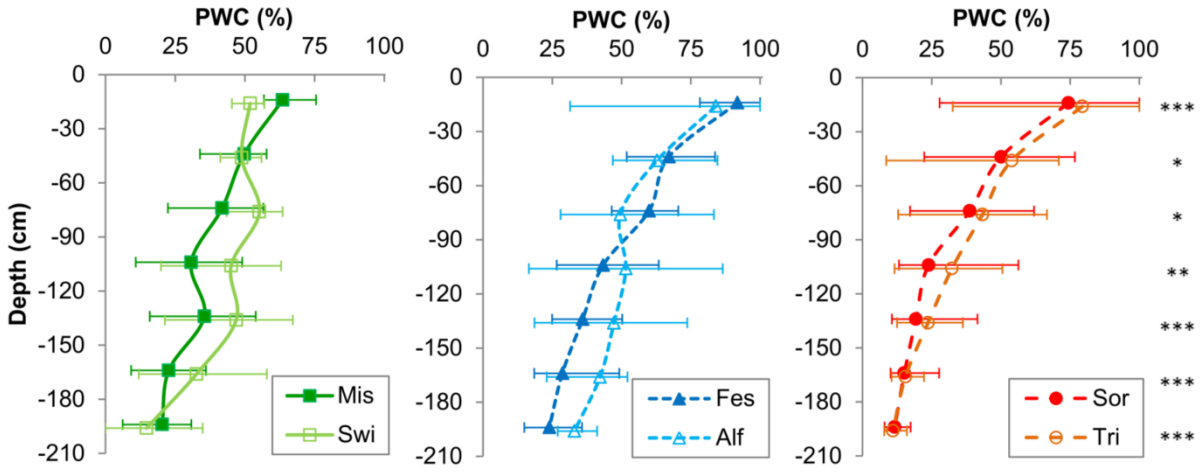


Figure 2.5. Proportional water capture (PWC) versus depth for each crop (Mis = miscanthus; Swi = switchgrass; Fes = fescue; Alf = alfalfa; Sor = sorghum; Tri = triticale). PWC is calculated at the date of the maximal soil water deficit (mean value over the period 2007-2013). Horizontal bars represent the range between the minimal and the maximal values found over the seven-year period. Asterisks indicate significant differences between crops in each soil layer (* = p<0.05; ** = p<0.01; *** = p<0.001).

PWC calculated at the date of maximal SWD were compared to PWC calculated at the end of the growing season, *i.e.* November 1 (data not shown). PWC changed very slightly for the three deeper layers (only 1% on average) but was reduced in the upper layers. This means that there was no or very little additional water retrieval in the deeper layers after the date of maximal SWD, even for crops for which maximal SWD was observed on average more than one month before the end of their growing period (switchgrass, fescue, alfalfa).

2.3.4 Root distribution and root intersection density

Figure 2.6 shows the root distribution of each crop observed in 2010 on the trench profiles. Miscanthus, switchgrass and alfalfa had a particularly deep root system: the maximum rooting depth was 300, 288 and 276 cm respectively. Sorghum had a more superficial root system (maximum rooting depth = 128 cm) and fescue and triticale were intermediate with a maximum rooting depth of 200 cm. The proportion of cells including roots decreased with depth, more or less according to the crops. This proportion decreased from 79% (sorghum) to 100% (fescue) in the 0-30 cm layer down to 0% (sorghum) to 10% (miscanthus) in the 180-210 cm layer. For the crops with a maximum rooting depth exceeding 210 cm, the proportion of cells including roots in the 210-300 cm layer was 7, 1 and 4% respectively for miscanthus, switchgrass and alfalfa.

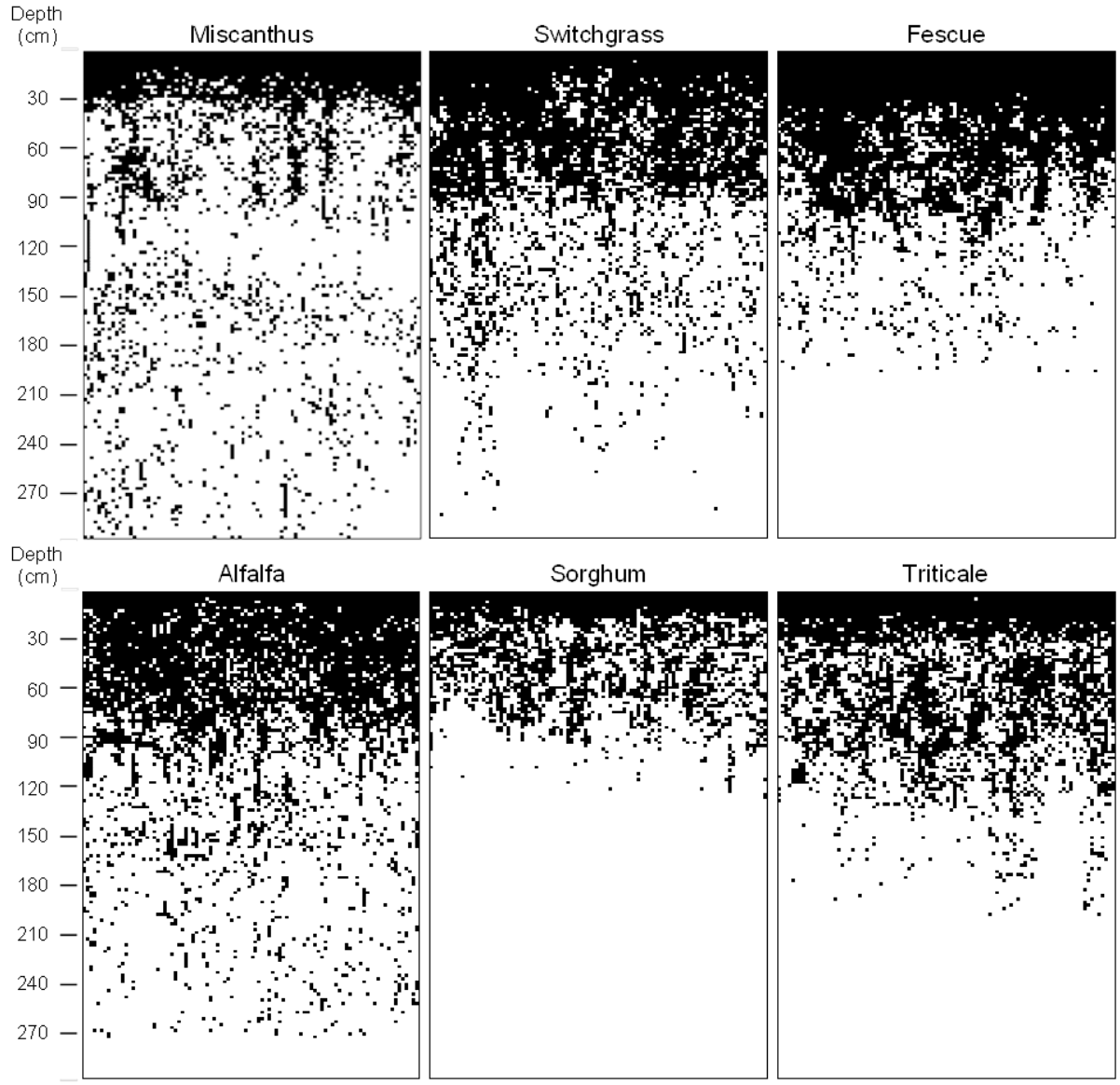


Figure 2.6. Spatial distribution of roots observed on the vertical trench wall (180 cm × 300 cm) for each crop in 2010. Each black dot represents a grid cell (1.9 × 1.9 cm) containing at least one root.

Root intersection density (RID) decreased drastically with depth for all crops (Table 2.3). It varied from 0.57 (sorghum) to 1.17 roots cm^{-2} (fescue) in the 0-30 cm layer, and became lower than 0.04 roots cm^{-2} for all crops in the 180-210 cm layer. The crop effect was significant in all soil layers. RID was significantly higher for fescue than sorghum, alfalfa and miscanthus in the first layer and higher for fescue, alfalfa and switchgrass than for miscanthus and sorghum in the 30-60 and 60-90 cm layers. In the 150-180 and 180-210 cm layers, miscanthus, switchgrass and alfalfa had a significantly higher RID than sorghum and triticale. Over 0-210 cm, the mean RID was 0.15 and 0.18 roots cm^{-2} respectively for sorghum and miscanthus, 0.24 and 0.26 roots cm^{-2} respectively for triticale and alfalfa and 0.30 and

0.32 roots cm^{-2} respectively for switchgrass and fescue. Only miscanthus, switchgrass and alfalfa produced roots below 210 cm, with a significantly lower RID for switchgrass.

Table 2.3. Root intersection density (mean number of root impacts per cm^2) measured for each soil layer and crop. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between crops in each soil layer.

Soil layer (cm)	Miscanthus		Switchgrass		Fescue		Alfalfa		Sorghum		Triticale	
0-30	0.800 (0.159)	*bc*	1.038 (0.107)	*ab*	1.173 (0.169)	*a*	0.669 (0.066)	*c*	0.571 (0.056)	*c*	0.867 (0.140)	*abc*
30-60	0.155 (0.037)	*b*	0.513 (0.082)	*a*	0.546 (0.009)	*a*	0.510 (0.113)	*a*	0.194 (0.030)	*b*	0.295 (0.084)	*b*
60-90	0.134 (0.055)	*b*	0.314 (0.018)	*a*	0.352 (0.095)	*a*	0.379 (0.035)	*a*	0.123 (0.013)	*b*	0.278 (0.057)	*a*
90-120	0.043 (0.016)	*bc*	0.101 (0.035)	*ab*	0.127 (0.071)	*ab*	0.126 (0.042)	*ab*	0.016 (0.010)	*c*	0.160 (0.058)	*a*
120-150	0.043 (0.034)	*a*	0.072 (0.039)	*a*	0.027 (0.013)	*a*	0.067 (0.039)	*a*	0.001 (0.001)	*b*	0.042 (0.017)	*a*
150-180	0.050 (0.006)	*ab*	0.057 (0.017)	*a*	0.020 (0.012)	*bc*	0.051 (0.014)	*ab*	0 (0)	*d*	0.014 (0.011)	*c*
180-210	0.031 (0.022)	*a*	0.020 (0.004)	*ab*	0.005 (0.002)	*bc*	0.022 (0.004)	*ab*	0 (0)	*c*	0.003 (0.004)	*c*
210-240	0.023 (0.010)	*a*	0.010 (0.004)	*b*	0 (0)	*c*	0.020 (0.002)	*a*	0 (0)	*c*	0 (0)	*c*
240-270	0.020 (0.012)	*a*	0.003 (0.002)	*b*	0 (0)	*b*	0.016 (0.009)	*a*	0 (0)	*b*	0 (0)	*b*
270-300	0.022 (0.016)	*a*	0.001 (0.001)	*b*	0 (0)	*b*	0.001 (0.000)	*b*	0 (0)	*b*	0 (0)	*b*

2.3.5 Relationship between root distribution and water uptake

We studied the relationship between RID measured in 2010 and PWC calculated for each soil layer at the date of the maximal SWD in 2010. Maximal SWD occurred on July 11 for triticale, on August 15 for switchgrass, fescue, alfalfa and sorghum and on October 31 for miscanthus. The year 2010 was the second driest year during the growing season, after 2009. PWC were therefore higher during year 2010 than the average for all crops at almost all depths.

A positive linear correlation was found between RID and PWC for all species ($r = 0.72$; $p < 0.001$). However, the relationship was not strictly linear but rather curvilinear asymptotic and differences appeared between crops (Figure 2.7). The highest PWC found in the 0-30 cm layer varied widely between crops, from 53% for switchgrass to 100% for fescue and triticale whereas the corresponding RID was similar for most crops. This was also true for the deeper soil layers where a large variability in PWC was observed for identical RID. The model derived from King et al. (2003) was fitted for each crop independently (Figure 2.7). Water capture was observed for sorghum in the layers 150-180 cm and 180-210 cm (PWC = 14 and 10% respectively), although no visible root was found in these layers. We used the mean of these two values (12%) for the y_0 parameter for all crops. Simulated PWC were in good agreement with observed data, with a mean RMSE of 7% (Table 2.4). The goodness of fit was equivalent for all crops except for fescue, which had the highest RMSE. The parameter values obtained after optimization were rather variable between crops (Table 2.4). The "resource capture coefficient" k was highest for fescue, followed by switchgrass and alfalfa and smaller for sorghum and triticale. Annual crops were characterized by an a value (highest PWC achievable by the crop) greater than for other crops, particularly perennials.

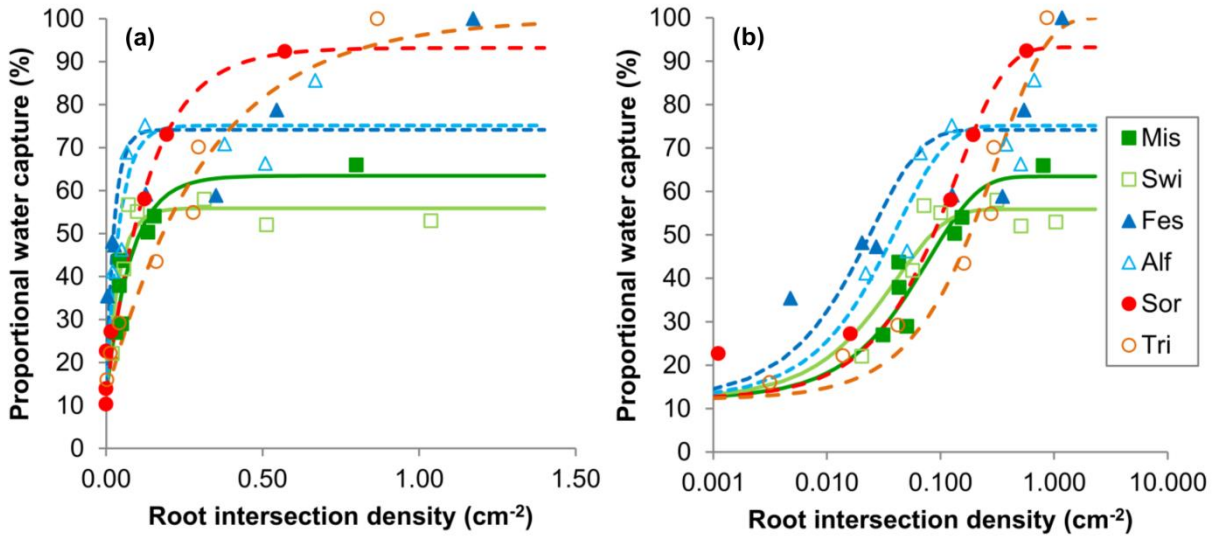


Figure 2.7. Relationship between proportional water capture (PWC) and root intersection density (RID) for each crop. PWC was calculated at the date of maximal soil water deficit in 2010: a) RID expressed in linear scale; b) RID expressed in logarithmic scale. Symbols are experimental data and lines are modelled data.

Table 2.4. Parameter and statistical criterion values obtained for each crop after fitting the model describing the relationship between root intersection density and proportional water capture (see Eq. 2 in text).

		Miscanthus	Switchgrass	Fescue	Alfalfa	Sorghum	Triticale
a	(%)	63.5	55.9	74.2	75.1	93.2	100.0
k	(cm^2)	12.6	24.4	39.6	23.9	7.3	3.1
RMSE	(%)	5.4	4.9	13.8	7.3	4.6	5.7

2.4 Discussion

2.4.1 Soil water deficit and water capture

Few studies have compared the evolution of soil water content during the growing season for different bioenergy crops. For example, alfalfa has been compared to annual crops (Entz *et al.*, 2001) but not to fescue or perennial C4 crops. Our study is the first one comparing perennial, semi-perennial and annual bioenergy crops. Entz *et al.* (2001) found that soil water content during summer (over 0-150 cm) was always lower for alfalfa than for annual crops during five years of cultivation. This is consistent with our study showing that the highest maximal SWD was observed for alfalfa six years out of seven. The differences in maximal SWD over 0-210 cm between miscanthus, switchgrass and annual crops were small and not significant in our study with a different ranking according to the year. McIsaac *et al.* (2010), who monitored soil moisture under miscanthus, switchgrass (cv. Cave-In-Rock) and a maize-soybean rotation, also found contrasting results between growing seasons. They found

that the minimal soil moisture under miscanthus and switchgrass was either equal (two years out of four) or lower (the other two years) than under annual crops. However, in contrast to our results, they observed that miscanthus resulted in lower minimal soil moisture than switchgrass during the four growing seasons.

We found that maximal SWD was correlated to the water balance (P+I-PET) but not to the aboveground biomass production. The biomass production of the semi-perennial crops was only 57% of the miscanthus production and 64% of the switchgrass production but their maximal SWD was higher. In fact, maximal aboveground biomass of perennial crops (in October) was even higher than the biomass at harvest (in February) due to leaf fall during winter (only for miscanthus) and carbon transfer from aboveground to belowground parts in autumn (Strullu *et al.*, 2011; Dohleman *et al.*, 2012). This suggests that miscanthus and switchgrass had higher water use efficiency than fescue and alfalfa. Beale *et al.* (1999) calculated water use efficiencies for miscanthus in UK with the maximal aboveground biomass reached by the crop during the growing season. They found comparable values to other C4 crops, such as maize, and higher values than C3 crops such as willow. Furthermore, it is possible that the timing of the growing periods of the crops affects water use efficiency due to differences in climate conditions. The lack of correlation between maximal SWD and aboveground biomass production observed during the seven years for each crop independently indicates that SWD was much more sensitive to the water balance than biomass production.

Our results also indicate that perennial C4 crops and semi-perennial forage crops, and particularly alfalfa, have the ability to take up significant amounts of water in deep soil layers (150-210 cm). Water uptake is even likely to occur below 210 cm for miscanthus, switchgrass and alfalfa since these crops have roots deeper than 210 cm. Our results are in agreement with Campbell *et al.* (1994) and Dardanelli *et al.* (1997) who showed that alfalfa growing in deep soil can withdraw water to a depth of 250 cm, and Finch and Riche (2008) who found significant soil water depletion down to 170 cm with miscanthus at two sites in England.

However, the ability of miscanthus and switchgrass to take up deep soil water did not lead to a significantly higher maximal SWD than annual crops because it was compensated by a lower SWD in the 0-30 cm layer. This lower SWD near the soil surface, which had not been emphasized in previous studies, was at least partly due to lower soil evaporation. Indeed, these two crops have a high and dense canopy during summer, when PET is maximal, which is likely to limit soil evaporation. Furthermore, the fallen leaves of miscanthus accumulating

at the soil surface form a 2-4 cm thick mulch (Amougou *et al.*, 2012) which enhances the reduction of soil evaporation.

2.4.2 Root distribution

We assumed that our protocol was appropriate to compare the root systems of the different crops. The root systems of annual crops measured at anthesis or post-anthesis were probably close to maximal development (Hoad *et al.*, 2001; Zhang *et al.*, 2004). Root distribution of perennial and semi-perennial crops was recorded at two periods during the year 2010 (June and September respectively). Root extension of perennial crops had probably reached steady state since the crops were 4 years old. Neukirchen *et al.* (1999) did not find any effect of the sampling date on root density when comparing three dates of measurements in a 5-year-old miscanthus. Semi-perennials were at the end of their second year of growth at the time of measurement: the recorded root distribution corresponded to well-established crops and was probably close to the maximal root development achieved during the 2010 growing season although we do not know if it was at steady state. The climate conditions in 2010, with a first part of the growing season (March-June) drier than the average, might have affected root development of annual and semi-perennial crops. However, this is unlikely because the available soil water was high in March (240 mm over 0-120 cm) and the biomass of these crops in 2010 was similar to the average (104% of the 2007-2013 mean biomass production).

In our conditions, *i.e.* in a deep loamy soil with no obstacles to rooting, crops exhibited large differences in rooting depth. Miscanthus, switchgrass and alfalfa had a particularly deep root system. The maximal rooting depth of miscanthus (≥ 300 cm) was deeper than that recorded in other field experiments: 200 cm for a 6-year-old crop in a silty clay loam in England (Riche and Christian, 2001) and 250 cm for a 3-year-old crop in a sandy loam in Germany (Neukirchen *et al.*, 1999). The difference with our study might result from differences in soil characteristics or maximum depth of observation. The maximum rooting depth of switchgrass measured in our experiment (288 cm) was intermediate between that observed by Riche and Christian (2001) for a 6-year-old crop in England (240 cm) and that reported by Ma *et al.* (2000) in a sandy loam in Alabama for a 7-year-old crop (330 cm). Evidence of a deep root system for alfalfa was also found in several studies (Campbell *et al.*, 1994; Dardanelli *et al.*, 1997). The maximum rooting depth of 200 cm observed for triticale was consistent with the highest values reported for winter cereals such as winter wheat (Hoad *et al.*, 2001; King *et al.*, 2003; Zhang *et al.*, 2004). Sorghum had the shallowest root system in

our conditions, with only 128 cm depth. This value was lower than that found by Robertson *et al.* (1993) for various grain sorghum cultivars in a sub-tropical environment in Australia (190 cm). Monti and Zatta (2009) found roots of fibre sorghum in Italy down to 120 cm depth. In our conditions, sorghum had a short growing period because it was sown after mid-May (May 21 in 2010) due to its susceptibility to low temperatures and this may have limited root development.

Root density has frequently been found to decrease exponentially with depth (Gregory, 2006). This exponential decrease is more or less verified for most crops, but not for alfalfa or miscanthus (Table 3). The root distribution observed for miscanthus, *i.e.* a rather low density in the upper layers with a drastic decrease below 30 cm and a constant and rather high density in the deeper layers, is consistent with the results of Neukirchen *et al.* (1999) and Riche and Christian (2001). However, it differs from the study of Monti and Zatta (2009) who found a surprisingly very low root density for miscanthus below 90 cm.

2.4.3 Relationship between root distribution and water capture

PWC was correlated to RID, meaning that root density was a limiting factor for extracting water, at least in deep layers. Indeed, soil-root water transfer occurs mainly in the few centimetres surrounding the roots, due to limitations in soil and/or root hydraulic conductivity (Garrigues *et al.*, 2006). However, the shape of the relationship (curvilinear asymptotic) showed that deep roots with a low density were relatively more efficient for recovering water than shallower, denser roots. Robertson *et al.* (1993) found the same type of relationship between water consumption and RLD for grain sorghum with a plateau above an RLD threshold. Zhang *et al.* (2004) also reported higher water uptake per unit of root length in depth than near the soil surface for rain-fed and irrigated winter wheat. When root density increases, competition between neighbouring roots is enhanced, reducing their relative efficiency.

Water capture equivalent to 15 mm in the 150-210 cm layer was observed for sorghum which had no roots in this layer. The deepest roots of sorghum might have not been observed due to spatial variability. It is also possible that upward soil water transfer occurred in these free root layers due to a hydraulic gradient caused by root uptake in the upper layers. Adding the y_0 parameter (PWC in free root layers) to the model allowed us to take this observation into account and increase the goodness of fit for annual crops (RMSE was divided by 2.5 on an average) with no impact for the other crops. We chose to take a common value for y_0

although it is likely that the upward water transfer depends on soil characteristics and decreases with depth when the distance to the deepest root increases.

The optimized parameters were rather variable between crops. The differences observed for *a* (highest PWC achievable by the crop), with lower values for perennial crops, could be partly explained by differences in soil evaporation between crops. However, the same tendency was observed when the model was fitted on data excluding the first soil layer. Very different values obtained for the "resource capture coefficient" *k* suggest large differences in water capture efficiency between crops. Monti and Zatta (2009) also found differences between crops but their ranking (miscanthus > sorghum > switchgrass) was different from ours (switchgrass > miscanthus > sorghum). In fact, their data on root density were very different from ours: low root density of miscanthus in depth compared to switchgrass and sorghum. We hypothesize that this discrepancy was due to a warmer climate, favourable to sorghum, in Italy and to the presence of a water table close to the soil surface which could penalize root growth of miscanthus.

Differences between crops for *k* can result from factors relative to root system or crop evaporative demand (growing period, morphological factors, etc.). The root system of annual crops like cereals grows simultaneously to the canopy and achieves its maximal development at anthesis (King *et al.*, 2003), contrary to perennial crops. Consequently, the deep roots of annual crops have less time than shallower roots to take up soil water. Robertson *et al.* (1993) hypothesized that incomplete water extraction in depth for grain sorghum under severe drought was due not only to lower root density but also to the lack of time for deeper roots to extract water between the arrival of roots in the soil layer and crop maturity. An extraction front travelling down the soil profile with time has been observed for annual crops (Robertson *et al.*, 1993; Dardanelli *et al.*, 1997; Dardanelli *et al.*, 2004) and is generally attributed to the growth of the root system. In 2009, the driest year of our experiment, an extraction front clearly appeared for sorghum: water depletion started approximately late June in the 30-60 cm layer (one month after sowing), late July in the 60-90 cm layer, mid-August in the 90-120 and 120-150 cm layers and late August for the two deeper layers. This difference in the timing of water extraction with depth could explain low values of *k* and high values of *a* observed for annual crops. Among root characteristics, root spatial arrangement could also explain differences between crops. For example, the degree of root clustering can significantly change soil water uptake for a given root density (Beudez *et al.*, 2013). Physiological properties such as root hydraulic conductivity could also influence the ability of roots to extract soil water (Nippert *et al.*, 2012).

2.5 Conclusions

This study provides an original monitoring of soil water utilization by different perennial and annual energy crops during seven years. As expected, perennial and semi-perennial crops were characterized by proportional water capture in deep soil layers higher than annual crops. Conversely, PWC was lower in the upper soil layer for miscanthus and switchgrass than for the other crops. Semi-perennial crops lead to a greater soil water deficit than the other crops, due to an important water uptake both in surface and deep layers. Contrary to our initial hypothesis, perennial C4 crops resulted in similar SWD than annual crops whereas perennials were more productive and had a deeper root system.

Our study also highlights the relationship between water uptake and root distribution of the crops. PWC was correlated to root density with a curvilinear asymptotic function but its parameters were crop-dependent. Therefore root density was not the only factor determining maximal water uptake. Since aboveground biomass was not correlated to SWD, other factors such as the timing and length of the growing period are likely to affect water use.

In the perspective of predicting the effect of bioenergy crops on water drainage, a complete water balance will have to be made in order to quantify the ratio between evapotranspiration and drainage for each crop. Our results already indicate that the risk of drainage reduction compared to annual crops is probably higher with semi-perennial crops like alfalfa than with perennial C4 crops. The impact of the crops on drainage will also depend on soil and climate characteristics. Areas with deep soils and low winter rainfall are probably more susceptible to exhibit large differences in drainage between crops. Further studies are needed to explore this effect of soil and climate variability. Finally, our dataset will be useful to test and improve soil-crop models in order to simulate the impact of bioenergy crops on drainage and aquifer loading under various environmental conditions.

3 Drainage and nitrate leaching assessed during seven years under perennial and annual bioenergy crops

Ce chapitre correspond à un article en préparation pour *Global Change Biology Bioenergy*.

List of authors

Fabien Ferchaud[1] and Bruno Mary[1]

[1] INRA, UR1158 AgroImpact, site de Laon, F-02000 Barenton-Bugny, France

Abstract

Since nitrogen excess in water is a threat to human health and aquatic ecosystems, it is important to assess and optimize the impact of bioenergy crops on water resource and quality. The aim of this study was to quantify drainage and nitrate concentration under perennial (*Miscanthus* × *giganteus* and switchgrass), semi-perennial (fescue and alfalfa) and annual (sorghum and triticale) bioenergy crops with two N fertilisation rates (N- and N+). Soil water and mineral N contents were measured twice a year (in mid-autumn and late winter) during 7 years. These measurements were used to assess drainage and nitrate concentration in drained water using the STICS model. Drainage varied greatly between treatments and years, from 0 to 227 mm yr^{-1}. Nitrate concentration was generally small, lower than 10 mg NO_3 l^{-1} in 80% of the situations. It was lower for perennials than other crops, except for miscanthus on the first year of measurement (1.5 year after establishment) when it reached 83 mg NO_3 l^{-1}. The mean weighted nitrate concentration over 7 years ranged between 2 and 23 mg NO_3 l^{-1}, for switchgrass N- and miscanthus N+ respectively. The comparison with nitrate concentrations measured in subsoil after 5 years suggests that the peak of nitrate produced after miscanthus establishment was subsequently recovered by the crop in deep layers. The mean amount of water drained during 7 years was higher for annual (133 mm yr^{-1}) than for semi-perennial crops (64 mm yr^{-1}). It ranged from 56 to 137 mm yr^{-1} under perennial crops, depending on crop and N rate. Drained water was strongly and negatively correlated with biomass production of perennial and annual crops. Semi-perennial crops produced less drainage than annual crops despite a similar biomass production. In our pedo-climatic conditions, the C4 perennial bioenergy crops performed best in terms of production, water resource and quality.

Keywords

Bioenergy, energy crops, nitrate losses, drainage, water balance, miscanthus, switchgrass

3.1 Introduction

Nitrogen (N) excess in water is a major concern in Europe as it poses direct threat to human and aquatic ecosystems (Sutton *et al.*, 2011). Nitrate concentrations in rivers, lakes, aquifers and coastal water are high in many regions, mainly as a result of diffuse pollution from agriculture (Grizzetti *et al.*, 2011). Nitrate in drinking water can harm human health and the N enrichment of aquatic ecosystem is responsible for toxic algal blooms, water anoxia and biodiversity loss (Grizzetti *et al.*, 2011). It also contributes to indirect nitrous oxide (N_2O) emissions through denitrification in riparian zones and hydrological networks (Butterbach-Bahl *et al.*, 2011).The European Union has adopted several regulations aiming at protecting waters such as the Nitrate Directive (Directive 91/676/EEC) and the Water Framework Directive (Directive 2000/60/EC). In this context, "good agricultural practices" (GAP), including improvement of N fertilisation practices and introduction of catch crops, have been promoted to reduce N leaching. Nevertheless, the implementation of GAP would not be sufficient to meet the requirements of European regulations in large areas of intensive arable farming such as the Seine Basin in France (Ducharne *et al.*, 2007) and greater changes in agricultural systems might be necessary (Thieu *et al.*, 2011).

The use of biomass as renewable carbon for bioenergy, biomaterials or biochemicals is expected to contribute to the energy transition in response to the challenges of climate change and depletion of fossil resources (IPCC, 2011). However, the development of biofuels produced from conventional food crops has raised concerns about their energy and greenhouse gas (GHG) balance and other environmental consequences (e.g. Crutzen *et al.*, 2008; Galloway *et al.*, 2008; Smith and Searchinger, 2012). New conversion technologies and biorefineries allow considering feedstocks that reach beyond current food crops (Ragauskas *et al.*, 2006; Somerville *et al.*, 2010). Perennial C4 crops such as miscanthus and switchgrass are viewed as promising bioenergy crops because of their high biomass production with low nutrient requirements (Don *et al.*, 2011; Cadoux *et al.*, 2014; Gabrielle *et al.*, 2014). These crops and other perennials such as short rotation coppices or C3 grasses are thought to have other environmental benefits such as reduced nitrate losses (e.g. McIsaac *et al.*, 2010; Lesur *et al.*, 2014; Pugesgaard *et al.*, 2014). Land conversion from arable cropping systems to perennial bioenergy crops could therefore help to improve water quality at the catchment scale (Ng *et al.*, 2010). However, perennial crops could also consume more water than annual crops because of their higher biomass production, longer growing season and deeper root system (McIsaac *et al.*, 2010; Pugesgaard *et al.*, 2014). This higher water consumption would reduce the amount of water drained and therefore the groundwater recharge (Vanloocke *et al.*,

2010). In order to evaluate the impact of bioenergy crops on water, it is therefore important to consider jointly the amount of water drained and the nitrate concentration in drained water.

Experimental comparisons of perennial and annual bioenergy crops regarding drainage and N leaching are scarce in the literature. Pugesgaard *et al.* (2014) compared winter wheat, grass-clover (mixture of ryegrass and clover) and willow managed for bioenergy production over three years in a sandy loam soil in Denmark. They found a lower drainage under grass-clover and willow than under winter wheat (171-191 mm yr^{-1} *vs.* 312 mm yr^{-1} respectively). Nitrate concentration was also much lower under perennial crops (12-31 mg l^{-1}) than under winter wheat (76 mg l^{-1}), showing the capacity of perennial crops to significantly reduce N losses compared to annual crops. Nitrogen leaching was also measured under miscanthus and switchgrass in comparison with conventional arable crops (McIsaac *et al.*, 2010; Smith *et al.*, 2013). These studies showed lower nitrate losses under perennial C4 crops than under annual crops. However, the first year or the two first years following miscanthus establishment have been shown to present a higher risk for N leaching than the subsequent years (Christian and Riche, 1998; Lesur *et al.*, 2014) and N fertilisation of perennial crops could increase N leaching (Christian and Riche, 1998; Davis *et al.*, 2014).

In an earlier paper, we measured soil water utilisation during the growing season for six different bioenergy crops in a long-term experiment. In this study, we aimed at (1) quantifying drainage and nitrate concentration in drained water for perennial, semi-perennial and annual bioenergy crops over 7 years, and (2) studying the interaction with N fertilisation. The study was based on the same experiment than the previous one and combined field measurements of soil water and soil nitrate content with modelling to calculate fluxes.

3.2 Materials and methods

3.2.1 Site and experimental design

The study is based on an ongoing long-term experiment established in 2006 at the INRA experimental station in Estrées-Mons, northern France (49.872°N, 3.013°E) called "Biomass & Environment" (B&E). The soil is a Haplic Luvisol (IUSS Working Group WRB, 2006). Detailed soil characteristics are given in Table 2.2. Before 2006, the field had been cultivated for many years with annual crops and the previous crop was winter wheat following spring pea.

The B&E experiment was initiated to study biomass production and the environmental impacts of a wide range of bioenergy crops. It allows comparing eight rotations: four with C4 perennial crops (monocultures), two with C3 semi-perennial forage crops and two with C3/C4

annual crops (Table 1). The perennial crops are miscanthus (*Miscanthus×giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in February (L). The semi-perennial crops are tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (× *Triticosecale* Wittmack). The experiment also includes two nitrogen treatments (N- and N+) with fertiliser-N rates depending on the crops (Table 3.1).

Table 3.1. Treatments of the B&E long-term experiment combining rotation and fertiliser-N rate (Mis = miscanthus, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fibre sorghum, Tri = triticale, CC = catch crop, E = early harvest (October), L = late harvest (February), N- = low fertiliser rate, N+ = high fertiliser rate, n.h. = not harvested)

Rotation	N rate	Crop and fertiliser-N rate (kg ha^{-1})								
		2006	2007	2008	2009	2010	2011	2012	2013	2014
Mis E		Mis n.h.	Mis E	Mis E	Mis E	Mis E	Mis E	Mis E	Mis E	Mis E
	N-	0	0	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120	120	120
Mis L		Mis n.h.	Mis L	Mis L	Mis L	Mis L	Mis L	Mis L	Mis L	Mis L
	N-	0	0	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120	120	120
Swi E		Swi n.h.	Swi E	Swi E	Swi E	Swi E	Swi E	Swi E	Swi E	Swi E
	N-	0	0	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120	120	120
Swi L		Swi n.h.	Swi L	Swi L	Swi L	Swi L	Swi L	Swi L	Swi L	Swi L
	N-	0	0	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120	120	120
Fes-Alf		CC/Fes	Fes	Fes	Alf	Alf	Fes	Fes	Fes	Alf
	N-	0	120	80	0	0	0	120	120	0
	N+	0	240	160	0	0	0	240	240	0
Alf-Fes		Alf	Alf	Alf	Fes	Fes	Alf	Alf	Alf	Fes
	N-	0	0	0	40	120	0	0	0	0
	N+	0	0	0	80	240	0	0	0	40
Sor-Tri		CC	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC
	N-	0	0	60	0	60	0	60	0	60
	N+	0	120	120	120	120	120	120	120	120
Tri-Sor		Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC	Maize*
	N-	0	60	0	60	0	60	0	60	0
	N+	0	120	120	120	120	120	120	120	120

*Fibre sorghum was replaced by silage maize (*Zea mays* L.) in 2014.

The 2.7 ha field was divided into two parts in order to facilitate cultural operations and limit competition between plants due to differences in canopy height (Figure 3.1): (i) a split-

block design in the west part for perennial crops with "rotations" in the main plots (miscanthus E, miscanthus L, switchgrass E, switchgrass L) and N fertilisation rates in the subplots (N- and N+), and (ii) a split-plot design in the east part for the other crops with rotations in the main plots (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilisation rates in the subplots (N- and N+). Both parts include three replicate blocks and 24 subplots of 360 m^2.

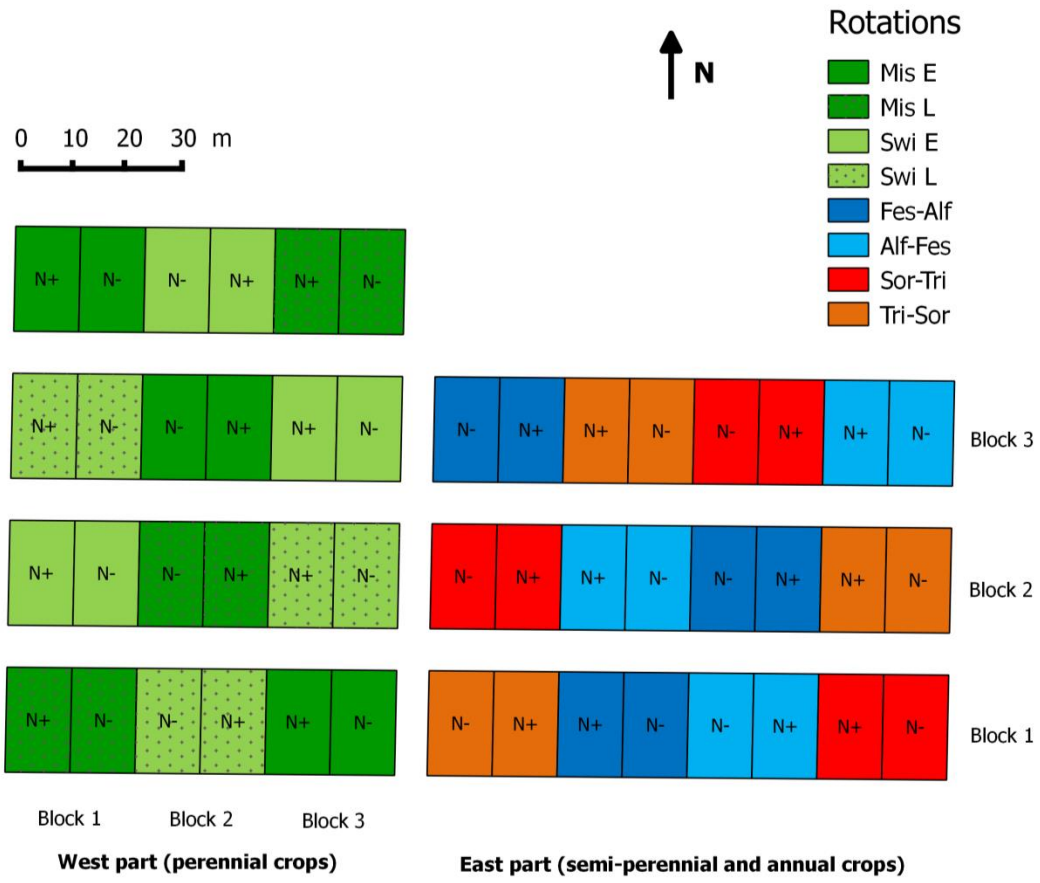


Figure 3.1. Map representing the experimental design of the B&E long-term experiment. All plots are 12 × 30 m (360 m^2) and the whole field is 2.7 ha

After winter wheat harvest in 2005, the field was mouldboard ploughed in early December and left bare during winter. Miscanthus was planted in April 2006 (1.5 rhizome m^{-2}) and switchgrass sown in June 2006 (seed rate = 15 kg ha^{-1}). Semi-perennial crops were sown in 2006, 2009, 2011 and 2014, usually in April. Before sowing, the previous crop (alfalfa or fescue) was destroyed in late autumn with a cultivator and a disc harrow in 2009 and mouldboard ploughed in 2011 and 2013. Fescue and alfalfa were harvested in two or three cuttings depending on years, with the last cut in October. Annual crops were cultivated under superficial tillage (12-15 cm deep). Sorghum was sown in late May and harvested in late September. Triticale was sown in mid-October and harvested in late July or early August.

A catch crop was sown every year in late August or early September between triticale and sorghum (oat in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2013). The N fertiliser was applied as UAN solution (urea ammonium nitrate). Perennial crops received a single annual application in late April. Fescue received N fertiliser at the beginning of each cycle of regrowth and seedling crops were not fertilised before the first cut, so that the total N rate varied according to the year. Sorghum was fertilised just before sowing (late May) and triticale in March and in late April. The experiment did not receive irrigation, except in May 2011 for semi-perennial and annual crops (58 mm in total) to facilitate establishment during a drought period. Further details about crop management are given by Cadoux *et al.* (2014).

3.2.2 Climatic data

Climatic data were obtained from an automatic weather station located on the experimental site. Over the period 2006-2014, mean annual temperature was 10.6 °C, annual rainfall (P) and Penman potential evapotranspiration (PET) were 699 and 715 mm respectively and annual global radiation was 4614 MJ m^{-2}. The water balance (P - PET) during the growing season (April to October) varied widely between years (Table 3.2), from -160 mm in the wettest year 2013 to -433 mm in the driest year 2009. The water balance during the drainage period, i.e. between November and March, was on average 191 mm and varied between 152 and 217 mm between years.

Table 3.2. Meteorological data: P = precipitation (mm), PET = Penman potential evapotranspiration (mm) recorded at Estrées-Mons over seven years.

Growing season: April-October				Drainage period: November-March			
	P	PET	P-PET		P	PET	P-PET
2006	450	702	-252	2006-07	297	81	217
2007	434	649	-216	2007-08	272	80	192
2008	491	626	-135	2008-09	216	63	152
2009	297	730	-433	2009-10	304	95	209
2010	369	667	-299	2010-11	259	72	186
2011	337	586	-249	2011-12	268	88	179
2012	461	596	-135	2012-13	254	68	185
2013	497	603	-106	2013-14	299	94	205

3.2.3 Crop production

The aboveground biomass at harvest was measured for each crop. On each harvest date, plants were collected manually and weighted. Details about sampling methodologies are given by Cadoux *et al.* (2014). The dry matter content was determined after drying representative subsamples at 65 °C for 96 h.

3.2.4 Soil water content and mineral nitrogen

Soil mineral nitrogen (SMN), *i.e.* soil nitrate and ammonium, along with soil water content (SWC) were measured in the three blocks each year in mid-autumn (early November) and late winter (mid-March). Soil cores were collected down to a depth of 150 cm with a hydraulic coring device. Soil cores were taken from 2006 to 2014 in all treatments, except in autumn for late harvested perennials (because the plots were not accessible to the coring device) and in autumn 2006 for all perennials.

The cores were divided into five layers (0-30, 30-60, 60-90, 90-120 and 120-150 cm). Composite soil samples were formed by mixing six soil cores in each plot. The samples were frozen immediately after collection until extraction and subsequent analysis. After thawing, gravimetric water content was determined and nitrate and ammonium were extracted using a KCl solution (1 M) and analysed by continuous flow colorimetry. Soil water content (mm) and soil nitrate and ammonium (kg N ha^{-1}) were calculated by taking account of the bulk density which was measured for each layer with steel cylinders (Table 2.2).

3.2.5 Nitrate concentration in subsoil

Soil cores of 80 mm diameter were extracted down to 390 cm on mid-November 2011, using a mechanical soil corer. One soil core was collected for each plot in N+ with a depth increment of 30 cm (13 layers). For each layer, gravimetric water content and nitrate content were determined using the same method as above. Nitrate concentration in the soil solution (mg NO_3 l^{-1}) was calculated as the ratio of nitrate content (mg N kg^{-1} soil) and the soil water content (l kg^{-1} soil) multiplied by 62/14.

3.2.6 Assessment of drainage and nitrate concentration in drained water

The SWC and soil nitrate content measured in early November were used as initial values to assess drainage and nitrate leaching below 150 cm during winter using the STICS model. STICS is a dynamic model which simulates the carbon, water and N balances of the soil-crop system with a daily time step by taking into account the impact of weather, soil, crop and management practices (Brisson *et al.*, 2003; Brisson *et al.*, 2008). The model has been

successfully evaluated for simulating water and N dynamics in various conditions including bare soils (Beaudoin *et al.*, 2008; Constantin *et al.*, 2012; Coucheney *et al.*, 2015). We performed a total of 256 simulations (36 plots × 7 years). The first winter (2006-07) was not simulated because of the lack of initial data for perennial crops. Input data for soil characteristics were estimated for each soil layer from direct measurements (Chap. 2). The water content at field capacity was defined as the median of the gravimetric measurements made in late winter. For the two upper layers, years presenting a negative water balance during the ten days before sampling were excluded to calculate the median. All plots were simulated as bare soils from November to early spring, assuming that the different crops had no significant growth during this period and influenced neither the water content nor the mineral N content in soil. This assumption is valid for perennials which were dormant and sorghum which was not sown. Fescue, alfalfa and triticale started their regrowth after mid-march, so that simulations were stopped on March 15 for these situations. Simulations were stopped at the date of emergence of perennial crops (on average April 3 for miscanthus E and April 9 for switchgrass E) or at the date of sowing of the other crops (on average April 17 for fescue and alfalfa and May 20 for sorghum). We assumed that most of the annual drainage occurred during this simulation period in our pedo-climatic conditions, in agreement with Beaudoin *et al.* (2005), although some late events in spring may have been ignored. For each plot and each winter, the model predicted the amounts of drained water and leached nitrate and the mean nitrate concentration of the drained water.

The ability of STICS to simulate the water and nitrate fluxes was evaluated by comparing the simulated and observed values of SWC and soil nitrate contents measured in mid-March in all plots. We computed the root mean square error (RMSE) and the mean deviation (MD) (Constantin *et al.*, 2012). Although the results were satisfactory for SWC, STICS was found in a first run to overestimate the soil nitrate content in most situations, except after alfalfa destruction. Therefore crop residues with a C:N ratio of 40 were incorporated into the soil on the first day of simulation in order to simulate immobilization of mineral N in the surface layer. The amount of residues was fitted for each preceding crop using a trial-error approach. After alfalfa destruction in 2011 and 2013, the C:N ratio of added residues was set at 10 in order to increase N mineralisation. A sensitivity analysis was performed to test the effect of varying the amount of residues added on nitrate leaching and nitrate concentration in drained water.

In an earlier paper, we showed that all crops in the experiment except sorghum had roots below 150 cm and we measured water capture between 150 and 210 cm depth using

water content reflectometers (Chap. 2). As a consequence, measurements in mid-autumn down to 150 cm underestimated the total soil water deficit (SWD), *i.e.* the difference between SWC at field capacity and actual SWC, and the drainage simulated with STICS at 150 cm overestimated the water recharge towards the aquifer. Drainage below 210 cm was calculated as the difference between the annual drainage simulated by STICS at 150 cm and the SWD in the 150-210 cm layer measured in early November. Since only N+ treatments were monitored with water content reflectometers, we calculated the ratio between SWD in the 120-150 cm layer and in the 150-210 cm layer and applied this ratio to the N- treatments, assuming that the distribution of water uptake within the soil profile was equivalent in N- and N+. For nitrate concentration, we assumed that it was identical at 150 and 210 cm. Nitrate leaching at 210 cm was then calculated annually using the corrected drainage at 210 cm and the simulated nitrate concentration at 150 cm.

3.2.7 Statistical analyses

All statistical analyses were performed using R (R Core Team, 2014). The influence of preceding crop, N rate, year and their interaction on drainage and nitrate concentration were evaluated by analysis of variance (ANOVA). ANOVA was also performed to assess the effects of rotation and N rate on mean SWC, soil nitrate and soil ammonium measured over seven years for each treatment and mean drainage and nitrate concentration. The effects of rotation and N rate on the nitrate concentration in the soil solution measured in autumn 2011 were evaluated for each soil layer using a third ANOVA.

Two linear mixed-effect models were used: the first one adapted to a split-block design (with blocks, rotation × blocks and nitrogen × blocks interactions as random factors) was used for perennial crops and the second, adapted to a split-plot design (with blocks and rotation × blocks interaction as random factors), was used for the other crops. The *lme* function from the *nlme* package was used to fit the models (Pinheiro *et al.*, 2014). Significant differences ($p<0.05$) between treatments were found with the *lsmeans* function (Lenth, 2014). The assumptions of ANOVA were checked by visually examining the residuals against predicted values and using the Shapiro-Wilk and Levene's tests. Log-transformed data or Box-Cox transformation were used if necessary to satisfy these assumptions.

3.3 Results

3.3.1 Soil water and mineral N contents in mid-autumn and late winter

The mean SWC (0-150 cm) measured over seven years in mid-autumn (2007-2013) varied significantly between rotations and N rates for perennial crops (Table 3.3). The highest value for perennial crops was observed for Swi E N- (476 mm) and the lowest for Mis E N+ (409 mm). Soils under perennial crops unfertilized had a higher water stock in mid-autumn than fertilized ones. SWC was higher under annual crops than semi-perennial crops (461 *vs.* 410 mm on average). The SWC in late winter was higher and much less variable. The mean value for semi-perennial and annual crops was 505 mm. A significant rotation × nitrogen interaction was found for perennial crops but differences between treatments were small: SWC ranged from 515 to 525 mm. These values were close to the estimated SWC at field capacity (527 mm for perennial and 516 mm for the other crops) and variations between treatments were probably due to small differences in soil texture. The temporal variability of SWC was also much higher in mid-autumn than in late winter (Table S1 and Table S4, Annexe 1). On average for all treatments, SWC in mid-autumn ranged from 390 to 490 mm, whereas SWC in late winter varied only from 501 to 527 mm.

The SMN content was on average 43 kg N ha^{-1} for all treatments in mid-autumn (2007-2013) and 46 kg N ha^{-1} in late winter (2008-2014). It was much higher at the beginning of the experiment in April 2006, with 183 and 133 kg N ha^{-1} for perennial and other crops respectively, but decreased markedly during the two first years. SMN consisted on average of 68% nitrate and 32% ammonium. In mid-autumn, more than half of the total SMN was located in the first layer and 81% was found in the two upper layers (0-60 cm). It was slightly less in late winter (46% and 74% in the 0-30 and 0-60 cm layer respectively). The soil nitrate content in mid-autumn was dependent on rotation for semi-perennial and annual crops and on rotation × nitrogen interaction for perennial crops. For perennial crops, it ranged from 11 to 35 kg N ha^{-1}, in Swi E N- and Mis E N+ respectively. For the other crops, it was lower for Fes-Alf (26 kg N ha^{-1}) than for the other rotations (37 kg N ha^{-1}). In late winter, soil nitrate was not different between Mis E and Mis L but slightly lower for Swi E than for Swi L. Between 2007 and 2013, soil nitrate in mid-autumn averaged for all treatments was not much variable over time (from 21 to 35 kg N ha^{-1}) (Table S2, Annexe 1). In late winter, it markedly increased in 2011 and 2014 after alfalfa destruction (Table S5, Annexe 1). Soil ammonium in mid-autumn was very stable over time with 10 to 16 kg N ha^{-1} on average for all treatments

(Table S3, Annexe 1). In late winter, it varied from 6 to 23 kg N ha^{-1} depending of the year (Table S6, Annexe 1).

Table 3.3. Mean soil water, nitrate and ammonium contents over 0-150 cm measured in early November (average of 7 years from 2007 to 2013) and mid-March (average of 7 years from 2008 to 2014). Values in brackets are standard deviations between blocks. Different letters indicate significant differences (p<0.05) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation (without interaction with rotations).

Rotation	N	Soil water content (mm)		Soil nitrate (kg N ha^{-1})		Soil ammonium (kg N ha^{-1})	
		Early November	Mid-March	Early November	Mid-March	Early November	Mid-March
Mis E	N-	434 (15) b+	516 (2) b	20 (1) b	16 (2) d	10 (1) a-	13 (2) b-
	N+	409 (4) b-	515 (1) b	35 (1) a	33 (2) a	15 (1) a+	17 (2) b+
Mis L	N-		518 (6) ab		17 (1) d		19 (4) a-
	N+		515 (2) b		33 (1) a		22 (5) a+
Swi E	N-	476 (4) a+	523 (4) ab	11 (1) c	11 (1) e	11 (2) a-	15 (3) ab-
	N+	446 (7) a-	520 (2) ab	19 (1) b	21 (1) c	15 (3) a+	18 (2) ab+
Swi L	N-		525 (4) a		14 (2) de		18 (2) ab-
	N+		518 (4) ab		28 (4) b		19 (1) ab+
Fes-Alf	N-	402 (6) C	506 (13) A	27 (8) C	35 (7) B	18 (2) A	16 (2) A
	N+	404 (4)	508 (11) A	25 (7)	35 (3)	17 (2) AB	16 (2)
Alf-Fes	N-	416 (15) B	503 (20) A	36 (2) AB	56 (4) A	15 (1) BC	13 (4) A
	N+	417 (7)	508 (13) A	35 (1)	58 (0)	16 (3) B	13 (2)
Sor-Tri	N-	465 (5) A	504 (9) A	37 (5) A	28 (2) B	8 (1) E	9 (1) B
	N+	455 (11)	499 (20) A	53 (9)	38 (3)	11 (1) D	10 (2)
Tri-Sor	N-	460 (5) A	504 (2) A	31 (8) B	38 (9) B	11 (1) D	9 (1) B
	N+	465 (13)	511 (6) A	30 (4)	38 (6)	11 (1) D	9 (2)

3.3.2 Nitrate concentration in soil and subsoil in 2011

Nitrate concentration in the soil solution was measured in N+ treatments down to 390 cm in November 2011, *i.e.* five years after the beginning of the experiment (Figure 3.2a,b). As generally observed for SMN in mid-autumn, nitrate concentration was higher in the upper layer (0-30 cm) and decreased with depth for all treatments in the five first layers. There was no significant difference between treatments whatever the soil layer under perennial crops, and no significant difference below 180 cm for the other crops. Nitrate concentration in the layer 0-180 cm was higher for Sor-Tri and Alf-Fes (*i.e.* after sorghum and alfalfa) than for the other two rotations. The high concentration in the upper layer after sorghum is attributed to a reduced N uptake in 2011 associated with a low biomass production due to a dry weather after

sowing which hampered the crop establishment. Nitrate concentration under perennial crops was very low between 60 and 300 cm (2 mg NO_3 l^{-1} on average). It increased below and reached 25 mg NO_3 l^{-1} on average in the 360-390 cm layer, with a large variability. Conversely, nitrate concentration under the other crops was fairly constant from 120 to 390 cm and averaged 8 mg NO_3 l^{-1}.

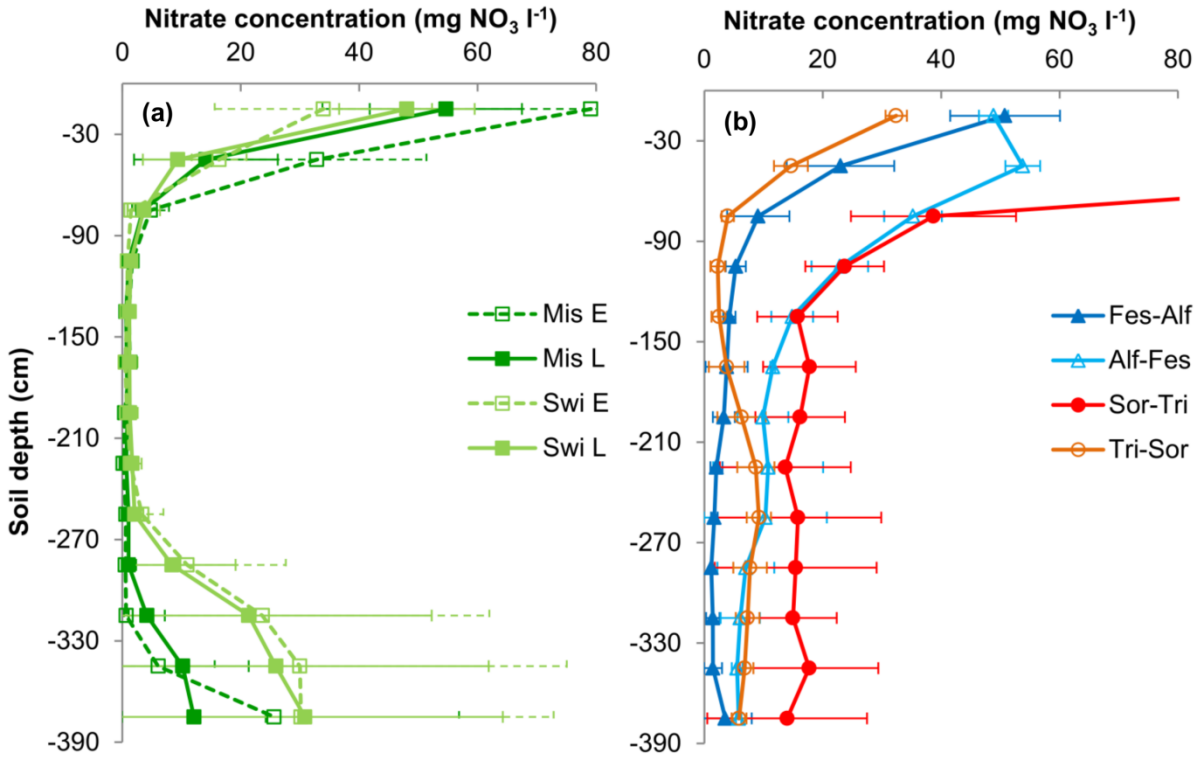


Figure 3.2. Nitrate concentration in soil solution versus depth measured in November 2011 in N+ treatments of perennial crops (a) and other crops (b). Bars represent the standard deviations between blocks.

3.3.3 Ability of STICS to reproduce SWC and soil nitrate in late winter

STICS was able to reproduce the SWC and the soil nitrate measured in late winter (Figure 3.3a,b). The RMSE was 10 mm for SWC and 16 kg N ha^{-1} for soil nitrate. The mean of observed and simulated SWC were almost identical (510 *vs.* 511 mm respectively) and the quality of fit was equivalent between the different layers (data not shown). The model slightly overestimated the soil nitrate content with a MD of 7 kg N ha^{-1} for the whole soil profile. Nevertheless, the RMSE and MD in the lower layer (120-150 cm) were small (RMSE = 3 and MD = 1 kg N ha^{-1}). The amount of nitrate measured in this layer was also the lowest (2 kg N ha^{-1} on average).

We performed a sensitivity analysis of the model to the amount of residues which were added to improve the prediction of soil nitrate in late winter. A 50% increase (resp. decrease) in the amount of added residues increased the amount of soil nitrate content by 2%

(resp. 19%) and the nitrate leaching by 1% (resp. 1%). Without adding residues, net N mineralisation is increased by 15 kg N ha^{-1}, soil nitrate in late winter is increased by 42% and nitrate leaching by 3% only. We conclude that nitrate leaching predicted by the model was almost unaffected by the tuning of net N mineralisation. This may be explained by the moderate rainfall during the drainage period which makes that the upper soil layer had a small contribution to nitrate leaching. The optimised value of net N mineralization was small for all treatments (5 kg N ha^{-1} on average between early November and mid-March) except during winters following the destruction of semi-perennial crops. N mineralization reached 25 kg N ha^{-1} after fescue and 67 kg N ha^{-1} after alfalfa destruction.

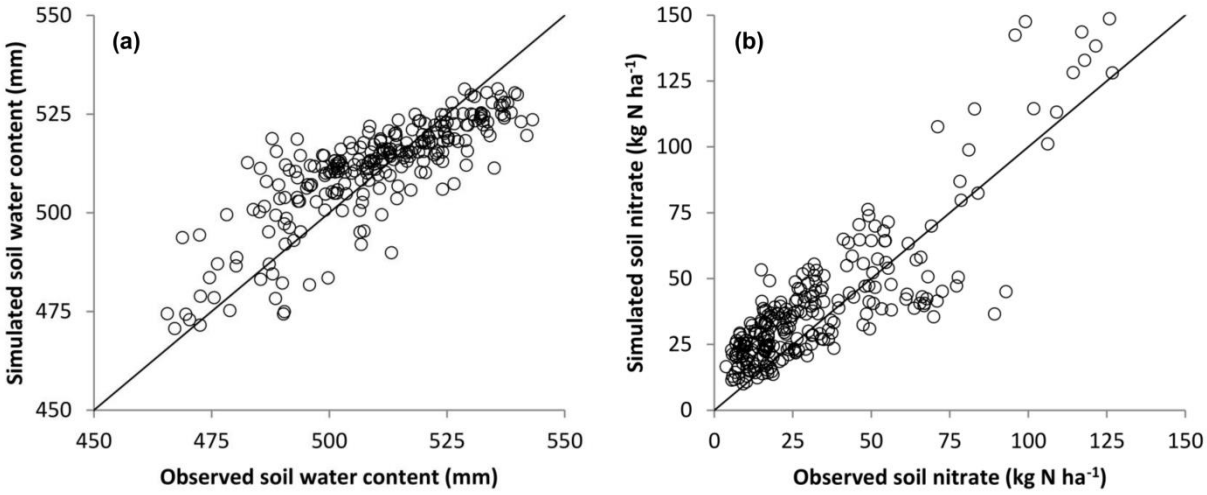


Figure 3.3. Comparison between observed and simulated values with STICS of (a) soil water content (a) and soil nitrate content (b) over 0-150 cm in mid-March for seven years (2007 to 2014). Each point represents one plot for a given year. The continuous lines are the 1:1 lines.

3.3.4 Evolution of drainage and nitrate concentration in drained water

Drainage varied greatly between treatments and years (Table 3.4). It was influenced by preceding crop, year and their interaction (Table 3.5). Significant effects of nitrogen and nitrogen × year interaction were also found for perennial crops. Indeed, the amount of drained water was similar in the two fertiliser treatments during the first years and became higher in the unfertilized treatment during the following years. Drainage varied widely between years, from 41 mm in 2009-10 to 192 mm in 2013-14. It also varied with crop rotation, from 227 mm after triticale in 2013-14 (Tri-Sor rotation) to 0 after alfalfa in 2009-10 and 2010-11 (Fes-Alf rotation). The estimated SWD in mid-autumn in the 150-210 cm layer varied between 1 and 54 mm. The reduction in drainage due to consideration of this SWD ranged from 16 mm in 2008-09 to 31 mm in 2009-10 (average of all treatments).

Table 3.4. Annual drained water (mm) calculated between 2007-08 and 2013-14. Values in brackets are standard deviations between blocks.

Rotation	N	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
Mis E	N-	71 (31)	41 (5)	38 (17)	71 (13)	84 (37)	70 (8)	208 (20)
	N+	62 (14)	46 (14)	24 (30)	51 (13)	9 (15)	40 (10)	157 (5)
Swi E	N-	129 (16)	134 (15)	55 (14)	138 (8)	122 (18)	106 (11)	208 (16)
	N+	126 (24)	118 (15)	28 (8)	64 (18)	61 (13)	70 (0)	188 (23)
Fes-Alf	N-	39 (18)	123 (45)	0 (0)	0 (0)	39 (7)	43 (8)	173 (3)
	N+	46 (36)	104 (66)	0 (0)	0 (0)	50 (22)	56 (10)	196 (9)
Alf-Fes	N-	55 (28)	98 (29)	6 (10)	11 (19)	123 (21)	29 (18)	160 (21)
	N+	54 (17)	102 (30)	0 (0)	14 (25)	110 (38)	20 (20)	141 (21)
Sor-Tri	N-	48 (19)	186 (10)	53 (0)	114 (6)	139 (20)	185 (16)	211 (18)
	N+	54 (14)	186 (20)	48 (22)	88 (14)	132 (19)	168 (43)	211 (23)
Tri-Sor	N-	171 (21)	127 (9)	134 (5)	115 (8)	108 (13)	107 (18)	231 (1)
	N+	173 (14)	123 (26)	103 (15)	109 (20)	101 (12)	91 (5)	222 (9)

Table 3.5. Factors influencing annual drainage and nitrate concentration in drained water over seven years (2007-08 to 2013-14). Asterisks indicate probability levels: * p<0.05; ** p<0.01; *** p<0.001; NS = not significant.

Factor or interaction		Drained water (mm)		Nitrate concentration (mg l^{-1})	
		Perennials	Other crops	Perennials	Other crops
Preceding crop	1	***	***	***	***
Nitrogen rate	2	***	NS	**	NS
Year	3	***	***	***	***
	1 x 2	NS	NS	NS	NS
	1 x 3	***	***	***	***
	2 x 3	***	NS	NS	NS
	1 x 2 x 3	NS	NS	NS	**

Nitrate concentration in drained water was generally less variable than drainage (Table 3.6) although it was dependent on preceding crop, year and their interaction. Nitrogen rate also influenced nitrate concentration under perennial crops and a significant interaction between the three factors (preceding crop, nitrogen and year) was found for the other crops. The highest value (98.2 mg NO_3 l^{-1}) was found after Mis E N+ in 2007-08 and the lowest (0.6 mg NO_3 l^{-1}) after fescue N- in 2012-13. Nitrate concentration under miscanthus E exhibited the highest temporal variability (Figure 3.4). It reached 83.3 mg NO_3 l^{-1} in 2007-08 (average of N- and N+), then decreased to 12.4 mg NO_3 l^{-1} in 2008-09 and remained below

10 mg NO_3 l^{-1} during the following years. Nitrate concentration under switchgrass E was lower and much more stable, varying between 1.1 and 6.1 mg NO_3 l^{-1} (average of N- and N+). Annual crops produced higher concentrations than perennial and semi-perennial crops did, except for miscanthus during the two first years, ranging from 4.5 to 15.3 mg NO_3 l^{-1} (average of the two rotations, N- and N+). A small increase in nitrate concentrations under semi-perennial crops was observed during the transitions between the two crops, *i.e.* in 2008-09, 2011-12 and 2013-14 (average 10.2 *vs.* 3.2 mg NO_3 l^{-1} in the other years).

Nitrogen leaching calculated annually was low. It varied from 0 (Fes-Alf in 2009-10 and 2010-11, no drainage) to 13.8 kg N ha^{-1} (Mis E N+ in 2007-08).

Table 3.6. Nitrate concentration (mg NO_3 l^{-1}) in drained water simulated between 2007-08 and 2013-14 (mean annual weighted concentration). Values in brackets are standard deviations between blocks.

Rotation	N	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
Mis E	N-	68.3 (26.9)	4.6 (3)	5.3 (1.2)	2.5 (0.1)	1.0 (0.4)	1.1 (0.4)	2.6 (0.3)
	N+	98.2 (7.4)	20.3 (17.8)	8.2 (2.9)	2.1 (0.9)	1.8 (0.8)	1.1 (0.2)	9.6 (5.3)
Swi E	N-	2.5 (0)	2.2 (0.4)	4.1 (0.9)	2.3 (1)	1.1 (0.5)	1.0 (0.2)	1.8 (0.7)
	N+	9.6 (5.7)	2.8 (0.5)	5.6 (0.4)	2.1 (0.6)	1.3 (0.8)	1.2 (0.4)	3.7 (0.2)
Fes-Alf	N-	2.6 (0.1)	2.8 (0.8)	5.9 (0.1)	3.0 (0.1)	4.8 (1.1)	0.6 (0.1)	4.2 (1.4)
	N+	2.7 (0.1)	2.5 (0.2)	5.0 (0.5)	2.9 (0.5)	3.3 (0.8)	0.7 (0.1)	3.4 (0.4)
Alf-Fes	N-	5.1 (2)	6.9 (2.5)	5.8 (2.9)	2.2 (0.9)	22.9 (9.9)	1.9 (0.9)	13.5 (8.4)
	N+	4.2 (1.3)	21.8 (21.8)	3.6 (0.1)	2.6 (0.4)	20.2 (2.4)	3.0 (2.2)	15.6 (7.4)
Sor-Tri	N-	4.8 (1.8)	6.7 (1.2)	8.0 (4.8)	5.0 (0.3)	17.7 (6.8)	14.2 (5.4)	6.7 (0.5)
	N+	45.2 (27.6)	10.1 (6.6)	8.3 (5.9)	5.5 (3.1)	27.5 (10.4)	13.2 (11)	15.9 (10.3)
Tri-Sor	N-	6.9 (4.9)	9.1 (3)	9.0 (2.3)	3.8 (1)	3.5 (1.1)	8.4 (3.3)	10.1 (0.9)
	N+	4.4 (1)	6.2 (2)	15.6 (9.9)	3.7 (0.9)	2.7 (0.7)	8.6 (8.7)	4.5 (2.8)

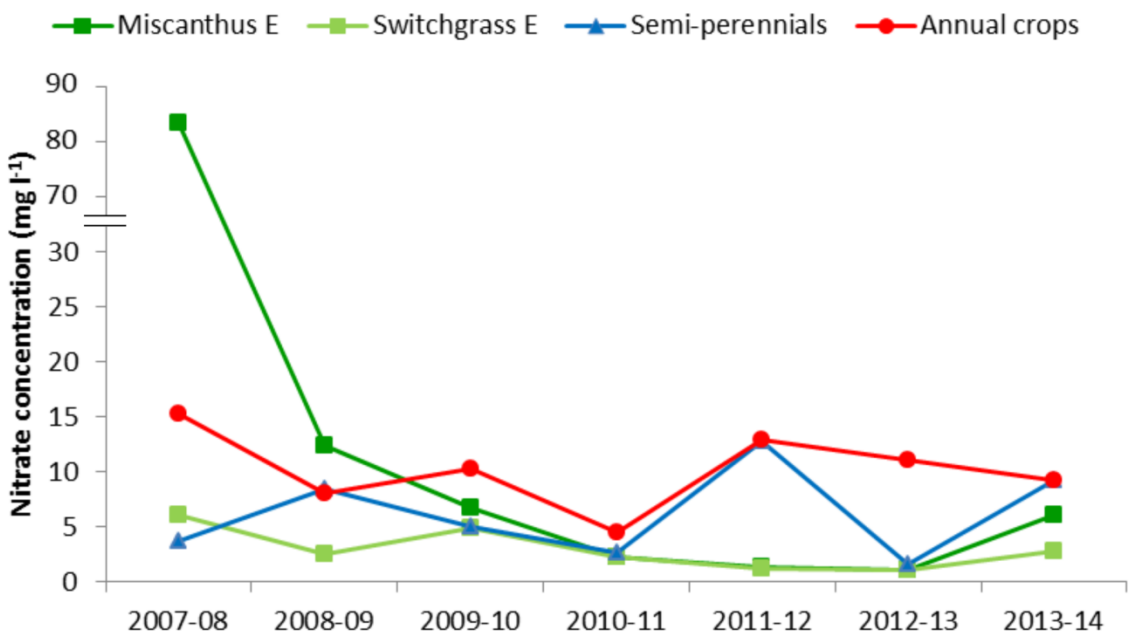


Figure 3.4. Evolution of the mean nitrate concentration in drained water (mg NO_3 l^{-1}) for miscanthus E, switchgrass E, semi-perennial (mean of Fes-Alf and Alf-Fes) and annual crops (mean of Sor-Tri and Tri-Sor). Data are averaged between N- and N+.

3.3.5 Comparison between observed and simulated nitrate concentrations in the subsoil

Nitrate concentrations observed in the subsoil in November 2011 were compared with the mean weighted nitrate concentration calculated over four years (from 2007-08 to 2010-11) for the N+ treatments. The SWC in the 210-390 cm layer in 2011 was 511 mm on average and exceeded the total amount of water drained over the four years (between 151 and 507 mm). Therefore, the thickness of the layer considered for defining the observed nitrate concentration in the subsoil was adjusted so that the amount of drained water equalled the amount of water contained in the layer. It ranged between 50 cm for Fes-Alf and Alf-Fes (210-260 cm) and 160 cm for Tri-Sor (210-370 cm). Excluding Mis E, we obtained a rather good, unbiased relationship between the two estimates (Figure 3.5): the regression equation between simulated and observed values was $y = 1.03\ x$ ($R^2 = 0.71$; $n = 5$). However, the simulated concentration for Mis E was much higher than the observed one: 41.9 *vs.* 0.4 mg l^{-1}. It is mainly due to the high losses simulated in 2007-08. This discrepancy suggests that most of the nitrate leached during this year was taken up later by the crop. This is consistent with the deep rooting system of miscanthus previously characterized on this site (Chap. 2).

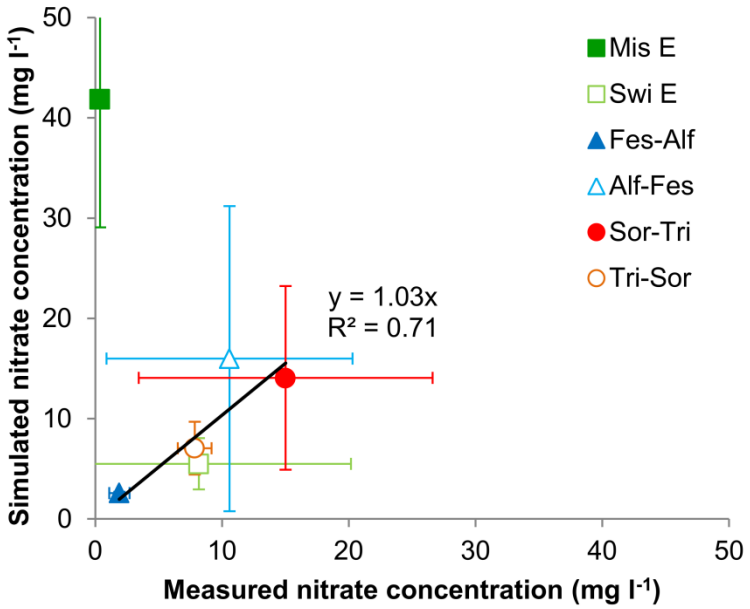


Figure 3.5. Comparison between observed and simulated nitrate concentrations in the subsoil (N+ treatments). The simulated concentration is the mean weighted nitrate concentration simulated over four years (2007-08 to 2010-11) using STICS model. The observed concentration was measured in November 2011 in the subsoil layer containing the amount of water drained during the four years. The upper depth of this layer was -210 cm and the lower depth varied between -260 and -370 cm. Bars represent the standard deviations. The black line is the linear regression without Mis E.

3.3.6 Mean drainage and nitrate concentration over seven years

The mean drainage and nitrate concentration calculated for each treatment over seven years are presented in Table 3.7. The amount of water drained annually ranged from 56 mm for Mis E N+ to 142 mm for Tri-Sor N-. The mean drainage was higher for annual (133 mm yr^{-1}) than for semi-perennial crops (64 mm yr^{-1}) and intermediate for perennial crops, varying from 56 to 137 mm yr^{-1} according to crop and N rate. Taking into account water consumption between 150 and 210 cm resulted in a decrease in drainage of 24, 29 and 17 mm yr^{-1} for perennial, semi-perennial and annual crops respectively.

The mean weighted nitrate concentration varied between 2 and 23 mg NO_3 l^{-1}. It was influenced by crop type and N rate in the case of perennials. Nevertheless, the higher concentrations for Mis E are mainly due to the year 2007-08 and are probably overestimated due to subsequent nitrate uptake by the crop in subsoil. If we exclude this year, the mean nitrate concentration was 2.5 ± 0.5 mg l^{-1} for Mis E N- and 8.1 ± 4.1 mg l^{-1} for Mis E N+. The lowest concentrations under the other crops were found for the Fes-Alf rotation (average 3.4 mg NO_3 l^{-1}) and the highest for Sor-Tri and Alf-Fes (average 13.8 mg NO_3 l^{-1}).

N leached calculated over seven years represented 2.0 kg N ha^{-1} yr^{-1} on average for all treatments. It was very low for Swi and Fes-Alf (< 1 kg N ha^{-1} yr^{-1}), highest for Sor-Tri N+ (5.0 kg N ha^{-1} yr^{-1}) and intermediate for the other treatments.

Table 3.7. Mean drainage and weighted nitrate concentration calculated over seven years in the different treatments. Values in brackets are standard deviations between blocks. Different letters indicate significant differences (p<0.05) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation (without interaction with rotations).

Rotation	N	Mean drainage (mm yr^{-1})		Mean nitrate concentration (mg NO_3 l^{-1})	
Mis E	N-	83 (13)	b+	10.4 (3.5)	a-
	N+	56 (11)	b-	23.0 (3.6)	a+
Swi E	N-	127 (9)	a+	2.0 (0.0)	b-
	N+	94 (13)	a-	4.1 (1.4)	b+
Fes-Alf	N-	59 (10)	B	3.8 (0.5)	C
	N+	65 (19)		3.1 (0.6)	
Alf-Fes	N-	69 (17)	B	12.4 (4.1)	A
	N+	63 (19)		16.9 (5.3)	
Sor-Tri	N-	134 (9)	A	9.6 (2.3)	A
	N+	127 (20)		16.4 (9.2)	
Tri-Sor	N-	142 (2)	A	7.6 (1.9)	B
	N+	132 (8)		6.1 (3.0)	

3.3.7 Relationship between biomass production and drainage

Figure 3.6 represents the relationship between biomass production and drainage for the different treatments over the seven-year period. Biomass production of perennial crops ranged from 12.8 to 26.5 t DM ha^{-1} yr^{-1}. It was higher for miscanthus E than for switchgrass E and for N+ than for N- treatments. Biomass production of semi-perennial crops was lower and ranged from 9.4 to 11.0 t DM ha^{-1} yr^{-1}. It was in the same order of magnitude for annual crops (between 9.4 and 12.1 t DM ha^{-1} yr^{-1}). Drainage under perennial and annual crops appears to be strongly and negatively linked with biomass production: $y = 5\ x + 187$ ($R^2 = 0.99$; $p < 0.001$); drainage was lower with more productive treatments. It is thus likely that the lower drainage in fertilized than unfertilized perennial crops was due to a higher biomass production and higher evapotranspiration. Semi-perennial crops had a different behaviour: they produced a much lower drainage than annual crops, in spite of a similar harvested biomass (10.3 *vs.* 10.7 t DM ha^{-1} yr^{-1}).

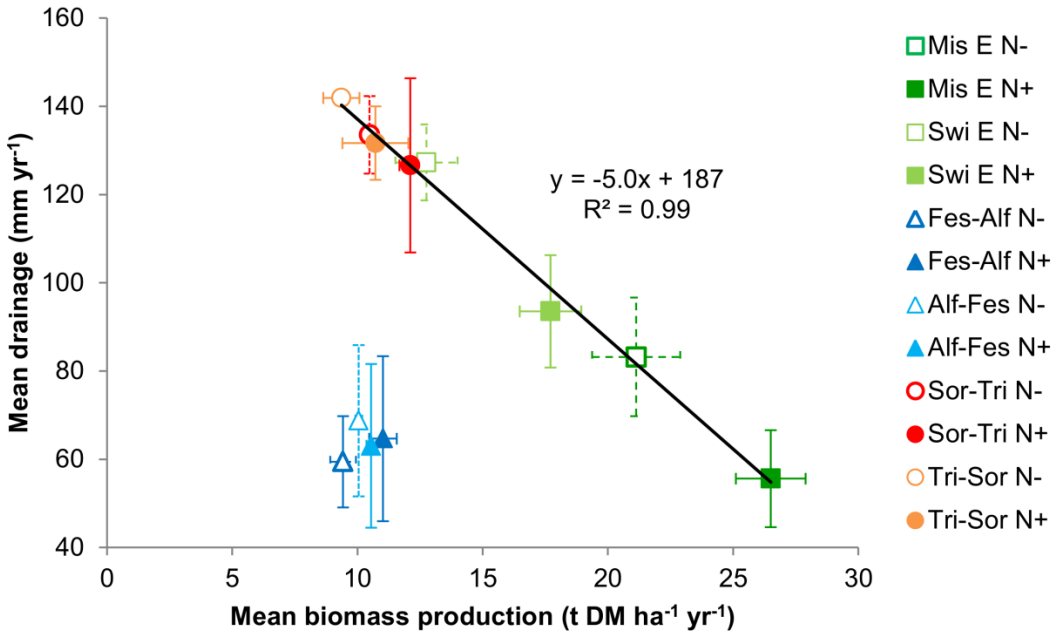


Figure 3.6. Relationship between mean water drainage and mean biomass production (average of seven years). Bars represent the standard deviations between blocks. The black line is the linear regression without semi-perennial crops. The slope represents the water consumption per unit of biomass produced (5 mm/t ha^{-1}).

3.4 Discussion

3.4.1 Water balance

Over seven years, large differences in drainage were found between treatments, with values ranging from 56 to 142 mm yr^{-1}. The differences were mainly due to the disparities in SWC at the end of the growing season, *i.e.* in early November. The length of the drainage period had a minor effect on these differences because the drainage in spring was small due to the rapid increase in evapotranspiration. Indeed, the longest drainage period occurred between triticale and sorghum but the mean drainage simulated between mid-March and the sowing of sorghum was only 19 mm yr^{-1}, corresponding to 13% of the total drainage. Assuming that water runoff was negligible (due to the very slight slope and moderate rainfall), we could calculate actual evapotranspiration (AET) between April 2007 and March 2014 by difference between precipitation and drainage, since the SWC variation was negligible. The mean AET varied between 546 and 624 mm yr^{-1} (for Tri-Sor N- and Mis E N+ respectively), with an inverse ranking to that of drainage.

The mean drainage for Mis E N+ was close to that of semi-perennial crops and much smaller than that of annual crops. This result may appear contradictory with an earlier paper based on the same experiment and using continuous SWC measurements with water content reflectometers. These authors observed that the maximal soil water deficit (SWD) occurring

during the growing season was highest for semi-perennial crops and equivalent for perennial and annual crops. The higher SWD in deep soil layers under perennials was compensated by a lower SWD in the upper layer. Nevertheless, when the crops were compared at the end of the growing season, *i.e.* in early November, the SWD under perennial crops was close to semi-perennial crops and higher than for annual crops. This was observed both with gravimetric measurements and reflectometers. Therefore the difference in drainage between perennial and annual crops is likely to be due to a higher AET during the last part of the growing season, which is consistent with the differences in crop phenology and harvest dates.

Few studies have compared drainage or AET of several bioenergy crops at the same site. Using soil moisture measurements over four growing seasons in central Illinois, McIsaac *et al.* (2010) estimated that AET from miscanthus was 104 mm yr^{-1} higher than for a maize-soybean rotation, which is consistent with our results. They also found that AET was equivalent for unfertilised switchgrass and annual crops. At the same site, Hickman *et al.* (2010) measured AET during one growing season with a residual energy balance approach. Evapotranspiration ranked in the following order: miscanthus > switchgrass > maize. Yimam *et al.* (2014) compared the annual AET of switchgrass and sorghum in two sites in Oklahoma using a soil water balance approach. AET ranged from 493 to 546 mm yr^{-1} and was greater for switchgrass than sorghum in two out of three site-years. In contrast, Abraha *et al.* (2015) measured AET by eddy covariance during three years in Michigan and found similar values for switchgrass and maize (555 mm yr^{-1} on average). In a study comparing annual and semi-perennial crops, Pugesgaard *et al.* (2014) calculated that drainage under grass-clover was 61% of the drainage under winter wheat (191 *vs.* 312 mm yr^{-1}).

Our results confirm that perennial and semi-perennial crops often consume more water than annual crops, resulting in lower drainage. This differentiation was probably enhanced by the soil type (deep soil with high available SWC). However, the relationship we observed between drainage and biomass production indicates that the higher water consumption of perennial crops was related to a higher biomass production. Semi-perennial C3 crops had approximately the same yields than annual crops but consumed more water: these crops had a lower water use efficiency (defined as the ratio between biomass production and annual AET) than the other crops.

3.4.2 N leaching and nitrate concentration

Nitrogen leaching as well as nitrate concentration in drained water were generally very low in our experiment. Over seven years, N leaching was only 2.0 kg N ha^{-1} yr^{-1} on average

for all treatments and nitrate concentration ranged between 2 and 23 mg l^{-1}. These values are much lower than those usually observed in arable cropping systems under similar climates. In a small catchment area in northern France with conventional cropping systems based on winter wheat, sugar beet, spring pea, winter barley and winter rapeseed, Beaudoin *et al.* (2005) reported mean N leaching of 27 kg N ha^{-1} yr^{-1} with a mean nitrate concentration of 49 mg l^{-1}. Benoit *et al.* (2014) compared N leaching in conventional and organic cropping systems on commercial arable farms of the Seine Basin (northern France). They found N leaching of 14-50 kg N ha^{-1} yr^{-1} in organic farming and 32-77 kg N ha^{-1} yr^{-1} in conventional farms, corresponding to mean nitrate concentrations of 53 and 106 mg l^{-1} respectively. In two long-term experiments also situated in the Seine Basin, Constantin *et al.* (2010) measured N leaching ranging from 13 to 36 kg N ha^{-1} yr^{-1} and nitrate concentration from 43 to 109 mg l^{-1}.

An important factor explaining the low nitrate losses in our study is probably the soil type. Indeed, several authors have shown that nitrate leaching is affected by soil type, with lower losses in fine (*i.e.* clayey or loamy) than coarse-textured (*i.e.* sandy) soils (Nieder *et al.*, 1995; Simmelsgaard, 1998; Beaudoin *et al.*, 2005; Lesur *et al.*, 2014). Beaudoin *et al.* (2005) found a negative relationship between N leaching (or nitrate concentration in drained water) and soil water content at field capacity (WFC). The mean nitrate concentration they measured in deep loamy soils (120 cm depth and mean WFC of 423 mm) was closer to our findings with 31 mg l^{-1}.

The small amount of N leached observed in annual crops could also be explained by crop types and management practices. The mean SMN in mid-autumn for annual crops (45 kg N ha^{-1} over 0-150 cm) was lower than that reported by Beaudoin *et al.* (2005): 57 kg N ha^{-1} in deep loamy soils (0-120 cm). This could result from (i) the absence of grain legume in our crop rotation, since SMN in autumn is enhanced after grain legumes such as pea (Beaudoin *et al.*, 2005; Benoit *et al.*, 2014); (ii) the establishment of catch crops before the spring crop (sorghum) which has been shown to reduce SMN in autumn and N leaching (Beaudoin *et al.*, 2005; Constantin *et al.*, 2010); and (iii) the moderate N fertilisation even in N+.

Nitrate concentration under semi-perennial crops was in the same order of magnitude than that measured under alfalfa by Benoit *et al.* (2014) or under grass-clover by Pugesgaard *et al.* (2014) in shallower and/or coarser soils: 24 and 12 mg l^{-1} respectively. The introduction of alfalfa into arable cropping systems has been shown to reduce nitrate losses (Beaudoin *et al.*, 1992; Benoit *et al.*, 2014). Nevertheless, nitrate concentration generally increased in our study during winters following alfalfa destruction. This was probably the result of an extra N

mineralisation induced by alfalfa destruction in autumn of approximately 65 kg N ha^{-1} during winter (*vs.* 25 kg N ha^{-1} after fescue destruction).

Under perennial crops, nitrate losses were generally very low and nitrate concentrations were most often smaller than under annual crops, except in 2007-08 for miscanthus. The higher losses observed for miscanthus in 2007-08 were due to a rather high SMN in autumn (50 and 81 kg N ha^{-1} in N- and N+ respectively) with half of it located in the deeper layers (60-150 cm). This could result from the high SMN at the beginning of the experiment in April 2006 and the slow crop growth during the first year: the aboveground biomass of miscanthus at the end of the first year was only 1.2 t DM ha^{-1}, limiting the N uptake by the crop. This effect of crop age has been observed in several studies. Lesur *et al.* (2014) assessed nitrate leaching during two winters in 38 commercial miscanthus fields. They found that crop age was the main factor influencing nitrate concentration which decreased from 31 mg l^{-1} after the first year to 7 and 3 mg l^{-1} after the second and third years respectively. High nitrate losses were measured by Christian and Riche (1998) following miscanthus establishment at Rothamsted (UK). The concentration dropped from 143 mg l^{-1} in the first winter to 13 and 9 mg l^{-1} in the second and third winters respectively in the unfertilised treatment. These high losses were probably favoured by previous crops (long-term grass removed four years earlier and winter pea as preceding crop) and by heavy winter rainfall. Smith *et al.* (2013) also observed higher N losses during the first winter following crop establishment than in subsequent years both for miscanthus and switchgrass. From the second or third year, Christian and Riche (1998), Smith *et al.* (2013) and Lesur *et al.* (2014) found nitrate concentrations similar to ours for unfertilised perennial crops. N leaching measured during three years for unfertilised miscanthus and switchgrass in Illinois by McIsaac *et al.* (2010) was also in the same order of magnitude than our measurements (5 kg N ha^{-1} yr^{-1} on average for both crops) and much smaller than for a maize-soybean rotation. This low N leaching for unfertilised miscanthus crops was confirmed by Davis *et al.* (2014) who found 2.6 kg N ha^{-1} on average over 6 sites in the US during the fifth year after establishment. In light of these results, it appears that the establishment phase of perennial crops presents a greater risk of nitrate losses than the mature phase. However, in deep soils such as in our study, nitrate lost during the first years and present in the subsoil can be taken up later by the crops thanks to their deep rooting depth (Chap. 2). This probably happened in our experiment for miscanthus as shown by the deep cores taken in subsoil in 2011. There is (apparently) no consensus on the effect of N fertilisation on nitrate leaching under miscanthus: in our experiment, fertilisation slightly increased nitrate concentration whereas Christian and Riche

(1998) and Davis *et al.* (2014) reported much larger losses in miscanthus fertilised with 120 kg N ha^{-1}: 30 kg N ha^{-1} in third year and 17 kg N ha^{-1} in fifth year respectively. These losses may be nevertheless overestimated due to the shallow depth of measurement (90 and 50 cm respectively) which was above the effective rooting depth of miscanthus in these sites. Furthermore, miscanthus was harvested late in these studies and N fertilisation had no effect on biomass production so that the input of 120 kg N ha^{-1} yr^{-1} probably far exceeded crop requirements in those sites. Nitrogen leaching in late harvest was not quantified in our study. It is expected to be close to that of early harvest because SMN in late winter was similar in early and late harvest treatments.

3.4.3 Perspectives for the development of bioenergy crops

This study confirms that perennial bioenergy crops managed properly can be effective to reduce nitrate losses compared to annual cropping systems. The establishment phase is however a riskier situation which has to be anticipated by lowering the amount of mineral nitrogen available at crop establishment (by avoiding legume crops or grassland as previous crops, growing a catch crop during the previous autumn, etc.). The high water consumption of semi-perennial C3 crops for a biomass production similar to annual crops can be a major drawback. For C4 perennial crops, there is a trade-off between biomass production and drainage. It seems possible that the efficiency of perennial crops to reduce nitrate losses would be greater in shallower soils where nitrate concentration remains low under perennial crops such as miscanthus (Lesur *et al.*, 2014) but increases greatly under arable cropping systems (Beaudoin *et al.*, 2005). The differences in drainage between crops could also be lower in these soils, but the biomass production is likely to be lower too. Further studies using experimental networks and/or soil-crop modelling are needed to explore these effects of soil (and climate) variability and help choosing the best locations for these crops.

4 Changes in soil carbon stocks under perennial and annual bioenergy crops

Ce chapitre correspond à un article publié dans *Global Change Biology Bioenergy* :

Ferchaud, F., Vitte, G., Mary, B., 2015. Changes in soil carbon stocks under perennial and annual bioenergy crops. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12249

List of authors

Fabien Ferchaud[1], Guillaume Vitte[1] et Bruno Mary[1]

[1] INRA, UR1158 AgroImpact, site de Laon, F-02000 Barenton-Bugny, France

Abstract

Bioenergy crops are expected to provide biomass to replace fossil resources and reduce greenhouse gas emissions. In this context, changes in soil organic carbon (SOC) stocks are of primary importance. The aim of this study was to measure changes in SOC stocks in bioenergy cropping systems comparing perennial (*Miscanthus* × *giganteus* and switchgrass), semi-perennial (fescue and alfalfa) and annual (sorghum and triticale) crops, all established after arable crops. The soil was sampled at the start of the experiment and five or six years later. SOC stocks were calculated at equivalent soil mass and $\delta^{13}C$ measurements were used to calculate changes in new and old SOC stocks. Crop residues found in soil at the time of SOC measurements represented 3.5 to 7.2 t C ha^{-1} under perennial crops *vs.* 0.1 to 0.6 t C ha^{-1} for the other crops. During the five-year period, SOC concentrations under perennial crops increased in the surface layer (0-5 cm) and slightly declined in the lower layers. Changes in $\delta^{13}C$ showed that C inputs were mainly located in the 0-18 cm layer. In contrast, SOC concentrations increased over time under semi-perennial crops throughout the old ploughed layer (*ca.* 0-33 cm). SOC stocks in the old ploughed layer increased significantly over time under semi-perennials with a mean increase of 0.93 ± 0.28 t C ha^{-1} yr^{-1}, whereas no change occurred under perennial or annual crops. New SOC accumulation was higher for semi-perennial than for perennial crops (1.50 *vs.* 0.58 t C ha^{-1} yr^{-1} respectively), indicating that the SOC change was due to a variation in C input rather than a change in mineralisation rate. Nitrogen fertilisation rate had no significant effect on SOC stocks. This study highlights the interest of comparing SOC changes over time for various cropping systems.

Keywords

Bioenergy crops, soil organic carbon, SOC, carbon sequestration, ^{13}C abundance, isotope, miscanthus, switchgrass

4.1 Introduction

Biomass can contribute to the energy transition towards low-carbon economies in response to the challenges of climate change and depletion of fossil resources (IPCC, 2011). The use of dedicated bioenergy crops is therefore expected to increase significantly (Chum *et al.*, 2011; Bentsen and Felby, 2012). The development of new conversion technologies and biorefineries allows considering a wide range of candidate crops (Ragauskas *et al.*, 2006; Somerville *et al.*, 2010). However, these crops will have to fulfil several requirements, including high productivity, low greenhouse gas (GHG) emissions and low environmental impacts (Tilman *et al.*, 2009; Karp and Richter, 2011). Different crop types such as short rotation coppices, perennial grasses, semi-perennial forage and annual crops are being investigated (Lewandowski *et al.*, 2003; Karp and Shield, 2008; Sanderson and Adler, 2008; Zegada-Lizarazu and Monti, 2011; van der Weijde *et al.*, 2013). Among them, perennial C4 crops such as miscanthus and switchgrass are viewed as promising bioenergy crops because of their high biomass production, low nutrient requirements and low GHG emissions (Don *et al.*, 2011; Cadoux *et al.*, 2014).

Among the environmental impacts of bioenergy crops, changes in soil organic carbon (SOC) stocks are of particular interest because they result in either carbon dioxide emissions or sequestration. Variation in SOC stock is a key term when calculating the GHG balance in bioenergy production (Don *et al.*, 2011). SOC changes occur as a result of modifications in land use, crop type and management practices, yielding a new equilibrium. Therefore, changes in SOC stocks due to bioenergy crops will depend not only on crop type and management, but also on the former land-use history. The conversion of forest or grassland to annual bioenergy crops leads to high SOC losses, creating a carbon debt that for several decades negates any reduction in GHG emissions as a result of the move away from fossil fuels to biofuel (Fargione *et al.*, 2008). On arable land, it is generally considered that SOC stocks decrease when crop residues are harvested rather than returned to the soil (Saffih-Hdadi and Mary, 2008; Powlson *et al.*, 2011). The same consequences can be expected with the introduction in crop successions of annual bioenergy crops, the whole aboveground biomass of which is harvested. In contrast, the conversion of arable land to grassland generally increases SOC stocks (Post and Kwon, 2000; Conant *et al.*, 2001; Soussana *et al.*, 2004). This increase can be explained by higher belowground inputs under grassland through root turnover and rhizodeposition which favour C storage, and slower SOC mineralisation due to the absence of soil tillage (Soussana *et al.*, 2004) although the actual effect of soil tillage on SOC stocks is questioned (Powlson *et al.*, 2014). Grassland management (mowing, grazing

intensity, nitrogen fertilisation) is known to affect SOC balance (Conant *et al.*, 2001; Soussana *et al.*, 2004; Soussana *et al.*, 2007) but SOC stock changes under forage crops such as fescue or alfalfa managed for bioenergy production have never been investigated. It is expected that the shift from annual cropping systems to perennial grasses such as miscanthus or switchgrass will increase SOC stocks for three reasons: (i) these crops allocate large amounts of C in belowground organs, either rhizomes (Garten *et al.*, 2010; Strullu *et al.*, 2011) or roots (Neukirchen *et al.*, 1999; Ma *et al.*, 2000), (ii) significant losses of aboveground biomass prior to harvest have been recorded when crops are harvested in winter, particularly for miscanthus (Amougou *et al.*, 2012), and (iii) SOC mineralisation might be reduced by the absence of soil tillage (Anderson-Teixeira *et al.*, 2013). Although there is an increasing body of work concerning the effect of perennial bioenergy crops on SOC stocks, C sequestration remains very uncertain as shown by the wide variability in experimental results (Anderson-Teixeira *et al.*, 2009; Don *et al.*, 2011). In their review, Poeplau and Don (2014) reported SOC change rates under miscanthus established on arable land ranging from -6.85 to +7.70 t ha^{-1} yr^{-1}. They could not identify possible explanatory variables (age of the crop, mean temperature *etc*.). Furthermore, very few studies have analysed the effects on SOC stocks of management practices of perennial crops, such as nitrogen fertilisation or harvest management (e.g. Follett *et al.*, 2012).

Some of the uncertainties regarding the effect of bioenergy crops on SOC stocks are probably due to methodological difficulties in measuring SOC stock changes. First of all, most of the published studies use a synchronic approach with paired plots: the soil in which a bioenergy crop has grown is sampled once, simultaneously with an adjacent reference plot. This approach can create a significant bias if the initial soil conditions are heterogeneous among the two plots. Using ^{13}C abundance, Poeplau and Don (2014) showed that the high variability reported in SOC stock changes after miscanthus plantation is probably due to this methodological bias. Secondly, the large spatial heterogeneity of perennial crops such as miscanthus makes it difficult to obtain representative soil samples (Zatta *et al.*, 2014). Thirdly, SOC stocks are most often calculated at an equal soil depth between treatments rather than at an equal soil mass, with a few exceptions such as Schmer *et al.* (2011). This may lead to bias in assessing SOC sequestration (Lee *et al.*, 2009). Finally, SOC stock changes are likely to vary with time. There is a need for long-term synchronic studies following SOC stock evolution over time and an opportunity to use ^{13}C signatures during the transition between C3 and C4 crops in order to quantify the contribution of "new" organic carbon to SOC stock changes.

In this study, we aimed at (1) comparing changes in SOC stocks of various bioenergy cropping systems with perennial, semi-perennial or annual crops established on arable land, and (2) studying the interaction with crop management, *i.e.* nitrogen fertilisation rate and harvest date of perennial crops. We analysed the first six years of an experiment which began in 2006 (Cadoux *et al.*, 2014). The originality of our approach consisted in comparing bioenergy crops at the same site, combining (1) a diachronic approach with the initial spatial variability fully characterised, (2) a calculation of SOC stocks on an equivalent soil mass (ESM) basis, and (3) the use of ^{13}C abundance to distinguish "new" and "old" SOC stock changes.

4.2 Materials and methods

4.2.1 Study site and experimental design

The study is based on an ongoing long-term experiment established at the INRA experimental station in Estrées-Mons, northern France (49.872°N, 3.013°E) called "Biomass & Environment" (B&E). The soil is a Haplic Luvisol (IUSS Working Group WRB, 2006). Soil characteristics are given in Table 4.1. Over the period 2006-2011, the mean annual temperature was 10.6 °C and annual rainfall and potential evapotranspiration were 673 and 737 mm respectively. Before 2006, the field had been cultivated for many years with annual crops, winter wheat (*Triticum aestivum*) being the most common crop. The soil was mouldboard ploughed annually, straw was not harvested and there was no organic fertilisation.

Table 4.1. Physical and chemical soil characteristics measured in 2006 for the two parts of the field trial: west (perennial crops) and east (semi-perennial and annual crops). Values in brackets are standard deviations between the 24 plots in each part

Part of the field trial	Soil layer (cm)	Clay <2 µm (g kg^{-1})	Fine silt 2-20 µm (g kg^{-1})	Coarse silt 20-50 µm (g kg^{-1})	Fine sand 50-200 µm (g kg^{-1})	Coarse sand 200-2000 µm (g kg^{-1})	$CaCO_3$ (g kg^{-1})	pH water
West	0-30	180 (27)	319 (14)	447 (22)	40 (8)	12 (2)	2 (1)	7.8 (0.2)
	30-60	233 (20)	311 (19)	409 (14)	39 (12)	6 (2)	2 (2)	7.8 (0.2)
East	0-30	148 (19)	331 (14)	471 (14)	34 (10)	14 (4)	3 (2)	7.9 (0.2)
	30-60	187 (35)	340 (61)	430 (60)	36 (15)	7 (3)	1 (1)	8.0 (0.2)

The B&E experiment was initiated to study biomass production and the environmental impacts of a wide range of bioenergy crops. It compares eight "rotations": four with C4 perennial crops (monocultures), two with C3 semi-perennial forage crops and two with C3/C4

annual crops (Table 4.2). The perennial crops are miscanthus (*Miscanthus×giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in February (L). The semi-perennial crops are fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (*Triticosecale* Wittmack). The experiment also includes two nitrogen treatments (N- and N+) with fertiliser-N rates depending on the crops (Table 1).

Table 4.2. Treatments of the B&E long-term experiment combining rotation and fertiliser-N rate (Mis = miscanthus, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fibre sorghum, Tri = triticale, CC = catch crop, E = early harvest (October), L = late harvest (February), n.h. = not harvested)

Rotation	N rate	Crop and fertiliser-N rate (kg ha^{-1})						
		2006	2007	2008	2009	2010	2011	2012
Mis E		Mis n.h.	Mis E	Mis E	Mis E	Mis E	Mis E	Mis E
	N-	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Mis L		Mis n.h.	Mis L	Mis L	Mis L	Mis L	Mis L	Mis L
	N-	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Swi E		Swi n.h.	Swi E	Swi E	Swi E	Swi E	Swi E	Swi E
	N-	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Swi L		Swi n.h.	Swi L	Swi L	Swi L	Swi L	Swi L	Swi L
	N-	0	0	0	0	0	0	0
	N+	0	120	120	120	120	120	120
Fes-Alf		CC/Fes	Fes	Fes	Alf	Alf	Fes	Fes
	N-	0	120	80	0	0	0	120
	N+	0	240	160	0	0	0	240
Alf-Fes		Alf	Alf	Alf	Fes	Fes	Alf	Alf
	N-	0	0	0	40	120	0	0
	N+	0	0	0	80	240	0	0
Sor-Tri*		CC	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC
	N-	0	0	60	0	60	0	60
	N+	0	120	120	120	120	120	120
Tri-Sor*		Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC	Sor
	N-	0	60	0	60	0	60	0
	N+	0	120	120	120	120	120	120

*Rotations with catch crops (oat in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2012) which were sown every year in late August or early September after triticale.

The 2.7 ha field was divided into two parts in order to facilitate cultural operations and limit competition between plants due to differences in canopy height (Figure 3.1): (i) a split-block design in the west part for perennial crops with rotations in the main plots (miscanthus

E, miscanthus L, switchgrass E, switchgrass L) and N fertilisation rates in the subplots (N- and N+), and (ii) a split-plot design in the east part for the other crops with rotations in the main plots (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilisation rates in the subplots (N- and N+). Both parts include three replicate blocks and 24 subplots of 360 m^2. Soil analyses performed in 2006 revealed a slightly higher clay content in the west than in the east part (180 ± 27 *vs.* 148 ± 19 g kg^{-1} in the 0-30 cm layer).

At the start of the experiment, the field was mouldboard ploughed at a depth of *ca.* 25 cm. After seedbed preparation with a cultivator, miscanthus was planted in April 2006 (1.5 rhizome m^{-2}) and switchgrass sown in June 2006 (seed rate = 15 kg ha^{-1}). Semi-perennial crops were sown in 2006, 2009 and 2011, usually in April. Before sowing, the previous crop (alfalfa or fescue) was destroyed in late autumn with a cultivator and a disc harrow (15 cm deep) in 2009 and mouldboard ploughed (*ca.* 22 cm deep) in 2011. Annual crops were cultivated under superficial tillage (12-15 cm deep) with a cultivator and a disc harrow. Fescue and alfalfa were harvested in two or three cuttings depending on years, with the last cut in October. Sorghum was harvested in late September and triticale in late July or early August. Further details about crop management are given by Cadoux *et al.* (2014).

4.2.2 Crop yields

Crop yields were measured every year from 2006 to 2011. On each harvest date, the aboveground biomass was collected manually, weighed, dried and ground before C content analysis. Details about sampling methodologies and analysis are given by Cadoux *et al.* (2014).

4.2.3 Soil sampling and analysis

The soil was sampled on two dates: May 2006 for the whole experiment, March 2011 for the perennial crops (west part of the field trial) and March 2012 for the other crops (east part of the field trial). Soil cores of 8 cm diameter were extracted with depth increments of 20 cm and inserted into plastic tubes using a powered soil corer (Humax soil sampler, Switzerland). In 2006, two soil cores were taken in each plot (one north and one south of the plot) down to 40 cm depth. In 2011 and 2012, six soil cores were taken in each plot down to 60 cm. All cores were located north of the plots inside a 2.6 m^2 micro-plot and taken in intra-row and inter-row zones. A specific coring strategy was developed for miscanthus in order to ensure a fully representative sampling scheme (Annexe 2). In each plot, two soil cores were taken in the rhizome area and four outside this area, which corresponded to the estimated fraction of the total field area covered by plant rhizomes (*ca*. 33%).

From 2005, the ploughing depth was reduced from *ca.* 30-35 cm to less than 25 cm in all treatments. The old ploughing depth (referred to below as *Y*) was identified in the soil cores on each sampling date by detecting changes in soil colour and structure. Soil cores removed from the plastic tubes in the laboratory were divided into three layers (0-20, 20-*Y* and *Y*-40 cm) in 2006 and into five layers (0-5, 5-20, 20-*Y*, *Y*-40 and 40-60 cm) in 2011 and 2012. Coarse residues (>2 mm), roots and rhizomes were then carefully removed from the soil by handpicking. The very fine roots could not be removed because it was a very time-consuming operation, especially for perennial crops which showed a large amount of roots in the cores. However, we could estimate that the C contained in very fine roots represented only 0.04 g C kg^{-1} soil on average and less than 0.20 g C kg^{-1} in all samples. Soil samples were dried at 38 °C for 96 h, crushed through a 2 mm sieve, subsampled and finely ground with a ball mill (PM 400, Retsch, Germany) before carbon analysis. 1368 soil samples were analysed for carbon concentration and ^{13}C abundance using an elemental analyser (EURO EA, Eurovector, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus Advantage, Thermo Electron, Germany).

Bulk densities were determined at each sampling date by two methods. The first was used for the 0-5 cm layer and consisted in pushing a steel cylinder (98 cm^3) into the soil and weighing the sample after oven drying for 48 h at 105 °C. The second method consisted of using a dual gamma probe (LPC-INRA, France) on the 5-10, 10-15, 15-20, 20-25, 25-30, 30-35 and 35-40 cm layers (see for example Pires and Pereira (2014)). In 2006, initial bulk densities were measured in six areas spread over the whole field with five replicates. The old ploughing depth was measured at the same locations in soil trenches of 3 m length with 30 measurements per trench. In 2011 and 2012, bulk densities were measured in each micro-plot used for soil sampling, with four replicates for the 0-5 cm layer and two replicates for the other layers. For the 40-60 cm layer, we assumed that bulk density did not vary with time and used measurements that were taken on six trench walls in 2007 with steel cylinders.

4.2.4 Crop residues and belowground biomass

Crop residues (>2 mm) remaining in soil were measured in 2011-2012 at the time of SOC measurements and in the same micro-plots. In 2011, residues present at soil surface after harvest were collected just before soil sampling. Stem bases and fragments (>10 mm) as well as fallen leaves (mulch) of miscanthus L were sampled in one micro-plot per plot. Small stem fragments (2 to 10 mm) and leaf debris (for miscanthus L) present at soil surface were collected in six areas of 27 × 27 cm within each micro-plot, corresponding to the location of

the soil cores. Stem fragments below soil surface (>2 mm) were collected in the 8 cm diameter cores. Residues from the six areas were pooled together, as well as residues from the six soil cores. The residues from semi-perennial and annual crops, buried by soil tillage, were collected in the soil cores in 2012. All residues were dried at 65 °C for 96 h, weighed and ground before analysis.

Belowground biomass (rhizomes and roots) of perennial crops was also measured in 2011. Rhizomes and roots collected in the soil cores were separated, weighed and washed. Samples from the six cores of each micro-plot and each layer were pooled, dried at 65 °C for 96 h, weighed and ground before analysis. For miscanthus, given the very large spatial variability of the rhizome biomass, a second method was used to quantify it more precisely. It consisted in extracting the entire rhizome of a median plant per plot, selected by the number of stems (see Strullu *et al.* (2011) for details). The C concentration of residues and belowground samples was determined using an elemental analyser (FLASH EA 1112 series, Thermo Electron, Germany).

4.2.5 Calculation of soil mass, SOC stocks and $\delta^{13}C$

SOC stocks were calculated on an equivalent soil mass (ESM) basis (Ellert and Bettany, 1995). Soil mass in the 0-*z* layer was calculated as:

$$M(z) = 10 \sum_{j=1}^{z} \rho(j) \quad (1)$$

where *M(z)* is the mass of dry soil (t ha^{-1}), *j* the soil depth (mm), *z* the calculation depth (mm) and $\rho(j)$ the bulk density (g cm^{-3}) at depth *j*.

The bulk density ρ, the depth of the old ploughed layer *Y* and the soil mass over the depth 0-*Y* measured in 2006 did not differ significantly between the west and east parts of the experiment. Their mean values were respectively ρ = 1.37 ± 0.05 g cm^{-3}, *Y* = 342 ± 17 mm and M_R = 4669 ± 135 t ha^{-1}. The latter value is called "reference" soil mass. Assuming that there was no erosion (due to the very slight slope and moderate rainfall), the soil mass over the depth 0-*Y* should remain constant in time and equal to M_R.

In 2011 and 2012, *Y* was identified in the soil cores to divide the layer 20-40 cm into 20-*Y* and *Y*-40 but a more precise estimate of *Y* was made using bulk density measurements: *M* was calculated from 0 to 60 cm depth by 1 mm increments using Eq (1) and *Y* was determined as the depth at which *M* equalled M_R.

Five soil layers (0-5, 5-20, 20-*Y*, *Y*-40 and 40-60 cm) were analysed separately. The cumulative SOC stock (t ha^{-1}) measured down to the layer *n* (*n* = 1 to 5) is:

$$SOC_m(n) = 0.001 \sum_{i=1}^{n} M(i) \cdot C_m(i) \quad (2)$$

where n is the soil layer, $M(i)$ the soil mass (t ha^{-1}) and $C_m(i)$ the SOC concentration measured in layer i (g kg^{-1} dry soil).

The ^{13}C signature of SOC was expressed as δ^{13}C (‰) relative to the international PDB (Pee Dee Belemnite) standard according to the equation:

$$\delta^{13}C_m(n) = 1000\left(\frac{R(n)}{R_{PDB}} - 1\right) \quad (3)$$

where $R(n)$ is the ^{13}C/^{12}C ratio measured in layer n and R_{PDB} is the ^{13}C/^{12}C ratio of the PDB standard. The mean weighted δ^{13}C of the SOC measured down to the layer n was calculated as follows:

$$\delta^{13}C_{mw}(n) = \frac{0.001}{SOC_m(n)} \sum_{i=1}^{n} M(i) \cdot C_m(i) \cdot \delta^{13}C_m(i) \quad (4)$$

where $\delta^{13}C_m(i)$ is the δ^{13}C measured in layer i (‰).

In 2006, SOC concentrations and δ^{13}C in each layer were measured at 96 soil sample points spread across the experimental field. This sampling strategy allowed to estimate these variables in the whole field taking a geostatistical approach, using the *gstat* package in R (Pebesma, 2004). A spherical semivariogram model was fitted for each variable and soil layer. Spatial interpolation was achieved on a grid of 4 m^2 cells using ordinary kriging and its quality evaluated by the cross-validation method. The data showed a clear spatial structure, and cross-validation provided good results with a root mean square error of 0.61 g kg^{-1} for SOC concentration and 0.4‰ for δ^{13}C in the 0-*Y* layer. Predicted values at the sampling sites in 2011 and 2012 were used rather than the average measured values to calculate initial SOC stocks and δ^{13}C of the different treatments.

In 2011 and 2012, the SOC concentration in each plot was calculated as the mean of the SOC concentrations of the different soil samples for a given layer. The δ^{13}C was also calculated as the mean of the different δ^{13}C analyses weighted by the SOC concentration. For miscanthus and switchgrass, the large belowground biomass found in some soil cores for the 0-5 and 5-20 cm layers resulted in a variable soil mass from one core to another. The SOC concentration and δ^{13}C were therefore weighted by the soil mass of the cores.

Calculations on an ESM basis were performed in all layers (Figure 4.1) using the reference soil masses calculated in 2006 (M_R = 667, 2667, 5553 and 8690 t ha^{-1} for 0-5, 0-20, 0-40 and 0-60 cm respectively). SOC stocks on an ESM basis down to layer n were calculated as follows:

$$SOC(n) = SOC_m(n) - 0.001\left(M(n) - M_R\right)\cdot C_m(n) \quad \text{if } M(n) \geq M_R \quad (5)$$

$$SOC(n) = SOC_m(n) + 0.001\left(M_R - M(n)\right)\cdot C_m(n+1) \quad \text{if } M(n) < M_R \quad (6)$$

Similarly, the mean weighted $\delta^{13}C$ down to layer *n* on an ESM basis was calculated as:

$$\delta^{13}C_w(n) = \frac{\delta^{13}C_{mw}(n)\cdot SOC_m(n) - 0.001\left(M(n) - M_R\right)\cdot C_m(n)\cdot \delta^{13}C_m(n)}{SOC(n)} \quad \text{if } M(n) \geq M_R \quad (7)$$

$$\delta^{13}C_w(n) = \frac{\delta^{13}C_{mw}(n)\cdot SOC_m(n) + 0.001\left(M_R - M(n)\right)\cdot C_m(n+1)\cdot \delta^{13}C_m(n+1)}{SOC(n)} \quad \text{if } M(n) < M_R \quad (8)$$

The SOC concentration and $\delta^{13}C$ in each soil layer *n* on an ESM basis are:

$$C(n) = 1000\,\frac{SOC(n) - SOC(n-1)}{M(n) - M(n-1)} \quad (9)$$

and

$$\delta^{13}C(n) = \frac{SOC(n)\cdot\delta^{13}C_w(n) - SOC(n-1)\cdot\delta^{13}C_w(n-1)}{SOC(n) - SOC(n-1)} \quad (10)$$

In the following, SOC concentrations, $\delta^{13}C$, mean weighted $\delta^{13}C$ and SOC stocks are presented on an ESM basis for all depths. Soil layers are called L1 to L5 (corresponding to 0-5, 5-20, 20-*Y*, *Y*-40 and 40-60 cm) and pooled soil layers for cumulative SOC stocks are L1-2 to L1-5.

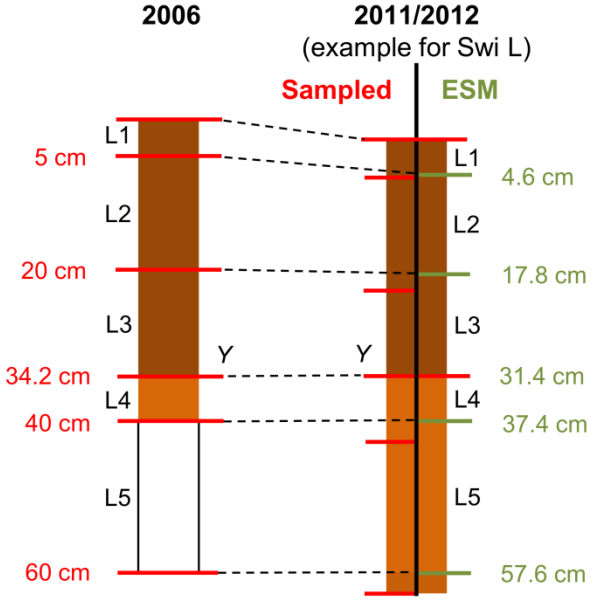


Figure 4.1. Soil layers (L) sampled in 2006 and 2011/2012 and used for soil organic carbon stocks calculations on equivalent soil mass (ESM) basis with corresponding soil depths. L5 was not sampled in 2006. L1 = 667 t ha^{-1}, L2 = 2000 t ha^{-1}, L3 = 2002 t ha^{-1}, L4 = 884 t ha^{-1} and L5 = 3137 t ha^{-1}. *Y* is the old ploughing depth.

4.2.6 Calculation of new/old SOC stocks

At the start of the experiment, SOC was derived from a mix of C3 (wheat, sugarbeet, *etc.*) and C4 (maize) crops, with a majority of C3 crops. In rotations which included only C4 (perennials) or C3 (semi-perennials) crops since 2006, it was possible to calculate the proportion of the final SOC stock derived from crop residues applied since the start of the experiment, *i.e.* the "new" SOC stock. According to Andriulo *et al.* (1999), the proportion α of new SOC in the total SOC is:

$$\alpha = \frac{\delta - \delta_0}{\delta_1 - \delta_0} \quad (11)$$

where δ is the final $\delta^{13}C$ measured in 2011 or 2012, δ_0 is the initial $\delta^{13}C$ measured in 2006 and δ_1 the $\delta^{13}C$ of the new crop. δ_1 was assessed as the average of all analyses of aboveground and belowground plant organs. The $\delta^{13}C$ values obtained were -12.7, -13.0, -28.5 and -30.2‰ for miscanthus, switchgrass, fescue and alfalfa respectively. For semi-perennial crops, a mean value for each rotation was calculated, taking into account the number of years for each crop in the rotation. The change in new SOC (ΔSOC_{New} in t ha^{-1}) was calculated as:

$$\Delta SOC_{New} = \alpha \cdot SOC \quad (12)$$

where SOC is the SOC stock in 2011 or 2012. The change in old SOC (ΔSOC_{Old} in t ha^{-1}) is:

$$\Delta SOC_{old} = SOC - SOC_0 - \Delta SOC_{new} \quad (13)$$

where SOC_0 is the SOC stock in 2006.

4.2.7 Statistical analysis

All statistical analyses were performed using R (R Core Team, 2014). For the harvested C content over the experimental period and the C content in crop residues and belowground biomass in 2011 or 2012, the effects of rotation, nitrogen and their interaction were evaluated by analysis of variance (ANOVA). ANOVA was also performed to assess the effects of rotation, nitrogen and their interaction on bulk densities in each layer in 2011 and 2012.

Rotation, nitrogen and soil layer effects on SOC concentrations and $\delta^{13}C$ were first tested in 2006, 2011 and 2012 and a second ANOVA was performed to evaluate rotation, nitrogen and year effects in each layer. Similarly, the effects of rotation, nitrogen and their interaction on SOC stocks and $\delta^{13}C$ signature were assessed each year using a first ANOVA, while year effects were assessed using a second ANOVA. The effects of rotation, nitrogen and their interaction on the change in new and old SOC stocks were evaluated using a third ANOVA.

Two linear mixed-effect models were used: the first one adapted to a split-block design (with blocks, rotation × blocks and nitrogen × blocks interactions as random factors) was used for perennial crops and the second, adapted to a split-plot design (with blocks and rotation × blocks interaction as random factors), was used for the other crops. The *lme* function from the *nlme* package was used to fit the models (Pinheiro *et al.*, 2014). Significant differences ($p < 0.05$) between treatments were found with the *lsmeans* function (Lenth, 2014). The assumptions of ANOVA were checked by visually examining the residuals against predicted values and using the Shapiro-Wilk and Levene's tests. Log-transformed data or Box-Cox transformation were used if necessary to satisfy these assumptions.

4.3 Results

4.3.1 Crop yields

The biomass production of the different crops was presented in an earlier paper (Cadoux *et al.*, 2014). The mean harvested biomass was 15.6 t DM ha^{-1} yr^{-1} for perennial crops in 2006-2010 and 9.5 t DM ha^{-1} yr^{-1} for the other crops in 2006-2011; the mean harvested C content was 7.31 and 4.16 t ha^{-1} yr^{-1} respectively (Table 4.3). Yields were higher in N+ than in N-, except for miscanthus L.

4.3.2 Crop residues and belowground biomass

The amount of crop residues found in soil at the time of SOC measurements was much higher in perennial than in other crops: 4.74 *vs.* 0.35 t C ha^{-1} on average respectively (Table 4.4). It was significantly affected by rotations, but not by N fertiliser rate. The residues of perennial crops were mainly located at soil surface, and residues below 5 cm depth were negligible. The soil cropped with Miscanthus L (harvested in February) contained many more residues (7.2 t C ha^{-1}) than the soils with other perennial crops (3.47-4.32 t C ha^{-1}). This is due to the presence of senescent leaves accumulated in mulch on the soil surface (2.86 t C ha^{-1}), whereas no significant leaf fall had been recorded in fields of switchgrass or miscanthus E.

Perennial belowground organs of miscanthus and switchgrass represented a large C pool in 2011 (Table 4.5). Total belowground biomass in L1-5 was 21.7 and 15.3 t DM ha^{-1}, corresponding to 9.90 and 6.78 t C ha^{-1} for miscanthus and switchgrass respectively. The larger part of this C was located in rhizomes for miscanthus and in roots for switchgrass. There was no significant effect of fertilisation on the belowground C content. Most of this carbon was located above 20 cm depth (in L1-2): 96% for miscanthus and 79% for switchgrass.

Table 4.3. Carbon content (t C ha^{-1}) in harvested biomass from 2006 to 2011. The mean harvested C content is calculated from 2006 to the last year before the soil sampling date (2010 for perennial crops, 2011 for the other crops). Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between rotations (lower case letters: perennial crops; upper case letters: other crops)

Rotation	Nitrogen	Carbon content in harvested biomass (t ha^{-1})						Mean harvested C content (t ha^{-1} yr^{-1})	
		2006*	2007	2008	2009	2010	2011		
Mis E	N-		10.48 (2.01)	10.86 (1.28)	11.11 (1.61)	12.29 (0.65)	7.41 (0.37)	8.95 (1.09)	a
	N+		10.52 (1.41)	12.21 (1.86)	12.79 (0.43)	13.06 (0.41)	12.58 (0.46)	9.72 (0.75)	
Mis L	N-		6.88 (2.17)	8.90 (0.85)	9.93 (1.04)	10.67 (1.08)	9.94 (1.21)	7.28 (0.85)	b
	N+		7.29 (1.73)	9.33 (1.11)	9.80 (0.88)	10.61 (1.40)	9.30 (0.91)	7.41 (0.80)	
Swi E	N-		8.97 (1.13)	8.69 (0.07)	6.98 (0.56)	4.30 (0.38)	3.43 (0.96)	5.79 (0.24)	b
	N+		8.89 (2.28)	9.17 (0.74)	9.06 (1.24)	8.46 (1.74)	6.38 (2.22)	7.12 (0.95)	
Swi L	N-		7.47 (0.47)	7.92 (0.34)	6.58 (0.29)	6.01 (0.60)	4.71 (0.37)	5.59 (0.30)	b
	N+		8.21 (1.24)	9.22 (0.76)	8.26 (0.56)	7.58 (0.41)	7.50 (0.69)	6.65 (0.34)	
Fes-Alf	N-		6.99 (0.46)	3.22 (0.04)	1.49 (0.09)	5.71 (0.88)	3.23 (0.44)	3.44 (0.27)	B
	N+		8.14 (0.25)	4.94 (0.07)	1.43 (0.29)	5.51 (0.80)	3.29 (0.18)	3.89 (0.21)	
Alf-Fes	N-	3.59 (0.28)	6.46 (0.46)	7.16 (0.12)	2.57 (0.49)	2.61 (0.19)	1.26 (0.07)	3.94 (0.01)	B
	N+	3.36 (0.52)	6.32 (0.26)	7.19 (0.23)	2.81 (0.41)	3.48 (0.35)	1.30 (0.27)	4.08 (0.16)	
Sor-Tri	N-		5.65 (0.96)	5.23 (0.17)	5.43 (0.76)	3.45 (0.38)	1.43 (0.14)	3.53 (0.10)	B
	N+		6.41 (0.18)	6.52 (0.22)	5.52 (0.65)	5.17 (0.51)	1.65 (0.37)	4.21 (0.27)	
Tri-Sor	N-	6.43 (0.25)	5.68 (0.10)	4.76 (1.01)	3.44 (0.53)	5.11 (0.82)	3.54 (0.19)	4.83 (0.26)	A
	N+	6.42 (0.23)	5.91 (0.47)	5.50 (1.07)	4.75 (0.36)	5.38 (1.90)	4.39 (0.37)	5.39 (0.50)	

*In 2006, perennial crops were not harvested because of the low biomass production during the first year of growth (0.5 and 2.5 t C ha^{-1} for miscanthus and switchgrass respectively in January 2007). Their aboveground biomass was cut and left on the soil surface

Table 4.4. Carbon content (t C ha^{-1}) in crop residues found at soil surface or in soil layers in 2011 for perennial crops and in 2012 for semi-perennial and annual crops (mean of treatments N- and N+). Values in brackets are standard deviations. Letters indicate significant differences (p<0.05) between rotations (lower case: perennial crops; upper case: semi-perennial/annual crops).

	Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes	Sor-Tri	Tri-Sor
Soil surface	2.44 (0.25) b	5.83 (0.16) a	2.50 (0.93) b	2.66 (0.12) b	0 (0) A	0 (0) A	0 (0) A	0 (0) A
L1	1.48 (0.59) a	1.35 (0.40) a	0.96 (0.13) a	1.66 (0.94) a	0.23 (0.03) B	0.07 (0.04) B	0.07 (0.15) B	0.46 (0.01) A
L2	0.06 (0.06) a	0.02 (0.04) a	0 (0) a	0 (0) a	0.15 (0.08) AB	0.04 (0.02) C	0.04 (0.03) BC	0.17 (0.02) A
L3	0 (0) a	0 (0) a	0 (0) a	0 (0) a	0.14 (0.01) A	0.03 (0.03) B	0 (0) B	0 (0) B
Total	*3.98 (0.69) b*	*7.20 (0.34) a*	*3.47 (0.84) b*	*4.32 (1.06) b*	*0.51 (0.07) A*	*0.14 (0.03) B*	*0.12 (0.02) B*	*0.63 (0.18) A*

Table 4.5. Carbon content (t C ha^{-1}) in roots and rhizomes of perennial crops measured in 2011 (mean of treatments N- and N+). Values in brackets are standard deviations. Letters indicate significant differences (p<0.05) between rotations (upper case: rhizome; lower case: roots).

Soil layer	Rhizome				Roots			
	Mis E	Mis L	Swi E	Swi L	Mis E	Mis L	Swi E	Swi L
L1	1.79 (0.37) A	2.10 (0.19) A	1.75 (0.13) A	2.06 (0.88) A	0.52 (0.10) c	0.62 (0.11) bc	1.21 (0.07) ab	1.23 (0.37) a
L2	5.36 (1.11) A	6.31 (0.56) A	0 (0) B	0 (0) B	1.26 (0.13) b	1.14 (0.31) b	2.28 (0.53) a	2.21 (0.56) a
L3	0 (0)	0 (0)	0 (0)	0 (0)	0.22 (0.04) b	0.21 (0.03) b	0.98 (0.16) a	0.73 (0.20) a
L4	0 (0)	0 (0)	0 (0)	0 (0)	0.04 (0.00) b	0.04 (0.02) b	0.16 (0.03) a	0.17 (0.04) a
L5	0 (0)	0 (0)	0 (0)	0 (0)	0.10 (0.04) b	0.10 (0.05) b	0.41 (0.04) a	0.38 (0.13) a
L1-5	*7.15 (1.48) A*	*8.41 (0.75) A*	*1.75 (0.13) B*	*2.06 (0.88) B*	*2.14 (0.20) b*	*2.11 (0.38) b*	*5.03 (0.73) a*	*4.72 (1.12) a*

4.3.3 Soil bulk densities

Bulk densities measured in spring 2006 were 1.39 g cm^{-3} on average in the 0-40 cm layer (Table 4.6). In 2011 and 2012, bulk densities varied significantly with rotation, but not with nitrogen fertilisation. The bulk density in the 0-40 cm layer was higher for perennial than for other crops (1.49 *vs.* 1.44 g cm^{-3} respectively), probably due to soil compaction occurring during the harvest of perennial crops (particularly for late harvest) and absence of soil tillage. No significant difference between treatments was found below 30 cm. In the 40-60 cm layer, the mean bulk density was 1.57 g cm^{-3}.

4.3.4 SOC concentration in the vicinity of belowground organs

It was hypothesised that perennial crops, and particularly miscanthus, would increase the spatial heterogeneity of SOC because of their heterogeneous plant cover and belowground organs. This hypothesis was evaluated by testing the correlations between the biomass of rhizomes and roots found in individual soil cores (*BG*, in g DM kg^{-1} of dry soil) and three variables measured in the same cores: the SOC concentration (g kg^{-1}), its $\delta^{13}C$ composition (‰) and the SOC derived from the new crop (g kg^{-1}). Significant positive correlations were found between *BG* and each of the three variables in layers L1 and L2 for miscanthus and in L1 for switchgrass (Table 4.7). The correlation with $\delta^{13}C$ was also significant for switchgrass in L2. The highest correlations were found for miscanthus in L2, which contained the highest amount of *BG* (154 g kg^{-1}): the regression equation for SOC concentration was $C = 0.023\ BG + 10.96$ ($r=0.54$, $p<0.001$, $n=72$). This shows that SOC concentration is higher (up to 32%) in the vicinity of belowground organs than in the rest of the soil, and that the increase is due to the supply of C4 plant material to the soil organic matter. These findings show the importance of a proper sampling protocol for perennial crops to make representative SOC stock calculations at plot scale.

Table 4.6. Soil bulk densities (g cm^{-3}) measured in 2006, 2011 and 2012 from 0 to 40 cm depth (mean of N- and N+). Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between rotations (lower case letters: perennial crops; upper case letters: semi-perennial and annual crops)

Soil layer (cm)	Year								
	2006	2011				2012			
		Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes	Sor-Tri	Tri-Sor
0-5	1.33 (0.08)	1.43 (0.03) b	1.50 (0.02) a	1.40 (0.01) b	1.45 (0.04) ab	1.35 (0.02) B	1.46 (0.06) A	1.39 (0.04) AB	1.25 (0.07) C
5-10	1.33 (0.08)	1.51 (0.02) a	1.57 (0.04) a	1.43 (0.01) b	1.52 (0.04) a	1.42 (0.01) B	1.50 (0.03) A	1.42 (0.01) B	1.28 (0.05) C
10-15	1.33 (0.07)	1.52 (0.03) b	1.58 (0.03) a	1.44 (0.01) c	1.53 (0.03) b	1.37 (0.03) C	1.45 (0.02) B	1.50 (0.03) A	1.43 (0.02) B
15-20	1.34 (0.06)	1.52 (0.03) ab	1.55 (0.01) a	1.45 (0.01) c	1.49 (0.02) bc	1.37 (0.04) B	1.43 (0.03) A	1.50 (0.00) A	1.48 (0.02) A
20-25	1.35 (0.07)	1.53 (0.01) a	1.52 (0.02) a	1.44 (0.02) b	1.49 (0.03) ab	1.36 (0.05) C	1.43 (0.04) B	1.50 (0.02) A	1.49 (0.03) A
25-30	1.39 (0.04)	1.52 (0.02) a	1.51 (0.02) a	1.45 (0.03) a	1.48 (0.04) a	1.43 (0.06) B	1.45 (0.03) AB	1.49 (0.02) AB	1.50 (0.02) A
30-35	1.50 (0.05)	1.52 (0.03) a	1.51 (0.03) a	1.44 (0.04) a	1.47 (0.06) a	1.49 (0.01) A	1.47 (0.02) A	1.49 (0.01) A	1.52 (0.03) A
35-40	1.53 (0.03)	1.49 (0.04) a	1.49 (0.04) a	1.45 (0.07) a	1.46 (0.06) a	1.50 (0.01) A	1.49 (0.04) A	1.48 (0.02) A	1.52 (0.05) A
0-40	*1.39 (0.05)*	*1.50 (0.01) a*	*1.53 (0.03) a*	*1.44 (0.02) b*	*1.48 (0.04) ab*	*1.41 (0.02) C*	*1.46 (0.02) AB*	*1.47 (0.01) A*	*1.44 (0.01) BC*

Table 4.7. Minimum and maximum values of belowground biomass (BG = roots + rhizomes) of miscanthus and switchgrass found in individual soil cores and the regression equation between BG and three variables: C (SOC concentration), $\delta^{13}C$ (SOC composition) and C_{New} (SOC concentration derived from the new crop). E, L, N- and N+ treatments were grouped together (n=72). *r* is the Pearson correlation coefficient. Significant correlations are marked with asterisks: * p<0.05; ** p<0.01; *** p<0.001

Crop	Soil layer	Belowground biomass (g DM kg^{-1} soil)		Variable	*r*		Regression equation
		Min.	Max.				
Miscanthus	L1	0.07	100.50	C (g kg^{-1})	0.41	***	y = 0.047 x + 13.70
				$\delta^{13}C$ (‰)	0.52	***	y = 0.042 x - 23.83
				C_{New} (g kg^{-1})	0.52	***	y = 0.062 x + 2.141
	L2	0.02	153.86	C (g kg^{-1})	0.54	***	y = 0.023 x + 10.96
				$\delta^{13}C$ (‰)	0.67	***	y = 0.024 x - 25.66
				C_{New} (g kg^{-1})	0.70	***	y = 0.025 x + 0.093
	L3	0.02	0.87	C (g kg^{-1})	0.22		
				$\delta^{13}C$ (‰)	0.17		
				C_{New} (g kg^{-1})	0.10		
	L4	0.00	0.31	C (g kg^{-1})	-0.05		
				$\delta^{13}C$ (‰)	0.02		
				C_{New} (g kg^{-1})	0.01		
Switchgrass	L1	0.34	87.80	C (g kg^{-1})	0.27	*	y = 0.042 x + 13.31
				$\delta^{13}C$ (‰)	0.50	***	y = 0.043 x - 23.23
				C_{New} (g kg^{-1})	0.43	***	y = 0.053 x + 2.862
	L2	0.26	8.74	C (g kg^{-1})	0.11		
				$\delta^{13}C$ (‰)	0.30	**	y = 0.092 x - 25.52
				C_{New} (g kg^{-1})	0.18		
	L3	0.08	3.07	C (g kg^{-1})	0.04		
				$\delta^{13}C$ (‰)	0.12		
				C_{New} (g kg^{-1})	0.05		
	L4	0.00	1.78	C (g kg^{-1})	0.15		
				$\delta^{13}C$ (‰)	0.26	*	y = 0.343 x - 25.34
				C_{New} (g kg^{-1})	0.29	*	y = 0.137 x + 0.03

4.3.5 Initial SOC concentrations and $\delta^{13}C$

In 2006, SOC concentrations and $\delta^{13}C$ in the different soil layers were mapped across the entire experimental field using ordinary kriging. Both variables showed a clear spatial structure (Figure 4.2 and Figure 4.3). In the old ploughed layer (L1, L2, L3), SOC concentrations were higher in the west than in the east part of the field, which was consistent with the differences observed for the clay content. Initial SOC concentrations in the 0-20 cm (L1-L2) layer were on average 11.4 ± 0.5 and 10.5 ± 0.2 g kg^{-1} for perennial and other crops respectively (Fig. 2). The spatial structure of $\delta^{13}C$ was more north-south oriented, so there was less difference between the mean initial values of the treatments (Fig. 3). The statistical analysis performed for each part of the experiment showed an effect of the soil layer (Table S1, Annexe 3). SOC concentrations in layer L3 were 6% lower on average than in layers L1 and L2. SOC concentrations below the old ploughed depth (L4) were about 50% lower than above it with a mean value of 5.4 g kg^{-1}. The mean $\delta^{13}C$ was -25.8‰ in L1 and L2 layers and slightly increased with depth to reach -25.4‰ in L4.

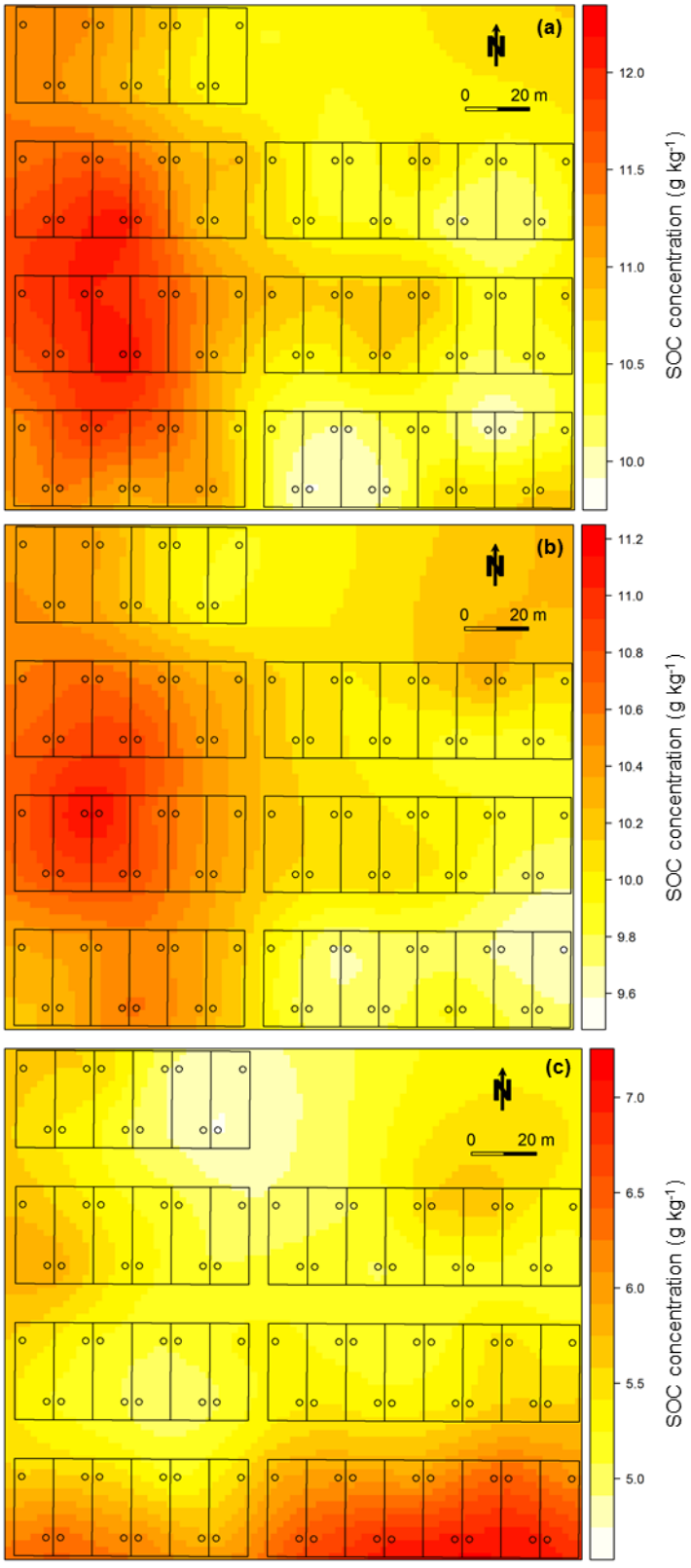


Figure 4.2. Map representing the spatial variability of soil organic carbon (SOC) concentration (g kg^{-1}) in 2006 for (a) 0-20 cm (L1-2), (b) 20-34.2 cm (L3) and (c) 34.2-40 cm (L4) obtained by ordinary kriging from the sampled points (open circles). Lines represent the outlines of the 48 plots

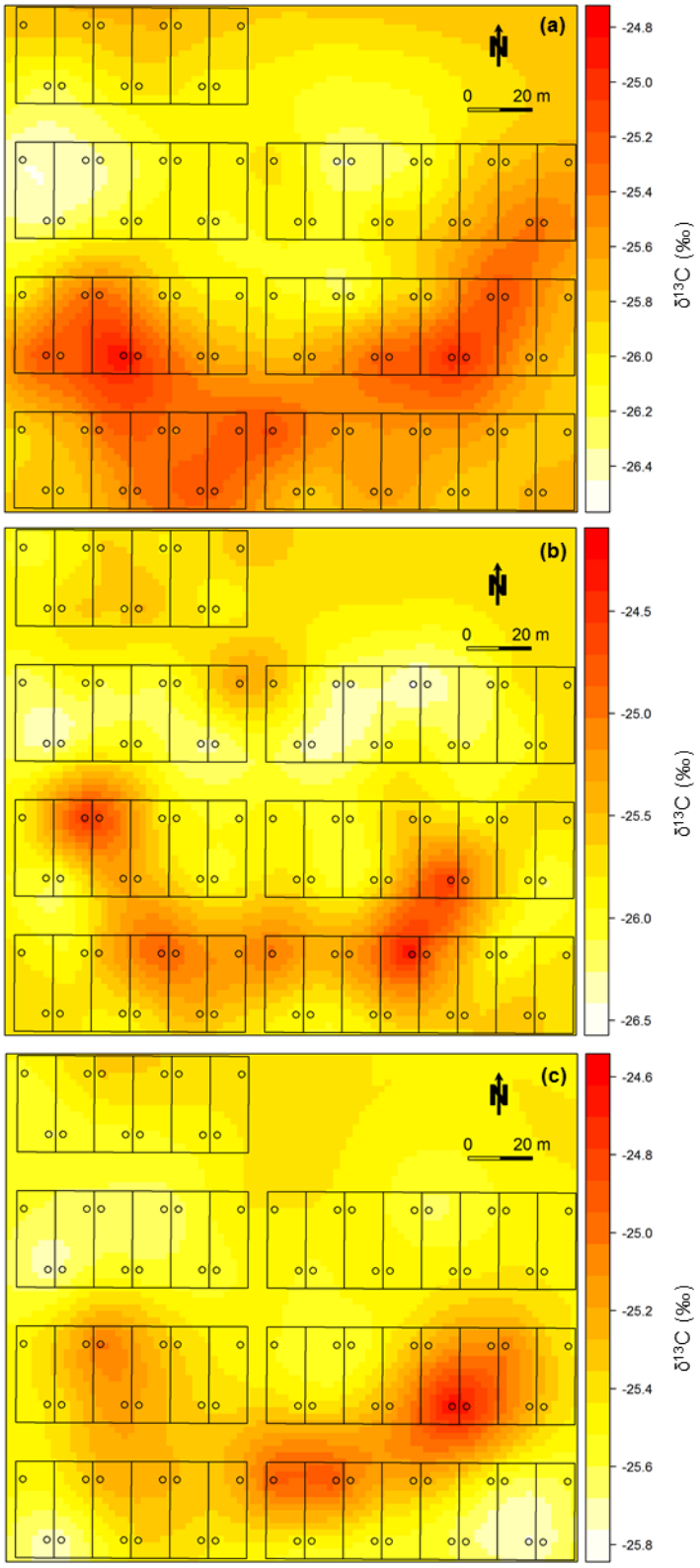


Figure 4.3. Map representing the spatial variability of $\delta^{13}C$ (‰) of soil organic carbon in 2006 for (a) 0-20 cm (L1-2), (b) 20-34.2 cm (L3) and (c) 34.2-40 cm (L4) obtained by ordinary kriging from the sampled points (open circles). Lines represent the outlines of the 48 plots

4.3.6 Changes in SOC concentrations

SOC concentrations under perennial crops in 2011 varied significantly among soil layers, but not between experimental treatments (Table S1, Annexe 3). They were much more stratified within the profile than in 2006 (Figure 4.4), with the highest concentrations in L1 (14.0 g kg^{-1} on average). The change between 2006 and 2011 was also tested for each layer. A significant increase in SOC concentration was observed between 2006 and 2011 for all treatments in L1. Conversely, there was a tendency to a slight decrease in SOC concentrations in the L2, L3 and L4 layers, although it was significant only for miscanthus L (L3 and L4) and switchgrass L (L2 and L4). SOC concentrations under semi-perennial and annual crops in 2012 also depended on soil layer. A stratified SOC distribution was observed in the old ploughed layer for annual crops, but not for semi-perennial crops. SOC concentrations significantly increased between 2006 and 2012 in L1 for the two annual rotations. An increase was also observed in L2 for the triticale-sorghum rotation. In L3, there was a tendency for a decrease, but it was only significant for the sorghum-triticale rotation. Under semi-perennial crops, SOC concentrations in layers L1, L2 and L3 had significantly increased between 2006 and 2012, from 10.3 to 11.5 g kg^{-1}. Finally, SOC concentrations in L5 in 2011 and 2012 were small and homogeneous (4.0 ± 0.4 g kg^{-1}), with no significant differences between treatments.

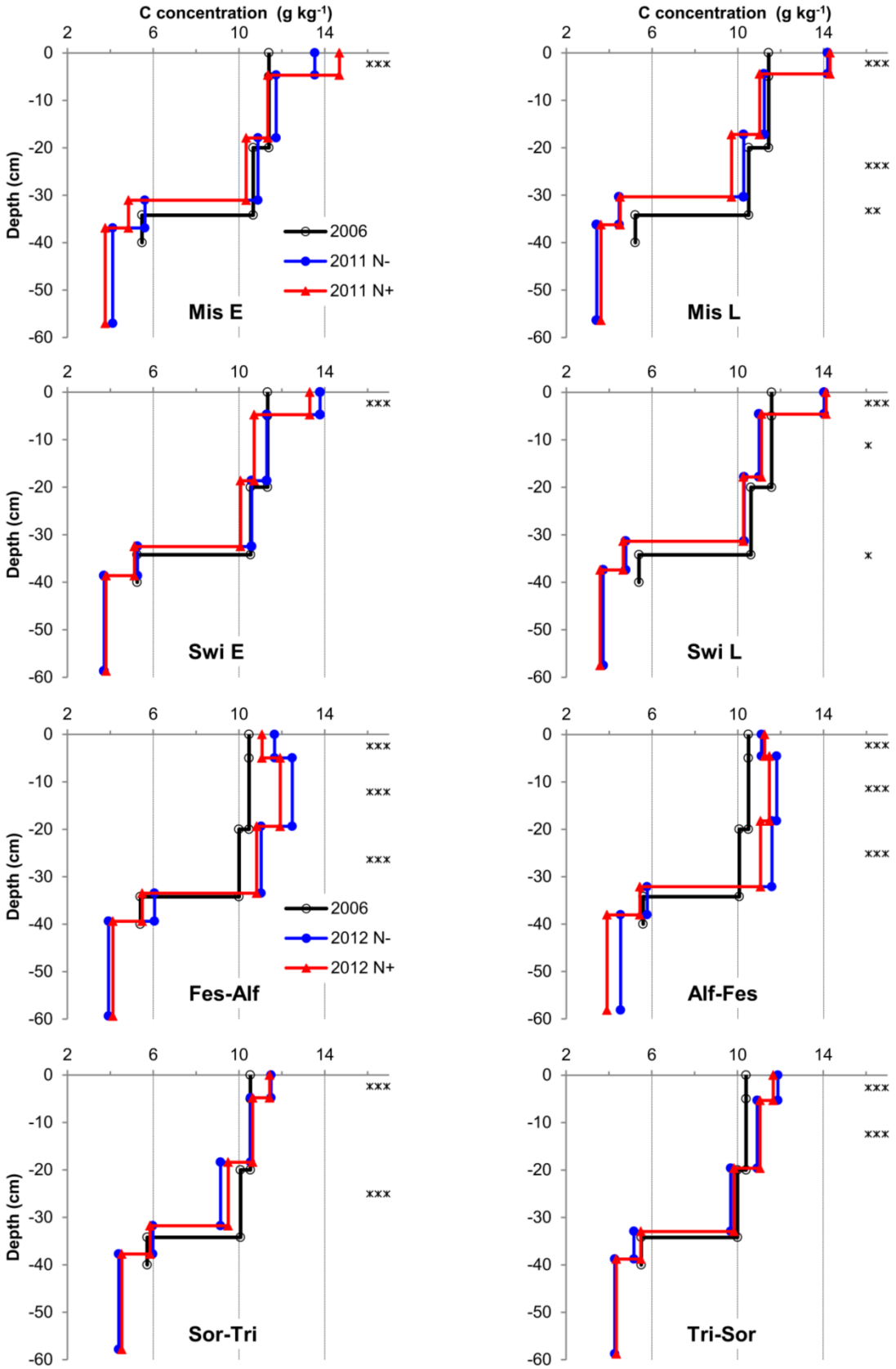


Figure 4.4. Profile of SOC concentration (g kg^{-1}) on ESM basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops). The 2006 data are averaged between N- and N+. Asterisks indicate significant changes between the two dates (* p<0.05; ** p<0.01; *** p<0.001)

4.3.7 Changes in $\delta^{13}C$

The $\delta^{13}C$ composition of SOC measured in 2011 under perennial crops varied markedly with depth (Table S1, Annexe 3): it was much higher in L1 (-23.0‰ on average) than in the other layers (Figure 4.5). It significantly increased between 2006 and 2011 in L1 and L2 for all treatments, indicating C inputs from C4 plants, but did not change significantly below *ca.* 18 cm except for switchgrass L. Soils under semi-perennial crops had a significantly lower $\delta^{13}C$ composition than soils under annual crops, which is consistent with the signature of C inputs (pure C3 for semi-perennial and mixed C3/C4 for annual crops). The effect of N fertilisation was complex: there was no effect on semi-perennials, but a tendency for annual rotations to have lower $\delta^{13}C$ in more fertilised treatments, which may result from a greater response of yield to N fertilisation in triticale than in sorghum. $\delta^{13}C$ did not differ significantly between soil layers for annual crops, but was significantly lower in the old ploughed layer (L1- L3) than below (L4-L5) for semi-perennials. The change in $\delta^{13}C$ between 2006 and 2012 in each layer depended on the rotation. $\delta^{13}C$ significantly decreased in the old ploughed layer for semi-perennial crops, and significantly increased in the same layers for the triticale-sorghum rotation.

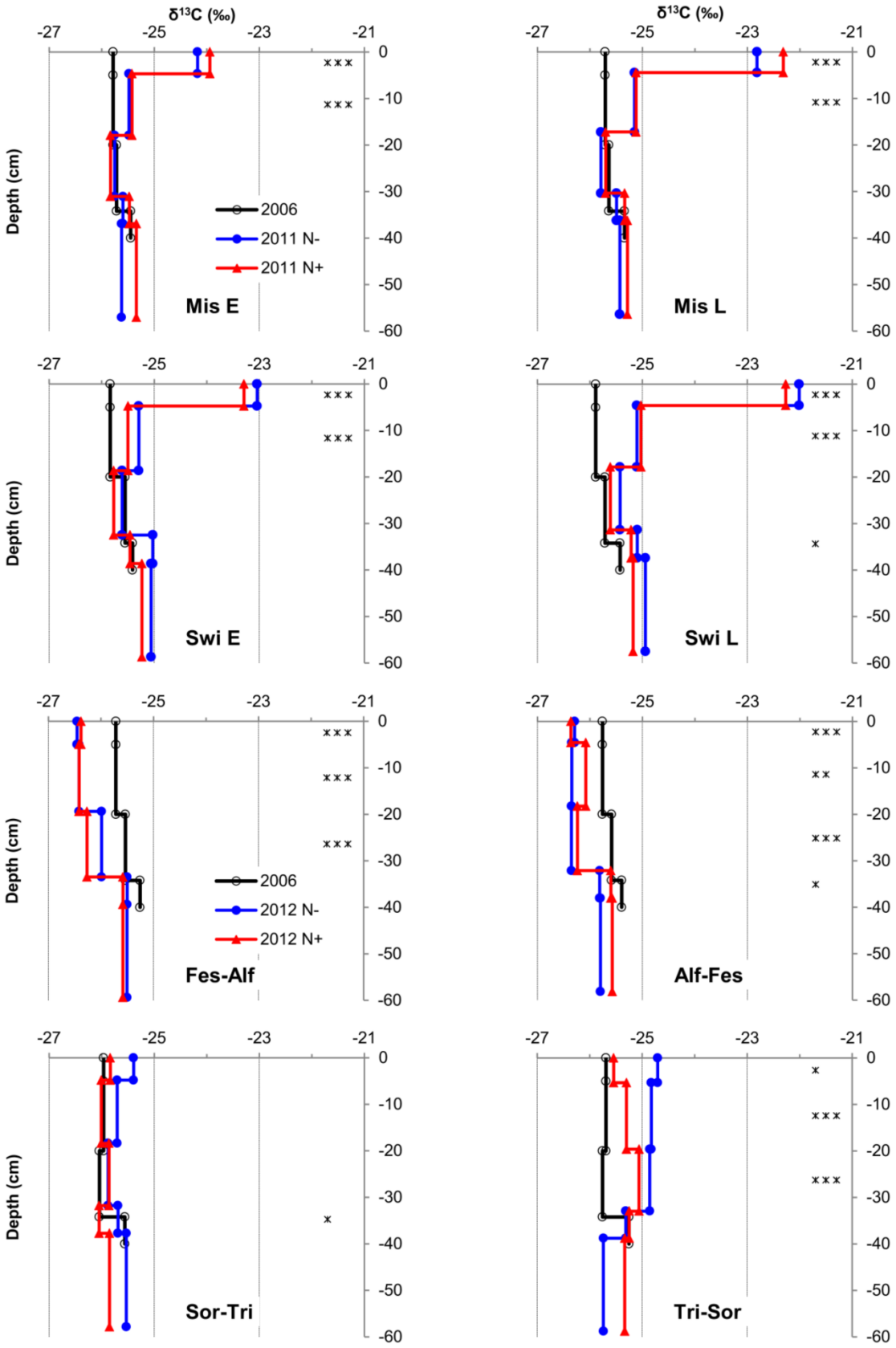


Figure 4.5. Profile of $\delta^{13}C$ (‰) of SOC on ESM basis measured in each treatment in 2006 and 2011 (perennial crops) or 2012 (other crops). The 2006 data are averaged between N- and N+. Asterisks indicate significant changes between the two dates (* p<0.05; ** p<0.01; *** p<0.001)

4.3.8 SOC stocks and mean weighted $\delta^{13}C$

The spatial distribution of SOC stocks and mean weighted $\delta^{13}C$ measured in 2006 in the old ploughed layer (L1-3) was heterogeneous, but well structured throughout the experimental field (Figure 4.6). SOC stocks varied from 45.9 to 54.4 t ha^{-1} and $\delta^{13}C$ from -26.4 to -24.8‰. The main gradient of SOC stocks was oriented west-east, consistently with SOC concentrations. As a result, the initial SOC stocks in L1-3 were significantly higher for perennial crops than for other crops (51.6 *vs.* 48.0 t ha^{-1}) (Table 4.8). In contrast, the ^{13}C signatures did not differ significantly between the two parts of the field. In each part, there was no difference between treatments except for the semi-perennial and annual crops for which a slight significant difference in initial SOC stocks (0.3 t C ha^{-1} in L1-3) was detected between N rates (Table S2, Annexe 3).

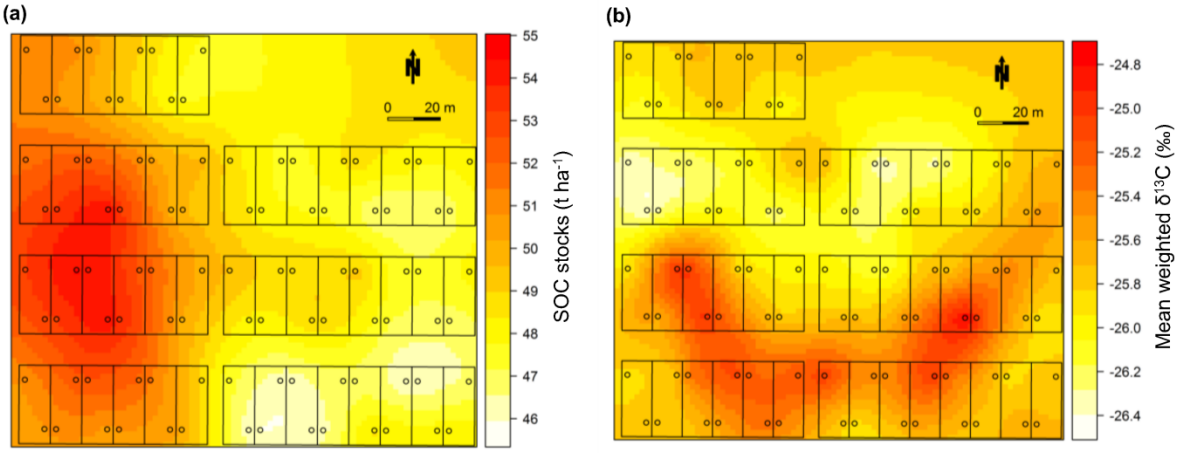


Figure 4.6. Map representing the spatial variability of (a) SOC stocks (t C ha^{-1}) and (b) mean weighted $\delta^{13}C$ (‰) measured in 2006 in the old ploughed layer, obtained by ordinary kriging from the sampled points (open circles). Lines represent the outlines of the 48 plots.

Table 4.8. Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted $\delta^{13}C$ ($\delta^{13}Cw$, ‰) measured at the start of the experiment (2006) in the two parts of the field: perennial and semi-perennial/annual crops. Values in brackets are standard deviations between the 24 plots of each part. Asterisks indicate significant differences (* p<0.001; NS = not significant). Bold values correspond to the old ploughed layer (L1-3)**

Soil layer	Soil mass (t ha^{-1})	Depth (cm)	SOC (t C ha^{-1})				$\delta^{13}C_w$ (‰)			
			Perennial crops		Semi-perennial and annual crops		Perennial crops		Semi-perennial and annual crops	
L1	667	5.0	7.62	(0.30)	6.98	(0.15) ***	-25.8	(0.3)	-25.8	(0.4) NS
L1-2	2667	20.0	30.47	(1.18)	27.90	(0.59) ***	-25.8	(0.3)	-25.8	(0.4) NS
L1-3	**4669**	**34.2**	**51.59**	**(1.71)**	**47.99**	**(0.98) *****	**-25.7**	**(0.3)**	**-25.9**	**(0.3) NS**
L1-4	5553	40.0	56.29	(1.84)	52.96	(0.76) ***	-25.7	(0.3)	-25.8	(0.3) NS

In 2011, SOC stocks did not differ significantly between treatments under perennial crops (Table S3, Annexe 3). The average SOC stock over the old ploughed layer was 52.3 t ha^{-1} (Table 4.9). The only significant effect of N rate was found in the deepest layer (L1-5) where the mean SOC stock was 69.1 t ha^{-1} in N- and 67.5 t ha^{-1} in N+. The $\delta^{13}C$ signature was affected by the rotation only in the upper soil layer: miscanthus L and switchgrass L had a significantly higher $\delta^{13}C$ value (-22.6 and -22.1‰) than miscanthus E (-24.1‰). For miscanthus, the difference could be attributed to the presence of leaf mulch in the L treatment but not in the E treatment. The mean $\delta^{13}C$ in the old ploughed layer was -25.0‰. It was not significantly different between N- and N+.

Table 4.9. Cumulative soil organic carbon stocks (SOC, t C ha^{-1}) and mean weighted δ^{13}C (δ^{13}Cw, ‰) on ESM basis measured (a) in 2011 for perennial crops and (b) in 2012 for semi-perennial/annual crops (mean of N- and N+). Values in brackets are standard deviations. Letters indicate significant differences (p<0.05) between rotations (lower case: SOC; upper case: δ^{13}Cw). Bold values correspond to the old ploughed layer.

Soil layer	Soil mass (t ha^{-1})	Perennial crops											
		Mis E			Mis L			Swi E			Swi L		
		Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)
L1	667	4.7	9.41 (0.69) a	-24.1 (0.7) A	4.4	9.51 (0.93) a	-22.6 (0.8) B	4.8	9.03 (0.72) a	-23.2 (0.2) AB	4.6	9.39 (0.72) a	-22.1 (0.9) B
L1-2	2667	17.9	32.49 (1.31) a	-25.1 (0.4) A	17.2	31.76 (2.44) a	-24.4 (0.6) A	18.6	31.04 (1.83) a	-24.7 (0.1) A	17.8	31.50 (1.19) a	-24.2 (0.6) A
L1-3	**4669**	**31.1**	**53.73 (2.21) a**	**-25.3 (0.4) A**	**30.4**	**51.76 (3.10) a**	**-24.9 (0.4) A**	**32.5**	**51.73 (2.65) a**	**-25.1 (0.1) A**	**31.4**	**52.10 (1.49) a**	**-24.7 (0.5) A**
L1-4	5553	36.9	58.36 (3.06) a	-25.4 (0.3) A	36.2	55.73 (3.51) a	-24.9 (0.4) A	38.6	56.32 (2.88) a	-25.1 (0.1) A	37.4	56.26 (1.47) a	-24.7 (0.5) A
L1-5	8690	57.1	70.70 (5.11) a	-25.4 (0.3) A	56.4	66.75 (4.22) a	-25.0 (0.4) A	58.7	68.10 (2.80) a	-25.1 (0.1) A	57.6	67.71 (1.72) a	-24.8 (0.5) A

Soil layer	Soil mass (t ha^{-1})	Semi-perennial and annual crops											
		Fes-Alf			Alf-Fes			Sor-Tri			Tri-Sor		
		Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)	Depth (cm)	SOC (t ha^{-1})	δ^{13}C$_w$ (‰)
L1	667	4.9	7.58 (0.39) a	-26.4 (0.3) A	4.6	7.46 (0.05) a	-26.3 (0) A	4.8	7.65 (0.25) a	-25.6 (0.2) B	5.3	7.86 (0.17) a	-25.1 (0.5) B
L1-2	2667	19.4	31.99 (1.49) a	-26.4 (0.5) A	18.2	30.74 (1.03) ab	-26.2 (0.4) A	18.4	28.82 (0.38) b	-25.8 (0.1) AB	19.6	29.83 (0.67) b	-25.1 (0.6) B
L1-3	**4669**	**33.5**	**53.87 (2.40) a**	**-26.3 (0.4) A**	**32.1**	**53.42 (0.22) a**	**-26.3 (0.3) A**	**31.8**	**47.48 (1.14) b**	**-25.8 (0.1) A**	**33.0**	**49.36 (1.39) b**	**-25.0 (0.7) B**
L1-4	5553	39.4	58.98 (1.54) a	-26.2 (0.3) A	38.1	58.37 (0.64) a	-26.2 (0.3) A	37.7	52.71 (1.04) b	-25.8 (0.1) A	38.8	54.07 (1.43) b	-25.0 (0.7) B
L1-5	8690	59.4	71.60 (2.29) a	-26.1 (0.3) A	58.1	71.59 (0.13) a	-26.1 (0.3) A	57.8	66.72 (2.17) b	-25.8 (0.1) A	58.8	67.60 (2.04) b	-25.1 (0.6) B

In 2012, SOC stocks under semi-perennial and annual crops were significantly affected by the rotation, as well as $\delta^{13}C$. SOC stocks were greater under semi-perennial than annual crops (53.6 *vs.* 48.4 t ha^{-1} respectively in L1-3), except in the first soil layer. As expected, $\delta^{13}C$ signatures were smaller in semi-perennials (pure C3) than in annual crops (mixed C3/C4).

A statistical analysis of the temporal variation in SOC stocks and mean weighted $\delta^{13}C$ was performed for each part of the experiment (Table S4, Annexe 3). Only semi-perennial crops showed a significant change between initial and final SOC stocks in the old ploughed layer (L1-3), with a mean increase of 0.93 ± 0.28 t ha^{-1} yr^{-1} (Figure 4.7a). The same conclusions were found for L1-4. However SOC stocks in L1-2 significantly increased in all rotations, except for sorghum-triticale. No significant effect of N fertilisation was found. Changes in $\delta^{13}C$ were significant in all rotations and soil layers, except for the sorghum-triticale rotation (Figure 4.7b). $\delta^{13}C$ in the old ploughed layer increased under perennial crops (between 0.08 ± 0.05 and 0.22 ± 0.01‰ yr^{-1}) and triticale-sorghum rotation (0.11 ± 0.04‰ yr^{-1}). It decreased under semi-perennial crops by -0.10 ± 0.02‰ yr^{-1} on average.

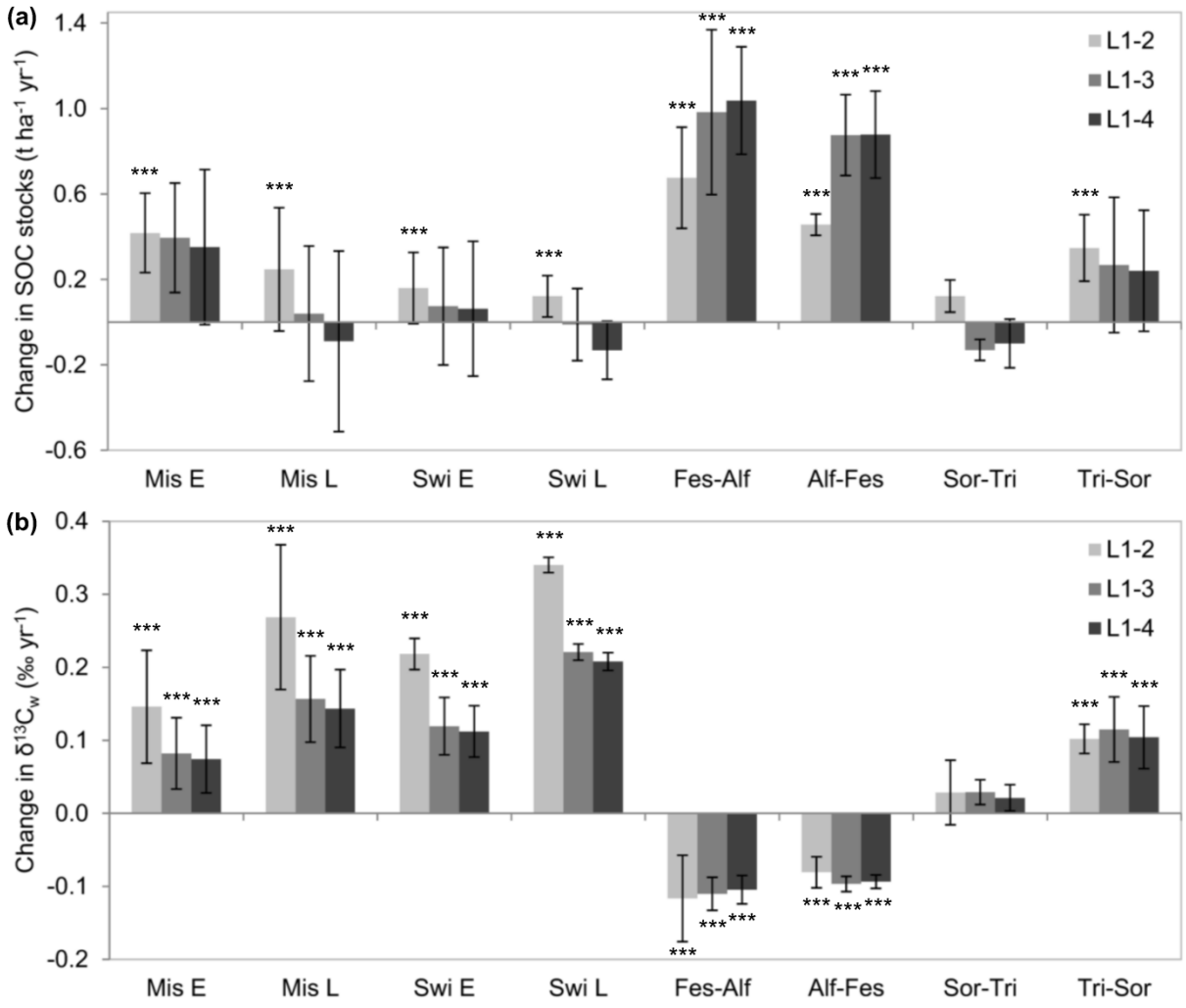


Figure 4.7. Change in (a) SOC stocks (t C ha^{-1} yr^{-1}) and (b) mean weighted $\delta^{13}C$ (‰ yr^{-1}) on ESM basis in layers L1-2 (2667 t ha^{-1}), L1-3 (4669 t ha^{-1}) and L1-4 (5553 t ha^{-1}) between 2006 and 2011 for perennial crops or 2012 for semi-perennial and annual crops. Bars represent the standard deviations. Asterisks indicate the probability of a significant change during the 5 or 6 year period: *** p<0.001

4.3.9 Changes in new and old SOC stocks

In 2011, the proportion of SOC in the old ploughed layer derived from the C4 perennial crops established five years earlier was 5.6% on average (Table 4.10). It was significantly higher for switchgrass L (8.6%) than for miscanthus E and switchgrass E (3.1 and 4.6% respectively), miscanthus L being intermediate (6%). This proportion was much higher in L1 (21.8% on average). In 2012, the SOC derived from the new C3 semi-perennial crops represented on average 16.9% of the SOC stock in the old ploughed layer and did not significantly differ between the two rotations.

Using these data, the rates of change in new and old SOC stocks during the experimental period were calculated (Table 4.11). The accumulation rate of new SOC in the old ploughed layer was significantly affected by the rotation. It was highest for semi-perennial

crops (1.50 ± 0.28 t ha^{-1} yr^{-1} on average). Within the perennial crops, the highest accumulation rate was found under switchgrass L (0.89 ± 0.03 t ha^{-1} yr^{-1}) and the lowest under miscanthus E (0.33 ± 0.18 t ha^{-1} yr^{-1}). Simultaneously, a decrease of old SOC stock was calculated in most situations. The rate of decrease in the old ploughed layer did not differ significantly between perennial and semi-perennial crops. The mean rate of decrease for all crops was -0.50 ± 0.45 t ha^{-1} yr^{-1}.

Table 4.10. Proportion of the soil organic carbon stock on an equivalent soil mass basis derived from the new crops (%) in 2011 for perennial crops and in 2012 for semi-perennial crops. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between rotations (lower case letters: perennial crops; upper case letters: semi-perennial crops). Bold values correspond to the old ploughed layer (L1-3: 4669 t soil ha^{-1})

Soil layer	Soil mass (t ha^{-1})	2011				2012	
		Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes
L1	667	13.1 (6.7) c	24.2 (5.4) ab	20.8 (1.1) bc	29.2 (3.5) a	20.1 (6.0) A	14.8 (6.8) A
L1-2	2667	5.6 (2.8) c	10.3 (3.9) ab	8.5 (0.8) bc	13.2 (0.7) a	20.2 (9.6) A	13.1 (4.4) A
L1-3	**4669**	**3.1** (1.8) **b**	**6.0** (2.3) **ab**	**4.6** (1.5) **b**	**8.6** (0.4) **a**	**18.5** (3.7) **A**	**15.2** (2.7) **A**
L1-4	5553	2.8 (1.7) b	5.6 (2.1) ab	4.4 (1.4) b	8.1 (0.5) a	17.5 (3.4) A	14.6 (2.4) A

Table 4.11. Change in new (C_{New}) and old (C_{Old}) SOC stocks (t C ha^{-1} yr^{-1}) calculated from the $\delta^{13}C$ analyses (Eq 11-13) on ESM basis between 2006 and 2011 for perennial crops or 2012 for semi-perennial crops (mean of N- and N+). Values in brackets are standard deviations. Letters indicate significant differences ($p<0.05$) between rotations. Bold values correspond to the old ploughed layer.

Soil layer	Soil mass (t ha^{-1})		Mis E	Mis L	Swi E	Swi L	Fes-Alf	Alf-Fes
L1	667	C_{New}	0.24 (0.11) c	0.47 (0.15) ab	0.38 (0.03) abc	0.55 (0.11) a	0.25 (0.07) bc	0.18 (0.08) c
		C_{Old}	0.12 (0.23) a	-0.09 (0.08) ab	-0.08 (0.07) ab	-0.22 (0.00) b	-0.16 (0.09) b	-0.11 (0.06) ab
L1-2	2667	C_{New}	0.36 (0.17) b	0.67 (0.30) ab	0.53 (0.03) ab	0.83 (0.07) ab	1.08 (0.50) a	0.68 (0.24) ab
		C_{Old}	0.06 (0.36) a	-0.42 (0.27) ab	-0.37 (0.15) ab	-0.71 (0.04) b	-0.40 (0.57) ab	-0.22 (0.19) ab
L1-3	**4669**	**C_{New}**	**0.33 (0.18) d**	**0.63 (0.28) cd**	**0.48 (0.16) cd**	**0.89 (0.03) bc**	**1.65 (0.28) a**	**1.36 (0.23) ab**
		C_{Old}	**0.06 (0.43) a**	**-0.59 (0.38) ab**	**-0.40 (0.24) ab**	**-0.91 (0.18) b**	**-0.67 (0.61) ab**	**-0.49 (0.42) ab**
L1-4	5553	C_{New}	0.32 (0.19) d	0.63 (0.27) cd	0.49 (0.15) cd	0.91 (0.04) bc	1.71 (0.31) a	1.42 (0.21) ab
		C_{Old}	0.03 (0.54) a	-0.72 (0.45) ab	-0.43 (0.26) ab	-1.04 (0.17) b	-0.68 (0.47) ab	-0.55 (0.40) ab

4.4 Discussion

4.4.1 Crop residues and belowground biomass

The amounts of crop residues found on the soil surface or within the soil were much higher for perennial crops than for the other crops. This difference is probably due to the absence of soil tillage and to more recalcitrant residues with perennial crops (Amougou *et al.*, 2011). In the case of miscanthus L, there was an additional C input through leaf fall during winter. The amount of mulch derived from fallen leaves (2.86 t C ha^{-1}) was almost identical to that measured by Amougou *et al.* (2012) one year earlier in the same experiment. The yearly input of leaves to the soil, estimated at 1.40 t C ha^{-1} by Amougou *et al.* (2012), was probably in equilibrium with the decomposition rate of the leaf mulch.

The belowground biomass of perennial crops represented a large C pool. The rhizome biomass of miscanthus was close to that measured in the same experiment by Strullu *et al.* (2011) in February 2010. For switchgrass, the rhizome biomass was higher than that reported by Garten *et al.* (2010), but the root biomass over 0-60 cm (4.9 t C ha^{-1}) was similar and represented the major part of the belowground C.

4.4.2 Changes in SOC concentrations and $\delta^{13}C$

The change in SOC concentration between 2006 and the second sampling date varied between soil layers, except for the semi-perennial crops, which showed a similar change from L1 to L3. This is probably due to differences in soil tillage between treatments. Indeed, in contrast to the other treatments, semi-perennial crops were mouldboard ploughed 18 months before the second sampling. SOC stratification appears in continuous reduced tillage systems, in contrast to conventional tillage systems *(e.g.* Dimassi *et al.*, 2014*;* Powlson *et al.*, 2014*)*. The $\delta^{13}C$ change observed under perennial crops showed that C inputs were greater in the upper layer. However, it was likely that rhizome and roots turnover and/or rhizodeposition made a significant contribution to the C inputs under perennial crops. Indeed, a significant change of $\delta^{13}C$ signature was observed in the L2 layer, which contained the highest belowground biomass. This hypothesis was also confirmed by the correlation found between the biomass of belowground organs in the soil samples and the SOC derived from the new crops. Zatta *et al.* (2014) found the same kind of relationship for a six year-old miscanthus with soil cores taken inside or outside the rhizome area.

4.4.3 Changes in SOC stocks

SOC stocks in 2006 displayed a high spatial heterogeneity. Therefore, the synchronic approach (comparison of treatments at a given time without estimating the initial SOC stocks), which is most often used, would have led to very different conclusions. For example, SOC stocks were 4.3 t ha^{-1} higher under miscanthus L in 2011 than under sorghum-triticale in 2012, which might have been interpreted as an important C sequestration with miscanthus, whereas a comparison with the initial values (in 2006) indicated that no significant sequestration occurred in either rotation (Fig. 5a). It was also important to apply the recommendation to compare stocks on an equivalent soil mass basis. Calculation to a fixed depth of 40 cm rather than an ESM of 5553 t ha^{-1} would have led to an overestimation of SOC change after five years by 2.5 t C ha^{-1} under miscanthus L.

No significant change in SOC stocks could be detected under perennial crops (using either 4669 or 5553 t ESM ha^{-1}), despite small standard deviations in measurements. A significant SOC increase was found in the upper layers (L1), offset by a decrease in the lower layers (L2-4). These results may appear to contradict the meta-analyses of Don *et al.* (2011) and Poeplau and Don (2014), who found a mean SOC increase of 0.66 ± 0.94 t ha^{-1} yr^{-1} and 0.40 ± 0.73 t ha^{-1} yr^{-1} respectively (mean ± standard deviation) under miscanthus harvested late and grown on former croplands, but these studies show a wide variability. In fact, studies dealing with young miscanthus plantations (< 10 years old) often fail to show a significant change in SOC stocks (Zimmermann *et al.*, 2012). Significant increases have generally been observed in older plantations (Hansen *et al.*, 2004; Dondini *et al.*, 2009; Felten and Emmerling, 2012; Dufossé *et al.*, 2014). However, Richter *et al.* (2015) found equal SOC stocks under a 14 year-old miscanthus and an arable reference plot. Published studies with switchgrass refer to relatively young plantations (< 9 years old). The observed SOC stock changes under switchgrass are generally positive, but highly variable and often non-significant (Liebig *et al.*, 2008; Schmer *et al.*, 2011; Dou *et al.*, 2013; Bonin and Lal, 2014). For example, Liebig *et al.* (2008) found a mean increase of 1.1 ± 1.4 t C ha^{-1} yr^{-1} across ten sites after five years (in the 0-30 cm layer), but only four sites showed a significant increase.

In our experiment, no significant change in SOC stocks was observed under annual crops (using 4669 t ESM ha^{-1} or more). The sorghum-triticale rotation showed a tendency to a decrease, but the inversed rotation did not. This result was unexpected because experimental and modelling studies generally show a decrease in SOC stocks when the whole aboveground biomass of annual crops is removed (Saffih-Hdadi and Mary, 2008; Powlson *et al.*, 2011). Other changes in crop management may have compensated for this effect. Indeed, a catch

crop has been grown every other year since 2006 and the introduction of catch crops has been shown to increase SOC stocks (Constantin *et al.*, 2010). The difference observed between the two rotations might also be due to the higher yields of the triticale-sorghum rotation over the period 2006-2011 (5.11 *vs.* 3.87 t C ha^{-1} yr^{-1}), probably leading to higher amounts of crop residues (stubble and roots) returned to the soil. Unlike the sorghum-triticale rotation, this rotation also showed a significant increase in $\delta^{13}C$, which was consistent with the higher sorghum production in this rotation (C4 crop).

The only significant change in SOC stocks in the old ploughed layer was observed for semi-perennial crops. This result was consistent with the meta-analyses on the effect of arable land conversion to grassland (Conant *et al.*, 2001; Soussana *et al.*, 2004). Soussana *et al.* (2004) estimated that the mean increase in SOC stocks after conversion is 0.49 ± 0.26 t ha^{-1} yr^{-1} over 20 years. The net increase rate in the fescue/alfalfa rotations of our experiment was 0.93 ± 0.28 t ha^{-1} yr^{-1} over 6 years, *i.e.* approximately two times greater than for grassland.

SOC stocks will have to be monitored over a longer period to confirm the differences between bioenergy crops and test the occurrence of long-term effects.

4.4.4 Changes in new and old SOC stocks

The variations in new and old SOC contents are indicators of C inputs (above and belowground plant materials) and outputs (C mineralised) respectively, and their balance determines the net change in SOC content.

C inputs under perennial crops were mainly located in the layer L1-2 (*ca*. 0-18 cm). This was consistent with other studies using ^{13}C abundance which found that new SOC was concentrated above 30 cm (Schneckenberger and Kuzyakov, 2007; Collins *et al.*, 2010; Felten and Emmerling, 2012; Cattaneo *et al.*, 2014; Poeplau and Don, 2014). In our experiment, the rate of new SOC accumulation in the old ploughed layer of 0.63 t ha^{-1} yr^{-1} under miscanthus L fell within the range of values compiled by Poeplau and Don (2014), *i.e.* 0.85 ± 0.68 t ha^{-1} yr^{-1} (0-30 cm). Results for switchgrass were also consistent with Collins *et al.* (2010) and Follett *et al.* (2012), who found new SOC accumulation of 1.0 and 0.5 t ha^{-1} yr^{-1} respectively at 0-30 cm. The increase in new SOC stocks was higher for semi-perennial than for perennial crops, indicating higher C inputs under these rotations. In contrast to perennial crops, semi-perennials were destroyed twice during the experimental period. This led to the return of the whole crop biomass (roots and aboveground below cutting height) to the soil, which could be significant. For example, Justes *et al.* (2001) estimated the root biomass of two-year-old alfalfa to be 2.8 t C ha^{-1}.

Reported changes in old SOC stocks under perennial crops are highly variable. Poeplau and Don (2014) calculated from experimental data a mean positive rate of change for old SOC of 0.83 ± 3.24 t ha^{-1} yr^{-1} under miscanthus L. In the absence of other carbon sources (sewage sludge, C3 weeds, *etc.*), this change should be negative. Poeplau and Don (2014) used RothC to simulate a mean change of -0.60 ± 0.43 t ha^{-1} yr^{-1} (0-30 cm), which matches our results for miscanthus L (-0.59 ± 0.38 t ha^{-1} yr^{-1}). The change in old SOC was not significantly different between perennial and semi-perennial crops. This result suggested that soil tillage associated with the periodic destruction of fescue and alfalfa did not increase SOC mineralisation. The increase in SOC stocks observed under semi-perennial crops was therefore due to a higher C input rather than to a change in mineralisation rate.

4.4.5 Effect of management practices

There have been few studies into the effects of bioenergy crop management practices on SOC stocks. In our study, N fertilisation had a significant effect only on crop yields, but not on crop residues, belowground biomass and SOC stocks measured in 2011-2012. This was in accordance with Cattaneo *et al.* (2014) for miscanthus, but in contrast with Follett *et al.* (2012) and Lee *et al.* (2007), who found that an increase in mineral N fertilisation enhances crop production and SOC stocks under switchgrass. In our experiment, N fertilisation effects on miscanthus E and switchgrass yields increased with time (Cadoux *et al.*, 2014), therefore future effects on crop residues and SOC stocks could be expected. The effect of the harvest date of perennial crops has been investigated for switchgrass but not for miscanthus. Follett *et al.* (2012) did not find any significant effect on SOC stocks under switchgrass, which is consistent with our findings. In our study, SOC stocks did not differ significantly between miscanthus E and L. This result was surprising because miscanthus L had higher C inputs to the soil due to leaf fall during winter. However, a large part of these leaves was found undecomposed at soil surface. New SOC accumulation was two times greater in the upper soil layer for miscanthus L than for miscanthus E, whereas old SOC change did not differ significantly between the two treatments. As for N fertilisation, significant effects of the harvest date of miscanthus on SOC stocks can be expected in the longer term and should be further studied. Finally, it is likely that crop management and particularly N fertilisation also have a strong effect on GHG balance as a result of their impact on N_2O emissions. A complete GHG budget including SOC stocks changes and measured N_2O emissions will need to be established.

5 The fate of cumulative applications of ^{15}N-labelled fertiliser in perennial and annual bioenergy crops

Ce chapitre correspond à un article en préparation pour *Agriculture Ecosystems & Environment*.

List of authors

Fabien Ferchaud[1], Guillaume Vitte[1], Jean-Marie Machet[1], Nicolas Beaudoin[1], Manuella Catterou[2] and Bruno Mary[1]

[1] INRA, UR1158 AgroImpact, site de Laon, F-02000 Barenton-Bugny, France

[2] Université de Picardie Jules Verne, Unit CNRS-FRE 3498 Edysan, 33 rue Saint Leu, F-80039 Amiens, France

Abstract

The fate of the nitrogen (N) fertiliser applied to bioenergy crops is a key issue to allow high biomass production while minimising environmental impacts due to N losses. The aim of this study was to follow the fate in the soil-plant system of N fertiliser applied to perennial (*Miscanthus* × *giganteus* and switchgrass), semi-perennial (fescue and alfalfa) and annual (sorghum and triticale) bioenergy crops. All crops except alfalfa received ^{15}N-labelled fertiliser during 4 or 5 successive years on the same subplots. Biomass production, N and ^{15}N removal at harvest were measured each year. The ^{15}N recovery in crop residues, non-harvested crop parts and soil was measured at the end of the ^{15}N-labelling period. Perennial crops had higher biomass production but generally lower ^{15}N recovery in harvested biomass than other crops, particularly when harvested late (end of winter). At the end of the 4 or 5-year period, the proportion of ^{15}N recovered in harvested biomass was 13-34% for perennials, 23-38% for semi-perennials and 34-39% for annual crops. Perennial crops stored large amounts of N in their belowground organs; the mean ^{15}N recovery in these organs was 12%, corresponding to a N storage flux of 14 kg N ha^{-1} yr^{-1}. The ^{15}N recovery in soil (including crop residues) was higher for perennials (average 36%) than semi-perennials (28%) and annual crops (19%), corresponding to a N immobilisation rate of 43, 15 and 12 kg N ha^{-1} yr^{-1} respectively. The mean overall ^{15}N recovery in the soil-plant system was 69% in perennials, 61% in semi-perennials to 56% in annual crops, suggesting that important fertiliser losses occurred through volatilisation and denitrification. Perennial bioenergy crops had the better efficiency by storing fertiliser-N in soil organic matter and living belowground biomass used as N reserves for succeeding years.

Keywords

Bioenergy, nitrogen fertiliser, ^{15}N, field experiment, nitrogen use efficiency, miscanthus, switchgrass

5.1 Introduction

Nitrogen (N) is a key input in crop production. The synthesis of N fertilisers from atmospheric N_2 by the Haber-Bosch process has enabled humankind to greatly increase agricultural production during the 20th century (Tilman *et al.*, 2002). In UE, manufactured fertiliser adds 11 million tonnes of reactive N annually to the agricultural area, but half of this N is lost to the surrounding environment (Sutton *et al.*, 2011). These losses contribute to a wide range of ecological and human health effects (Galloway *et al.*, 2003; Galloway *et al.*, 2008; Sutton *et al.*, 2011).

Bioenergy production from crops has been supported to contribute to the production of renewable energy in response to the challenges of climate change and depletion of fossil resources (Don *et al.*, 2011). However, the use of conventional food crops to produce biofuels has raised a lot of concerns about its environmental consequences (e.g. Galloway *et al.*, 2008; Smith and Searchinger, 2012). The large N requirements of these first generation bioenergy crops may be harmful to the greenhouse gas balance of biofuels (Crutzen *et al.*, 2008). The development of new conversion technologies and biorefineries allows considering a wide range of new dedicated bioenergy crops (Ragauskas *et al.*, 2006; Sanderson and Adler, 2008; Somerville *et al.*, 2010). Among them, perennial C4 crops such as miscanthus and switchgrass are considered as promising because of their high biomass production with low nutrient requirements and expected low greenhouse gas emissions (Don *et al.*, 2011; Jørgensen, 2011; Monti *et al.*, 2012; Cadoux *et al.*, 2014). However, even with these crops, N fertilisation can still be necessary to maintain high yields and soil fertility on the long term (Cadoux *et al.*, 2012; Monti *et al.*, 2012; Cadoux *et al.*, 2014). Nitrogen use efficiency of bioenergy crops is therefore a key issue to allow high biomass production while minimising environmental impacts due to N losses.

There are various ways to define and measure N efficiency. Two different approaches are widely used in the literature: (1) the apparent recovery which is based on the difference in N uptake between a crop receiving N fertiliser and a reference plot without N applied (e.g. Cassman *et al.*, 2002) and (2) the actual recovery or ^{15}N recovery which is the fraction of labelled N that is taken up by a crop following application of ^{15}N-labelled fertiliser (Hauck and Bremner, 1976). Both methods can give similar or dissimilar results whether or not an "added nitrogen interaction" (ANI) occurs, *i.e.* whether or not the uptake of inorganic soil N is different between fertilised and unfertilised treatments. "Pool substitution" between fertiliser-N and soil mineral N can cause a positive ANI and therefore lead to a higher apparent than actual recovery (Jenkinson *et al.*, 1985). Nevertheless, only the ^{15}N method

allows to determine the fate of the fertiliser-N in the different compartments of the agroecosystem (crop, soil, ...) and therefore the overall losses of fertiliser-N (Gardner and Drinkwater, 2009). Few studies have analysed the fate of ^{15}N-labelled fertiliser applied to dedicated bioenergy crops. Christian *et al.* (2006) and Pedroso et al. (2014) analysed the effect of a single ^{15}N fertilisation pulse during 3 successive years on miscanthus and switchgrass respectively. They found a rather low ^{15}N recovery in the harvested biomass (14-37% for miscanthus and 18-39% for switchgrass) and that belowground organs represented an important N sink. Pedroso *et al.* (2014) also pointed out the effect of crop management, *i.e.* harvest date, on the ^{15}N recovery and partitioning. However, no study has compared the ^{15}N recovery of different bioenergy crops at the same site.

Most experiments have measured ^{15}N recovery during one growing season (Gardner and Drinkwater, 2009). A few of them have followed the fate of the residual ^{15}N, *i.e.* the labelled fertiliser N remaining in soil (mainly in organic form) and crop residues after harvest. In arable cropping systems, a small proportion of this residual ^{15}N is re-mineralised each year from the soil organic matter pool and can be recovered by the following crops or lost through N leaching or gaseous losses (Glendining *et al.*, 2001; Macdonald *et al.*, 2002; Sebilo *et al.*, 2013). The amount of ^{15}N remaining in the soil-plant system after the year of application is likely to be greater with perennial bioenergy crops because of the presence of perennial organs. The ^{15}N stored in perennial organs can be used by the crop in the subsequent years and partly recovered at harvest, as shown by Christian *et al.* (2006) for miscanthus. Using cumulative applications of ^{15}N-labelled fertiliser in the same plots over several growing seasons could allow, (1) to integrate part of this long term fate of the residual ^{15}N, and (2) to reduce the variability in plant N uptake and fertiliser-N losses due to climate conditions and agronomical context (*i.e.* age of the crop, other stresses, etc.).

The aim of this study was to (1) determine the fate of ^{15}N-labelled fertiliser applied to perennial, semi-perennial and annual bioenergy crops in the soil-plant system, and (2) study the interaction with crop management, *i.e.* harvest date of perennial crops and N fertiliser rate for all crops. The originality of our approach consisted in comparing different crops at the same site and using cumulative applications of ^{15}N over four or five years.

5.2 Materials and methods

5.2.1 Site and experimental design

The study is based on an ongoing long-term experiment established in 2006 at the INRA experimental station in Estrées-Mons, northern France (49.872 N, 3.013 E) called

"Biomass & Environment" (B&E). The soil is a Haplic Luvisol (IUSS Working Group WRB, 2006). Soil characteristics are given in Table 4.1. The mean annual temperature was 10.6 °C over the period 2006-2011; the mean rainfall and potential evapotranspiration were 673 and 737 mm yr^{-1} respectively. Before 2006, the field had been cultivated for many years with annual crops, winter wheat being the most common crop.

The experiment was initiated to study the production and the environmental impacts of a wide range of bioenergy crops. It compares eight "rotations": four with C4 perennial crops (monocultures), two with C3 semi-perennial forage crops and two with C3/C4 annual crops (Table 5.1). The perennial crops are miscanthus (*Miscanthus*×*giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in February (L). The semi-perennial crops are tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (× *Triticosecale* Wittmack). The experiment also includes two nitrogen treatments (N- and N+) with fertiliser-N rates depending on the crops (Table 5.2).

The 2.7 ha field was divided into two parts in order to facilitate cultural operations and limit competition between plants due to differences in canopy height (Figure 3.1): (i) a split-block design in the west part for perennial crops with "rotations" in the main plots (miscanthus E, miscanthus L, switchgrass E, switchgrass L) and N fertilisation rates in the subplots (N- and N+), and (ii) a split-plot design in the east part for the other crops with rotations in the main plots (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilisation rates in the subplots (N- and N+). Both parts include three replicate blocks and 24 subplots of 360 m^2. Soil analyses performed in 2006 revealed a slightly higher clay content in the west than in the east part (180 ± 27 *vs.* 148 ± 19 g kg^{-1} in the 0-30 cm layer, Table 4.1).

Table 5.1. Rotations of the B&E long term experiment (Mis = miscanthus, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fibre sorghum, Tri = triticale, CC = catch crop; E = early harvest, L = late harvest, n.h. = not harvested).

Rotation	2006	2007	2008	2009	2010	2011
Mis E	Mis n.h.	Mis E	Mis E	Mis E	Mis E	Mis E
Mis L	Mis n.h.	Mis L	Mis L	Mis L	Mis L	Mis L
Swi E	Swi n.h.	Swi E	Swi E	Swi E	Swi E	Swi E
Swi L	Swi n.h.	Swi L	Swi L	Swi L	Swi L	Swi L
Fes-Alf	CC/Fes	Fes	Fes	Alf	Alf	Fes
Alf-Fes	Alf	Alf	Alf	Fes	Fes	Alf
Sor-Tri*	CC	Sor	Tri/CC	Sor	Tri/CC	Sor
Tri-Sor*	Sor	Tri/CC	Sor	Tri/CC	Sor	Tri/CC

*Rotations with catch crops (oat in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2011) which were sown every year in late August or early September between triticale and sorghum.

Table 5.2. Nitrogen fertilisation rates applied to the B&E long term experiment using ^{15}N-labelled UAN.

Rotation	N	N fertiliser rate (kg ha^{-1})						Total N applied 2007-10/11 (kg ha^{-1})	Mean N rate 2007-10/11 (kg ha^{-1} yr^{-1})	^{15}N excess atom fraction (%)
		2006	2007	2008	2009	2010	2011			
Mis E	N-	0	0	0	0	0		0	0	
	N+	0	120	120	120	120		480	120	0.395
Mis L	N-	0	0	0	0	0		0	0	
	N+	0	120	120	120	120		480	120	0.395
Swi E	N-	0	0	0	0	0		0	0	
	N+	0	120	120	120	120		480	120	0.395
Swi L	N-	0	0	0	0	0		0	0	
	N+	0	120	120	120	120		480	120	0.395
Fes-Alf	N-	0	120	80	0	0	0	200	40	0.395
	N+	0	240	160	0	0	0	400	80	0.197
Alf-Fes	N-	0	0	0	40	120	0	160	32	0.395
	N+	0	0	0	80	240	0	320	64	0.197
Sor-Tri	N-	0	0	60	0	60	0	120	24	0.790
	N+	0	120	120	120	120	120	600	120	0.395
Tri-Sor	N-	0	60	0	60	0	60	180	36	0.790
	N+	0	120	120	120	120	120	600	120	0.395

At the start of the experiment, the field was mouldboard ploughed at a depth of *ca.* 25 cm. After seedbed preparation, miscanthus was planted in April 2006 (1.5 rhizome m^{-2}) and switchgrass sown in June 2006 (seed rate = 15 kg ha^{-1}). Semi-perennial crops were sown in 2006, 2009 and 2011, usually in April. Before sowing, the previous crop (alfalfa or fescue) was destroyed in late autumn with a cultivator and a disc harrow (15 cm deep) in 2009 and

mouldboard ploughed (*ca.* 22 cm deep) in 2011. These crops were harvested in two or three cuttings depending on years, with the last cut in October. Annual crops were cultivated under superficial tillage (12-15 cm deep). Sorghum was sown in late May and harvested in late September. Triticale was sown in mid-October and harvested in late July or early August. The N fertiliser was applied as UAN solution (urea ammonium nitrate) containing 390 g N l^{-1} (50% urea, 50% NH_4NO_3). Perennial crops received a single annual application in late April. Fescue received N fertiliser at the beginning of each cycle of regrowth and seedling crops were not fertilised before the first cut, so that the total N rate varied according to the year. Sorghum was fertilised just before sowing and triticale in March at mid-tillering for N- and N+ treatments and in late April at the beginning of stem elongation for N+. Further details about crop management are given by Cadoux *et al.* (2014).

^{15}N-labelled UAN fertiliser, uniformly labelled on urea, NH_4 and NO_3, was applied to a subplot of 36 m^2 located north of each plot from 2007 to 2010 (perennial crops) or 2011 (other crops). Simultaneously, the unlabelled UAN was added at the same rate in the rest of the plot. The labelled UAN solution was applied with a CO_2-pressurised hand sprayer to mimic the concentration and volume of liquid fertiliser applied in the rest of the plot. The isotopic excess of the labelled fertiliser varied between treatments in order to apply the same amounts of ^{15}N per surface area in all treatments (Table 5.2).

5.2.2 Sampling and analytical procedures

5.2.2.1 Aboveground biomass at harvest

Harvested crop production was measured every year from 2006 to 2011. On each harvest date, the aboveground biomass was collected manually in one micro-plot inside the ^{15}N-labelled subplot. The size of the micro-plot depended on the crops, according to the amount of biomass produced per unit area and stand homogeneity: 3.84 m^2 for miscanthus (six plants), 2.5 m^2 for switchgrass, *ca.* 3.6 m^2 for sorghum and *ca.* 5 m^2 for fescue, alfalfa and triticale. The cutting height was 7 cm for all crops. The fresh biomass was weighted and a representative subsample was dried at 65 °C for 96 h to determine the dry matter content and ground before analysis. In order to better take into account canopy variability of miscanthus, the measured biomass was corrected by the number of stems determined in a wider undisturbed area of 25 m^2 according to Strullu *et al.* (2011). The N concentration and ^{15}N abundance were determined using an elemental analyser (FLASH EA 1112 series, Thermo Electron, Bremen, Germany) coupled to an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Electron, Bremen, Germany).

5.2.2.2 Soil

The soil was sampled on two dates: at the beginning of the experiment in May 2006 to measure initial ^{15}N excess and at the end of the ^{15}N-labelling period, *i.e.* in March 2011 for perennial crops (west part of the field trial) and March 2012 for other crops (east part of the field trial). Soil cores of 8 cm diameter were extracted with depth increments of 20 cm and inserted into plastic tubes using a powered soil corer (Humax soil sampler, Switzerland). In 2006, two soil cores were taken in each plot down to 40 cm depth. In 2011 and 2012, six soil cores were taken in each plot down to 60 cm. All cores were located inside the labelled subplot in a 2.6 m^2 micro-plot and taken in intra-row and inter-row zones.

From 2006, the ploughing depth was reduced from ca. 30-35 cm to less than 25 cm in all treatments. The old ploughing depth (referred to below as *Y*) was identified in the soil cores on each sampling date by detecting changes in soil colour and structure. Soil cores removed from the plastic tubes in the laboratory were divided into five layers (0-5, 5-20, 20-*Y*, *Y*-40 and 40-60 cm) in 2011 and 2012. Coarse residues (>2 mm), roots and rhizomes were then carefully removed from the soil by handpicking. Soil samples were dried at 38 °C for 96 h, crushed through a 2 mm sieve, subsampled and finely ground with a ball mill (PM 400, Retsch, Germany) before analysis. Soil samples were analysed for nitrogen concentration and ^{15}N abundance using an elemental analyser (EURO EA, Eurovector, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus Advantage, Thermo Electron, Germany). Bulk densities were also determined at each sampling date either with steel cylinders or a dual gamma probe (LPC-INRA, France). Full details of the methodology are given in Chap. 4.

5.2.2.3 Crop residues and living biomass

In order to make a complete ^{15}N balance in the soil-plant system, crop residues and "living biomass" were sampled at the same time and location than the final soil sampling. Crop residues included dead plant parts accumulated in soil or at soil surface whereas living biomass was composed of living aboveground material in the case of alfalfa, fescue and triticale and living belowground material (roots and rhizomes) for all crops.

Crop residues from perennials present at soil surface were collected just before soil sampling in 2011. Stem bases and fragments (>10 mm) as well as fallen leaves (mulch) of miscanthus L were sampled in the whole micro-plot. Small stem fragments (2 to 10 mm) and leaf debris (for miscanthus L) present at soil surface were collected in six areas of 27 × 27 cm within each micro-plot, corresponding to the location of the soil cores. Stem fragments below soil surface (>2 mm) were collected in the 8 cm diameter cores. Aboveground residues from

the six areas were pooled together, as well as belowground residues from the six soil cores. The residues from semi-perennial and annual crops, buried by soil tillage, were collected in the soil cores in 2012. All residues were dried at 65 °C for 96 h, weighed and ground before analysis.

The aboveground living biomass of fescue and alfalfa was cut just above the soil surface before soil sampling in 2012. Triticale and catch crop (before sorghum) plants were pulled from the soil in order to collect aboveground and belowground biomass and washed to eliminate soil contamination. Samples were dried at 65 °C for 96 h, weighed and ground before analysis. Roots of all crops and perennial crop rhizomes collected in the six cores of each micro-plot were pooled for each layer, washed and dried at 65 °C for 96 h, weighed and ground before analysis. Given the very large spatial variability of the rhizome biomass of miscanthus, the method proposed by Strullu *et al.* (2011) was used to quantify it more precisely. It consisted in counting the number of stems of all plants in a given subplot and extracting the entire rhizome of the plant having the median number of stems.

The N concentration and ^{15}N abundance of all samples were determined using an elemental analyser (FLASH EA 1112 series, Thermo Electron, Bremen, Germany) coupled to an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Electron, Bremen, Germany).

5.2.3 Calculations

5.2.3.1 Biomass, N content and apparent N recovery

For each sampling, the measured biomass was expressed in tons of dry matter per hectare and the N content (kg N ha^{-1}) was obtained by multiplying the biomass by the N concentration.

The annual crop production and harvested nitrogen measured in labelled subplots were compared to the measurements achieved in unlabelled subplots of the experiment and presented in an earlier paper (Cadoux *et al.*, 2014). We found a good relationship between the two estimates although the N content was slightly lower in labelled subplots (y = 0.98 x, R^2 = 0.93 for biomass production; y = 0.94 x, R^2 = 0.87 for harvested N). This difference was considered acceptable. The apparent recovery of fertiliser N (R_A, in %) was calculated as follows:

$$R_A = \frac{T_N - T_0}{F}$$

where T_N and T_0 are the amounts of N in the fertilised (N+) and unfertilised (N-) aboveground crop biomass at harvest (kg N ha^{-1}), respectively, and F is the amount of fertiliser N applied (kg N ha^{-1}). This calculation was applicable only to the crops whose N- treatment was unfertilised, *i.e.* for perennial crops and sorghum.

5.2.3.2 Actual ^{15}N recovery

The amount of N derived from the ^{15}N-labelled fertiliser in a given crop part or soil layer (*Ndff*, in kg N ha^{-1}) was calculated according to Hauck and Bremner (1976):

$$Ndff = T \cdot \frac{(p-q)}{(f-q)}$$

where T is the amount of N in the labelled crop part or soil layer (kg N ha^{-1}), p the ^{15}N excess atom fraction in the labelled crop part or soil layer, q the ^{15}N excess atom fraction in control crop or soil that did not received labelled N and f the ^{15}N excess atom fraction of the labelled fertiliser. The ^{15}N recovery was calculated as the ratio between *Ndff* and the amount of N applied. The mean q value in aboveground biomass at harvest was derived from the analyses made in the unlabelled plots in 2007 and 2009. For the final crop residues and living biomass, q was either obtained from corresponding unlabelled samples (N- treatments for perennial crops) or using the mean value calculated for aboveground biomass. The q values in soil samples were obtained with the measurements made in 2006 in the corresponding plots and soil layers.

The *Ndff* in soil samples were calculated in each treatment on an equivalent soil mass (ESM) basis (Ellert and Bettany, 1995). The "reference" soil masses used for calculations were those measured in 2006 (667, 2000, 2002, 884 and 3137 t ha^{-1} for 0-5, 5-20, 20-*Y*, *Y*-40 and 40-60 cm respectively). Detailed calculations are given in Chap. 4 for soil organic carbon stocks and carbon isotopic composition. The same equations were applied here replacing carbon concentration by N concentration and δ^{13}C by ^{15}N excess atom fraction. In the following, soil layers on ESM basis are called L1 to L5.

5.2.4 Statistical analyses

All statistical analyses were performed using R (R Core Team, 2014). The effects of rotation, nitrogen and their interaction were evaluated by analysis of variance (ANOVA) for the different variables. Two linear mixed-effect models were used: the first one adapted to a split-block design (with blocks, rotation × blocks and nitrogen × blocks interactions as random factors) was used for perennial crops and the second, adapted to a split-plot design (with blocks and rotation × blocks interaction as random factors), was used for the other crops. The

lme function from the *nlme* package was used to fit the models (Pinheiro *et al.*, 2014). Significant differences ($p < 0.05$) between treatments were identified with the *lsmeans* function (Lenth, 2014). The assumptions of ANOVA were checked by visual examination of the residuals against predicted values and using the Shapiro-Wilk and Levene tests. Log-transformed data or Box-Cox transformation were used if necessary to satisfy these assumptions.

5.3 Results

5.3.1 Crop production and N removal at harvest

The mean harvested biomass was calculated from 2007 (first year with all crops present and beginning of ^{15}N applications) to the end of the period during which ^{15}N-labelled fertiliser was applied, *i.e.* 2010 for perennial crops and 2011 for the other crops. It represented 19.0 t DM ha^{-1} yr^{-1} in perennial crops and 10.3 t DM ha^{-1} yr^{-1} in other crops (Table 5.3). Rotation, fertiliser-N rate and their interaction had a significant effect on biomass production for both crop types. Among perennial crops, Mis E N+ was the most productive, yielding 26.6 ± 2.6 t ha^{-1} yr^{-1}. Miscanthus produced generally more than switchgrass, particularly in the early harvest (E) treatments. An interaction between harvest date and N fertilisation was observed: biomass production was significantly higher in N+ than in N- for E treatments whereas N fertilisation had no significant effect for L treatments. The difference between N- and N+ in E treatments tended to increase with time (Table S1, Annexe 4). Among semi-perennial and annuals crops, Tri-Sor N+ was the most productive treatment with 12.6 ± 1.3 t ha^{-1} yr^{-1}. The higher level of fertilisation (N+) significantly enhanced biomass production in annual crops compared to N-, but not in semi-perennials crops. Fescue alone had a small and significant response to N rate (+1.5 t ha^{-1} yr^{-1} in N+). The harvested biomass of alfalfa was markedly lower in establishment years than in other years (4.4 *vs.* 14.2 t ha^{-1} yr^{-1} respectively) and sorghum had a particularly low production in 2011 (4.1 t ha^{-1}).

The amount of N exported at harvest varied widely, from 38 ± 10 kg ha^{-1} yr^{-1} in Mis L N- in 2007-2010 to 228 ± 15 kg ha^{-1} yr^{-1} in Alf-Fes N+ in 2007-2011 (Table 5.3). It was significantly affected by rotation, fertiliser-N rate and their interaction. Fertilised perennial crops exported systematically higher amounts of N than unfertilised ones (+24 kg ha^{-1} yr^{-1} on average). The amount of N exported at harvest was greater in early than in late harvest treatments, particularly with miscanthus. It tended to increase with time for miscanthus and decrease for switchgrass (Table S2, Annexe 2). It was high for semi-perennial crops (204 kg ha^{-1} yr^{-1} on average) but did not change significantly with N fertilisation. On the

contrary, fertilized annual crops (N+) exported more N than low fertilized ones (+50 kg ha^{-1} yr^{-1} on average).

Table 5.3. Mean harvested biomass (t DM ha^{-1} yr^{-1}) and nitrogen exported (kg ha^{-1} yr^{-1}) from 2007 to 2010 (perennial crops) or 2011 (other crops). Values in brackets are standard deviations. Different letters indicate significant differences (p<0.05) between treatments (lower case: perennial crops; upper case: other crops).

Rotation	N	Mean harvested biomass (t DM ha^{-1} yr^{-1})		Mean N exported (kg N ha^{-1} yr^{-1})	
Mis E	N-	24.2 (3.0)	b	100 (10)	b
	N+	26.6 (2.6)	a	135 (18)	a
Mis L	N-	19.0 (2.2)	cd	38 (10)	e
	N+	18.7 (1.6)	cdef	52 (5)	cd
Swi E	N-	15.6 (0.6)	dfh	70 (4)	cd
	N+	18.1 (1.4)	ceg	95 (5)	b
Swi L	N-	14.8 (0.8)	gh	51 (6)	de
	N+	15.1 (1.3)	efgh	71 (2)	c
Fes-Alf	N-	9.8 (0.2)	BC	180 (6)	C
	N+	10.4 (0.3)	B	190 (12)	BC
Alf-Fes	N-	8.9 (0.4)	C	217 (12)	AB
	N+	9.6 (0.2)	BC	228 (15)	A
Sor-Tri	N-	9.8 (0.3)	BC	71 (6)	E
	N+	11.9 (0.5)	A	122 (20)	D
Tri-Sor	N-	9.3 (0.6)	BC	66 (7)	E
	N+	12.6 (1.3)	A	114 (13)	D

5.3.2 Crop residues and living biomass

The amount of crop residues found at soil surface or within soil at the end of the ^{15}N-labelling period was much higher in perennial than in other crops: 13.2 *vs.* 2.4 t DM ha^{-1} on average respectively (Table 5.4). It did not change significantly with N fertilisation. The amount of aboveground residues was particularly important in Mis L because of the presence of senescent leaves accumulated in mulch at soil surface. The total living biomass was also much higher in perennial crops: rhizomes and roots (0-60 cm) of perennial crops represented 12.9 to 24.7 t DM ha^{-1} (18.5 t ha^{-1} on average) in March 2011 whereas the total living biomass of the other crops was only 0.4 to 6.7 t DM ha^{-1} in 2012.

Table 5.4. Crop residues and living biomass (t DM ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops).

	Mis E		Mis L		Swi E		Swi L	
	N-	N+	N-	N+	N-	N+	N-	N+
Aboveground crop residues	5.3 (1.3) b	6.5 (1.3) b	16.1 (1.2) a	13.3 (1.2) a	5.5 (2.4) b	6.9 (2.3) b	6.6 (0.9) b	6.8 (0.6) b
Belowground crop residues	6.6 (4.8) a	4.1 (2.3) a	5.7 (2.2) a	4.5 (1.5) a	3.4 (0.1) a	4.0 (1.3) a	5.8 (3.9) a	4.6 (2.4) a
a) Total crop residues	*11.9 (3.5) b*	*10.6 (2.6) b*	*21.9 (1.6) a*	*17.8 (2.7) a*	*8.9 (2.4) b*	*10.8 (1.2) b*	*12.5 (4.7) b*	*11.4 (2.5) b*
Rhizome	17.0 (6.4) a	14.2 (0.9) a	19.7 (1.3) a	16.8 (2.9) a	4.6 (2.3) b	3.0 (2.7) b	5.9 (1.7) b	3.4 (3.6) b
Roots (0-20 cm)	4.7 (0.4) b	3.3 (0.6) b	4.1 (1.6) b	3.6 (1.7) b	7.5 (1.5) a	7.6 (2.9) a	8.9 (3.6) a	6.7 (3.1) a
Roots (20-40 cm)	0.9 (0.2) b	0.4 (0.1) b	0.7 (0.2) b	0.5 (0.1) b	2.8 (0.5) a	2.7 (0.6) a	2.4 (0.9) a	1.9 (0.3) a
Roots (40-60 cm)	0.3 (0.1) b	0.2 (0.1) b	0.2 (0.2) b	0.3 (0.1) b	1.1 (0.2) a	0.8 (0.1) a	1.0 (0.3) a	0.9 (0.3) a
b) Total living biomass	*22.9 (6.2) ab*	*18.1 (0.5) ab*	*24.7 (3.0) a*	*21.2 (3.3) a*	*16.0 (3.2) b*	*14.2 (6.1) b*	*18.2 (6.4) b*	*12.9 (6.3) b*
Total (a+b)	*34.8 (9.7) b*	*28.8 (2.1) b*	*46.6 (3.0) a*	*39.0 (4.0) a*	*24.9 (5.7) b*	*25.0 (5.5) b*	*30.7 (11.1) b*	*24.3 (8.9) b*
	Fes-Alf		Alf-Fes		Sor-Tri		Tri-Sor	
	N-	N+	N-	N+	N-	N+	N-	N+
a) Total crop residues	*3.5 (1.2) A*	*4.0 (1.7) A*	*0.8 (0.2) B*	*0.6 (0.2) B*	*1.6 (0.3) B*	*1.6 (0.6) B*	*3.3 (0.8) A*	*3.7 (0.7) A*
Aboveground living biomass	3.6 (0.5) A	3.9 (0.7) A	1.1 (0.6) B	1.1 (0.6) B				
Belowground living biomass	2.4 (0.7) A	2.8 (0.3) A	1.5 (1.2) A	1.3 (0.6) A				
b) Total living biomass	*6.0 (1.2) A*	*6.7 (0.9) A*	*2.6 (1.7) B*	*2.4 (1.2) B*	*0.4 (0.0) C*	*0.4 (0.1) C*	*1.2 (0.5) BC*	*1.2 (0.2) BC*
Total (a+b)	*9.5 (2.3) A*	*10.6 (0.9) A*	*3.4 (1.7) BC*	*3.1 (1.4) BC*	*2.0 (0.3) C*	*2.0 (0.5) C*	*4.5 (1.1) B*	*5.0 (0.9) B*

Table 5.5. N content in crop residues and living biomass (kg ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant effect of N fertilisation (without interaction with rotations).

	Mis E				Mis L				Swi E				Swi L			
	N-		N+		N-		N+		N-		N+		N-		N+	
Aboveground crop residues	14 (4)	e	27 (6)	cde	71 (9)	b	85 (3)	a	20 (9)	de	34 (13)	c	23 (3)	cde	35 (2)	bc
Belowground crop residues	20 (13)	a	18 (7)	a	24 (9)	a	30 (11)	a	13 (1)	a	19 (4)	a	23 (16)	a	28 (18)	a
a) Total crop residues	*34 (10)*	*b-*	*44 (1)*	*b+*	*95 (2)*	*a-*	*115 (13)*	*a+*	*33 (10)*	*b-*	*54 (10)*	*b+*	*46 (18)*	*b-*	*63 (18)*	*b+*
Rhizome	63 (24)	b-	147 (39)	b+	167 (8)	a-	247 (66)	a+	30 (18)	c-	34 (27)	c+	52 (17)	c-	51 (51)	c+
Roots (0-20 cm)	35 (2)	b	38 (2)	b	40 (13)	ab	56 (24)	ab	34 (5)	b	58 (11)	B	52 (19)	a	79 (32)	a
Roots (20-40 cm)	6 (1)	bc	4 (0)	c	7 (2)	bc	7 (2)	bc	12 (1)	b	22 (5)	a	13 (4)	b	21 (4)	a
Roots (40-60 cm)	2 (1)	c	1 (1)	c	2 (1)	c	3 (1)	bc	4 (1)	bc	5 (1)	ab	4 (1)	bc	7 (2)	a
b) Total living biomass	*106 (27)*	*b-*	*190 (37)*	*b+*	*216 (11)*	*a-*	*313 (71)*	*a+*	*79 (19)*	*b-*	*119 (33)*	*b+*	*120 (39)*	*b-*	*159 (77)*	*b+*
Total (a+b)	*140 (36)*	*b-*	*234 (37)*	*b+*	*311 (11)*	*a-*	*428 (73)*	*a+*	*113 (27)*	*b-*	*173 (27)*	*b+*	*166 (57)*	*b-*	*221 (95)*	*b+*
	Fes-Alf				Alf-Fes				Sor-Tri				Tri-Sor			
	N-		N+		N-		N+		N-		N+		N-		N+	
a) Total crop residues	*21 (7)*	*A*	*30 (14)*	*A*	*7 (2)*	*B*	*5 (1)*	*B*	*5 (0)*	*B*	*6 (2)*	*B*	*11 (2)*	*A*	*23 (10)*	*A*
Aboveground living biomass	82 (4)	A	90 (31)	A	18 (9)	B	21 (12)	B								
Belowground living biomass	44 (13)	A	50 (8)	A	43 (37)	A	34 (20)	A								
b) Total living biomass	*126 (16)*	*A*	*140 (39)*	*A*	*61 (46)*	*B*	*55 (32)*	*B*	*15 (0)*	*B*	*15 (4)*	*B*	*26 (7)*	*B*	*27 (7)*	*B*
Total (a+b)	*147 (24)*	*A*	*170 (25)*	*A*	*68 (48)*	*B*	*60 (33)*	*B*	*20 (0)*	*C*	*22 (3)*	*C*	*37 (8)*	*BC*	*50 (16)*	*BC*

The N content in crop residues was significantly higher in N+ than in N- for perennial crops but not for the other crops (Table 5.5). It was higher in Mis L than in the other perennial treatments (105 *vs.* 48 kg N ha^{-1} respectively on average). Residues of semi-perennial and annual crops contained 5 to 30 kg N ha^{-1}. Large amounts of nitrogen were stored in rhizomes and roots of perennial crops: 264 kg ha^{-1} in Mis L and 148 kg ha^{-1} in Mis E. A greater amount of N in the L treatment was also found for switchgrass but was not significant ($p<0.05$). N fertilisation increased N stocks in belowground organs of miscanthus and switchgrass by 65 kg ha^{-1} on average. Nitrogen was mainly stored in rhizomes for miscanthus (73%) and in roots for switchgrass (65%). Regarding the other crops, the N content in the living biomass ranged from 21 kg ha^{-1} (Sor-Tri: triticale sown in October 2011) to 158 kg ha^{-1} (Fes-Alf: fescue sown in April 2011) and did not differ significantly between N- and N+.

5.3.3 *Ndff* and ^{15}N recovery in the exported biomass

The amount of N derived from fertiliser exported at harvest was calculated each year from 2007 to 2011 (Table 5.6). From 2008 onwards, the *Ndff* could derive either from the fertiliser applied during the same year or from preceding applications because the ^{15}N-labelled fertiliser was applied every year in the same subplots. In unfertilised crops (alfalfa or sorghum N-) following fertilised ones, the *Ndff* was low (between 0.4 and 2.8 kg ha^{-1}), except in 2011 for fescue and alfalfa N+ (5.6 kg ha^{-1} on average), showing that the carry over effect of fertiliser was much smaller than its direct effect. The *Ndff* tended to increase with time for miscanthus but not for switchgrass. On average over the whole period, *Ndff* in the exported biomass represented 26, 18 and 28 kg N ha^{-1} yr^{-1} for perennial, semi-perennial and annual crops respectively. This corresponds to 28-30% of the exported N for perennial crops, 3-17% for semi-perennials and 11-37% for annual crops. The exported N derived from other sources (soil and atmosphere) was greater: 62, 186 and 65 kg ha^{-1} yr^{-1} for perennial, semi-perennial and annual crops respectively.

The actual ^{15}N recovery in the harvested biomass was on average 21.8% for perennial and 33.5% for the other crops (Table 5.6). Perennial crops harvested late had a significantly lower recovery than the early harvested: 13.2 ± 1.4% for Mis L *vs.* 34.1 ± 8.5% for Mis E. The lower ^{15}N recovery in the Alf-Fes than in the Fes-Alf rotation (24.9 *vs.* 39.7%) could be due to the lower yields of fescue (6.8 *vs.* 11.7 t DM ha^{-1} yr^{-1}) in this rotation. The ^{15}N recovery was significantly higher in N+ than in N- treatments (mean difference of 4.4%). For each crop independently of the rotation, the mean ^{15}N recovery was 29.9 and 33.6% for fescue N- and

N+ respectively, 31.4% for sorghum N+ and 32.3 and 46.0% for triticale N- and N+ respectively.

Table 5.6. *Ndff* in the exported biomass (kg N ha^{-1}) during each year, N derived from fertiliser and other sources and ^{15}N recovery (%) in the exported biomass over the whole period. Values in brackets are standard deviations. Different letters indicate significant differences (p<0.05) between treatments (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant mean effect of N fertilisation (without interaction with rotations).

Rotation	N	*Ndff* in the exported biomass (kg N ha^{-1})					N exported in 2007-2010/11 (kg N ha^{-1} yr^{-1})		^{15}N recovery in exported biomass (%)
		2007	2008	2009	2010	2011	From fertiliser	From other sources	
Mis E	N+	30 (8)	28 (8)	45 (12)	60 (19)		41 (10)	94 (11)	34.1 (8.5) a
Mis L	N+	8 (1)	11 (3)	17 (0)	28 (3)		16 (2)	36 (4)	13.2 (1.4) c
Swi E	N+	37 (6)	19 (8)	24 (2)	32 (12)		28 (4)	67 (1)	23.3 (3.2) b
Swi L	N+	23 (1)	23 (4)	14 (3)	20 (0)		20 (2)	51 (3)	16.6 (1.4) c
Fes-Alf	N-	48 (1)	24 (6)	0 (0)	2 (0)	3 (1)	15 (1)	165 (5)	38.3 (2.9) A-
	N+	96 (2)	61 (7)	1 (0)	2 (1)	5 (1)	33 (2)	157 (11)	41.1 (1.9) A+
Alf-Fes	N-	0 (0)	0 (0)	8 (1)	25 (8)	3 (3)	7 (2)	210 (13)	22.6 (6.1) B-
	N+	0 (0)	0 (0)	20 (3)	61 (9)	6 (6)	17 (2)	211 (15)	27.3 (2.5) B+
Sor-Tri	N-	0 (0)	17 (6)	1 (1)	21 (4)	1 (1)	8 (2)	63 (6)	33.8 (7.0) A-
	N+	37 (12)	46 (7)	49 (0)	57 (5)	39 (9)	46 (7)	77 (14)	38.2 (5.5) A+
Tri-Sor	N-	23 (2)	1 (0)	18 (6)	1 (0)	17 (1)	12 (1)	54 (6)	33.6 (2.6) A-
	N+	59 (5)	34 (4)	54 (1)	30 (9)	60 (7)	47 (4)	67 (10)	39.4 (3.6) A+

The ^{15}N recovery was compared to the apparent recovery (R_A) calculated for perennial crops and sorghum (crops with an unfertilised control) (Figure 5.1). The two methods gave very similar results: the regression equation was $y = 0.95\ R_A$ ($R^2 = 0.69$). This good agreement confirmed the veracity of the low efficiency of fertiliser-N detected with the ^{15}N data.

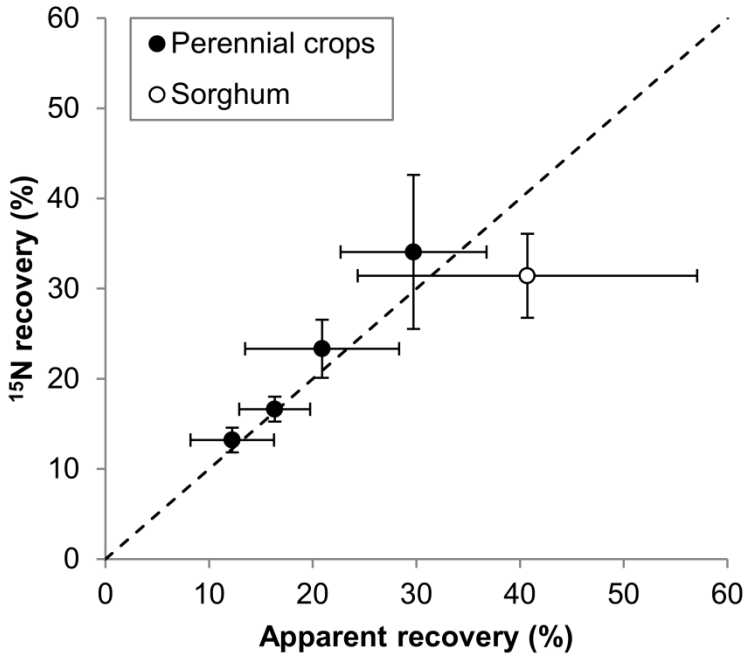


Figure 5.1. Relationship between the ^{15}N recovery and the apparent recovery (%) in the exported biomass of perennial crops (2007-2010) and sorghum N+ (2007-2011). The dashed line represents the 1:1 line. Bars represent the standard deviations.

5.3.4 ^{15}N recovery in crop residues and living biomass

A significant share of the ^{15}N fertiliser was found in residues of perennial crops (4.2% on average) whereas it was almost negligible for the other crops (0.3%, Table 5.7). As expected, the ^{15}N recovery in crop residues was higher in Mis L (6.6%) than in the other perennial crops. The ^{15}N recovery in the living biomass of perennial crops (belowground organs) was also important. It was higher in Mis L (17.5%) than in other perennials (9.8% on average). The *Ndff* was mainly located in rhizomes for miscanthus and in roots for switchgrass. In spite of their deep rooting system (Chap. 2), miscanthus and switchgrass allocated a very small fraction of fertiliser in roots below 40 cm (0.2% on average). Finally the ^{15}N recovery in the living biomass of semi-perennial and annual crops (above and belowground) was low: 0.3 to 1.2%. The whole ^{15}N recovery in crop residues and living biomass ranged from 11.2 to 24.1% in perennial crops and 0.5 to 1.3% in the other crops. The *Ndff* represented 27-32% of the total N content in crop residues and living biomass of perennial crops, and 1-15% for the other crops.

Table 5.7. ^{15}N recovery (%) measured in crop residues and living biomass in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: other crops).

	Mis E		Mis L		Swi E		Swi L	
	N-	N+	N-	N+	N-	N+	N-	N+
Aboveground crop residues		1.5 (0.4) b		4.7 (0.2) a		2.2 (1.0) b		2.3 (0.1) b
Belowground crop residues		1.0 (0.4) a		1.9 (0.7) a		1.2 (0.2) a		1.8 (1.4) a
a) Total crop residues		*2.6 (0.1) c*		*6.6 (0.6) a*		*3.4 (1.1) bc*		*4.1 (1.5) b*
Rhizome		8.4 (2.8) ab		13.8 (2.8) a		2.3 (1.9) c		3.7 (3.7) bc
Roots (0-20 cm)		2.3 (0.1) b		3.2 (1.0) ab		4.0 (0.8) ab		5.2 (2.1) a
Roots (20-40 cm)		0.2 (0.1) b		0.3 (0.0) b		1.3 (0.3) a		1.2 (0.2) a
Roots (40-60 cm)		0.0 (0.0) b		0.1 (0.1) b		0.3 (0.1) a		0.5 (0.2) a
b) Total living biomass		*10.9 (2.8) b*		*17.5 (2.5) a*		*7.9 (2.2) b*		*10.6 (5.4) b*
Total (a+b)		*13.4 (2.7) b*		*24.1 (2.4) a*		*11.2 (1.8) b*		*14.7 (6.9) b*
	Fes-Alf		Alf-Fes		Sor-Tri		Tri-Sor	
	N-	N+	N-	N+	N-	N+	N-	N+
a) Total crop residues	*0.1 (0.0) B*	*0.1 (0.1) B*	*0.2 (0.1) B*	*0.1 (0.0) B*	*0.1 (0.0) B*	*0.1 (0.0) B*	*0.5 (0.0) A*	*0.8 (0.5) A*
Aboveground living biomass	0.6 (0.1) A	0.5 (0.2) A	0.4 (0.1) A	0.4 (0.2) A				
Belowground living biomass	0.2 (0.0) A	0.2 (0.0) A	0.6 (0.4) A	0.8 (0.5) A				
b) Total living biomass	*0.7 (0.1) AB*	*0.7 (0.2) AB*	*1.0 (0.5) A*	*1.2 (0.8) A*	*0.3 (0.1) B*	*0.4 (0.2) B*	*0.5 (0.2) B*	*0.4 (0.1) B*
Total (a+b)	*0.8 (0.1) A*	*0.8 (0.2) A*	*1.2 (0.6) A*	*1.3 (0.8) A*	*0.5 (0.1) A*	*0.5 (0.1) A*	*1.0 (0.3) A*	*1.2 (0.6) A*

Table 5.8. ^{15}N recovery (%) measured in soil layers in 2011 for perennial crops and in 2012 for semi-perennial/annual crops. Layers L1, L2, L3, L4 and L5 correspond to ca. 0-5, 5-18, 18-32, 32-38 and 38-58 cm respectively. Values in brackets are standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: semi-perennial and annual crops).

Layer	Soil mass (t ha^{-1})	Mis E	Mis L	Swi E	Swi L	Fes-Alf		Alf-Fes		Sor-Tri		Tri-Sor	
		N+	N+	N+	N+	N-	N+	N-	N+	N-	N+	N-	N+
L1	667	20.4 (6.2)	16.5 (2.2)	15.4 (3.5)	17.3 (2.5)	2.0 (0.3)	1.9 (0.2)	5.0 (3.4)	3.0 (0.5)	6.7 (1.8)	4.5 (0.6)	7.8 (2.2)	5.3 (1.2)
L2	2000	9.6 (1.6)	8.5 (2.6)	9.0 (2.2)	11.7 (2.3)	14.6 (1.9)	12.4 (2.2)	15.3 (4.9)	14.0 (5.3)	12.8 (2.7)	6.0 (2.0)	9.7 (0.6)	9.0 (0.8)
L3	2002	2.7 (0.2)	2.6 (1.1)	2.7 (0.5)	3.7 (0.6)	7.6 (1.6)	8.1 (2.7)	11.3 (7.9)	9.0 (3.3)	3.3 (0.3)	1.7 (0.4)	2.0 (0.6)	1.6 (0.3)
L4	884	0.3 (0.1)	0.3 (0.1)	0.5 (0.1)	0.7 (0.3)	0.2 (0.2)	0.3 (0.0)	0.7 (0.2)	0.8 (0.3)	0.5 (0.1)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)
L5	3137	1.6 (0.8)	0.8 (0.2)	1.3 (0.7)	1.2 (0.4)	0.7 (0.3)	0.6 (0.2)	1.6 (0.6)	1.8 (0.9)	1.2 (0.1)	0.6 (0.2)	0.6 (0.1)	0.5 (0.2)
L1-5	*8690*	*34.6 (7.0)*	*28.7 (6.0)*	*28.9 (5.8)*	*34.6 (3.0)*	*25.1 (1.8)*	*23.3 (1.8)*	*33.9 (2.0)*	*28.5 (3.8)*	*24.4 (4.6)*	*12.9 (2.5)*	*20.4 (2.1)*	*16.6 (1.3)*
		a	*a*	*a*	*a*	*BC*	*BC*	*A*	*AB*	*BC*	*E*	*CD*	*DE*

5.3.5 ^{15}N recovery in soil

The average ^{15}N recovery of labelled fertiliser in all soil layers (L1-5, *ca.* 0-58 cm depth) was 31.7% for perennial crops and 23.1% for the other crops (Table 5.8), corresponding to 38 and 13 kg N ha^{-1} yr^{-1} respectively. Under perennial crops, the ^{15}N recovery did not differ between treatments whatever the soil layer and was mainly located (85%) in the upper two layers (*ca.* 0-18 cm). Under semi-perennial and annual crops, the ^{15}N recovery in soil was significantly affected by the rotation, the fertiliser-N rate and their interaction. It was higher under semi-perennial than annual crops (27.7 *vs.* 18.6% respectively on average in L1-5) and higher in N- than in N+ (+5.6% on average) although the difference was only significant for Sor-Tri. Similarly to perennial crops, 83% of the fertiliser recovered under annual crops was found in the upper soil layers (*ca.* 0-19 cm). It was only 62% under semi-perennial crops due to the soil ploughing event in 2011 which incorporated a part of the labelled N below 19 cm.

5.3.6 Overall ^{15}N recovery

The overall ^{15}N recovery in the soil-plant system at the end of the ^{15}N-labelling period, *i.e.* the sum of the labelled N exported in the harvested biomass during the four or five year period and stored in living biomass, crop residues and soil at the end of the period, is presented in Figure 5.2. It ranged from 51.6 ± 4.4% (Sor-Tri N+) to 82.1 ± 6.5% (Mis E N+). It was significantly higher for Mis E N+ than for the other perennial crops (82.1 *vs.* 65.2% respectively on average). Overall ^{15}N recovery in semi-perennial and annual crops did not differ between treatments and was 58.3% on average. The unrecovered ^{15}N is attributed to losses towards the groundwater and the atmosphere, *i.e.* leaching, volatilization and denitrification. It represented a large part of the fertiliser: 17.9% for Mis E N+, 34.8% for other perennial crops and 41.7% for semi-perennial and annual crops.

The *Ndff* exported at harvest represented 20% (Mis L N+) to 74% (Sor-Tri N+) of the overall recovery (50% on average for all treatments). The *Ndff* stored in living biomass in 2011 or 2012 was 17% of the overall recovery for perennial crops and only 1% for the other crops. The *Ndff* stored in crop residues was 6% and <1% of the overall recovery for perennials and other crops respectively. Finally, the *Ndff* stored in soil in 2011 or 2012 ranged between 25% (Sor-Tri N+) and 59% (Alf-Fes N-) of the overall recovery (42% on average for all treatments).

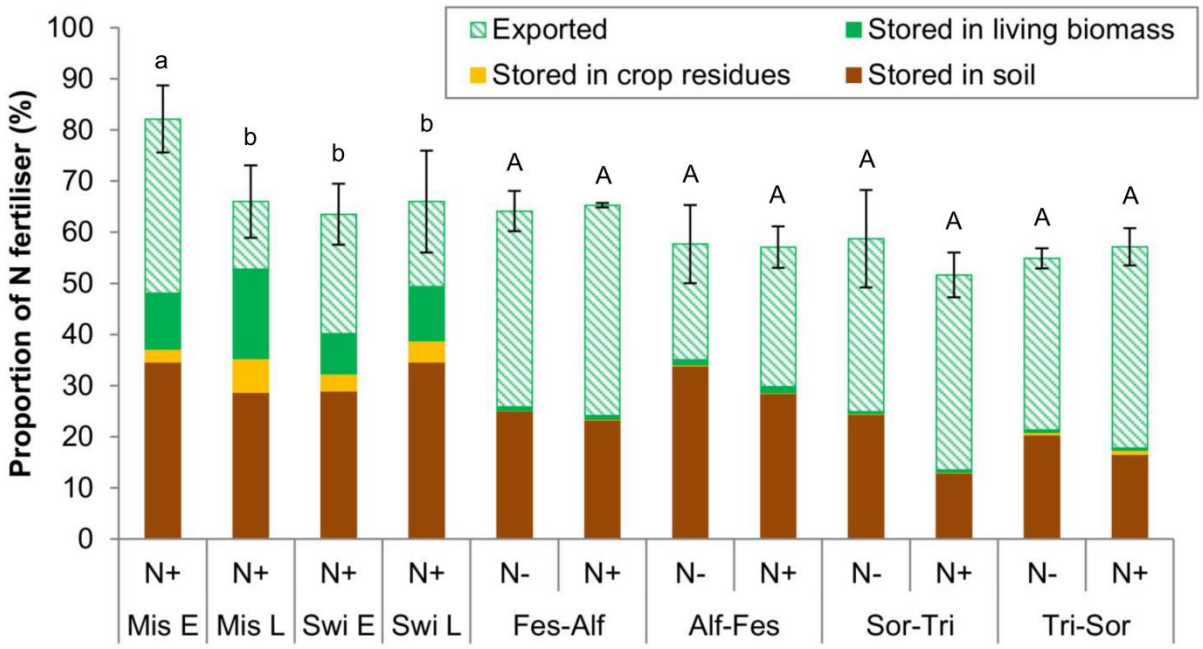


Figure 5.2. Overall ^{15}N recovery (%) measured for perennial (2007-2010) and semi-perennial/annual crops (2007-2011). Bars represent the standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: semi-perennial and annual crops).

5.4 Discussion

5.4.1 Crop production and nitrogen removal at harvest

5.4.1.1 Crop production

Perennial C4 crops were the most productive crops in this experiment. The crop yields were in the range of those reviewed in the literature by Gabrielle *et al.* (2014), except for fibre sorghum which had a lower production in our experiment compared to literature data originating from southern Europe. The marked sensitivity of sorghum to low temperatures probably reduces its potential in northern Europe.

The interactive effect of harvest date and N fertilisation on the yield of perennial crops was probably the result of the harvest date on belowground N reserves. Early harvest impedes a complete N translocation from aboveground to belowground organs in autumn, reducing N reserves for the succeeding year (Strullu *et al.*, 2011; Pedroso *et al.*, 2014). However, yields of fertilized, early harvested treatments were higher than those of fertilized, late harvested treatments of miscanthus because the aboveground biomass decreased in autumn and winter due to C translocation towards rhizomes and the important leaf fall (Strullu *et al.*, 2011; Strullu *et al.*, 2014a).

5.4.1.2 N removal at harvest

As already shown previously (Cadoux *et al.*, 2014), N exported by late harvested perennial crops was particularly low because N concentration in the aboveground biomass was very low at the end of winter. This is due to N translocation in autumn (Garten *et al.*, 2010; Strullu *et al.*, 2011; Cadoux *et al.*, 2012; Pedroso *et al.*, 2014). N exported by perennial crops harvested early was closer to that observed for annual crops due to the higher N concentrations and yields than in late harvest, as a result of incomplete N and C translocation. Semi-perennial crops showed the highest N removal with high N concentrations, particularly for alfalfa. This result is in accordance with previous studies showing high N concentrations in the harvested biomass of these crops (Sweeney *et al.*, 1996; Lloveras *et al.*, 2001; Da Silva Perez *et al.*, 2010; Kanapeckas *et al.*, 2011). However, a large part of the N removed by alfalfa probably originated from the atmosphere through symbiotic N fixation (Anglade *et al.*, 2015).

5.4.2 N content of crop residues and living biomass

5.4.2.1 Crop residues

Although the biomass of crop residues was much higher in perennial than other crops, the difference was smaller for their N content because residues of perennial crops had a higher C:N ratio than other crops (85 *vs.* 21). The greatest amount of crop residues was found in miscanthus L: 19.9 t DM ha^{-1} and 105 kg N ha^{-1} (average of N- and N+). About half of this amount was contained in senescent leaves accumulated in a thick mulch at soil surface (8.1 t DM ha^{-1} and 50 kg N ha^{-1}) and the rest was located in stem residues. The values obtained in our study for the leaf mulch were almost identical to those measured by Amougou *et al.* (2012) one year earlier in the same experiment and close to the measurements of Christian *et al.* (2006) on a 4-year-old miscanthus in late harvest (6.9 t DM ha^{-1} and 57 kg N ha^{-1}). The biomass and amount of N in switchgrass residues (10.9 t DM ha^{-1} and 49 kg N ha^{-1}) were very close to the measurements reported by Garten *et al.* (2010) for a 4-year-old switchgrass (10.7 t DM ha^{-1} and 52 kg N ha^{-1}).

5.4.2.2 Living biomass

Perennial crops were also characterized by a large amount of N stored in living belowground organs. The biomass and N content of the miscanthus rhizomes observed in our experiment were comparable to those reported by Himken *et al.* (1997) (16 t DM ha^{-1} and 179-227 kg N ha^{-1}) and higher than the values reported by Christian *et al.* (2006) (9.9 t DM

ha^{-1} and 140 kg N ha^{-1}) also in a 4-year-old plantation with late harvests. The root biomass and N content found in our experiment were intermediate between those reported by Christian *et al.* (2006) and Neukirchen *et al.* (1999). For switchgrass, the amount of N stored in the rhizome was higher than that reported by Garten *et al.* (2010) but the root N content over 0-60 cm was similar. To our knowledge, the combined effects of N fertilisation and harvest date on the belowground N content of switchgrass have not been studied in other experiments. Pedroso *et al.* (2014) compared a two-harvest system to a single post-anthesis harvest system and found that the two-harvest system increased the N removal at harvest by 51 kg N ha^{-1} yr^{-1} and reduced belowground N stock over 0-100 cm by 36%, in accordance with our results.

The belowground N content of semi-perennial crops measured in 2012 over 0-60 cm (47 and 38 kg N ha^{-1} for fescue and alfalfa respectively) was smaller than that of perennial crops. However, these crops were re-sown in spring 2011 and the dry spring of that year caused difficulties in alfalfa establishment. Indeed, the root biomass measured in 2012 was twice lower than that reported by Thiébeau *et al.* (2011) at the end of the first year of growth.

5.4.3 ^{15}N recovery in the soil-plant system

5.4.3.1 ^{15}N recovery in crops

Perennial crops harvested late were characterized both by a low ^{15}N recovery in the harvested biomass and a high ^{15}N recovery in living belowground organs. This is consistent with the observations made for N and attributed to the important N remobilisation from aboveground to belowground organs occurring in autumn. Christian *et al.* (2006) also observed that the greatest part of the labelled fertiliser taken up by miscanthus was located in the belowground biomass at the end of winter. Using their results, we could calculate that the ^{15}N recovery in the cumulative harvested biomass over 3 years was 28.4%. This is much higher than the 13.2% observed in our experiment for miscanthus L. A similar difference between the two studies was observed for the total N removed at harvest (73 *vs.* 38 kg N ha^{-1} yr^{-1}) which suggests a greater N remobilisation in autumn in our experimental conditions. Pedroso *et al.* (2014) found a similar effect of the harvest mode on a 2-year-old switchgrass: ^{15}N recovery at harvest increased from 18.4 to 39.1% with a two-harvest system compared to a single post-anthesis harvest system, and simultaneously the ^{15}N recovery in belowground organs decreased from 27.0 to 10.4%. The ^{15}N recovery in harvested biomass in the single harvest treatment was consistent with the 16.6% observed in our study (switchgrass L) whereas the ^{15}N recovery in belowground organs was higher than ours (27.0 *vs.* 10.6%). The main difference is due to the high ^{15}N recovery in deep roots (*ca.* 10% below 60 cm). Finally,

the ^{15}N recovery in the whole plant including crop residues measured in our study for miscanthus L (37.3%) was smaller than the 55.8% which can be calculated using the data reported by Christian *et al.* (2006) in a 4-year-old crop. For switchgrass L, there was also a large gap between our result (31.3%) and the value reported by Pedroso *et al.* (2014) (47.8%) that could be partly due to the difference in root sampling depth.

Concerning semi-perennial crops, the ^{15}N recovery in the harvested biomass ranged between 22.6 and 41.1%, with a significant difference between the two rotations. In the literature, the ^{15}N recovery by forage crops has been mainly studied in perennial ryegrass (*Lolium perenne*) or natural grassland. Reported values of ^{15}N recovery at harvest range generally between 50 and 60% (Whitehead and Dawson, 1984; Webster and Dowdell, 1985; Bristow *et al.*, 1987; Triboi, 1987; Stevens and Laughlin, 1989; Jenkinson *et al.*, 2004), but smaller values have been observed by Dawson and Ryden (1985) (11 to 48%), Ledgard *et al.* (1988) (26 to 60%) and Kumar and Goh (2002) (37%). Dawson and Ryden (1985) and Ledgard *et al.* (1988) showed an effect of the date of application, the ^{15}N recovery at harvest being higher for spring than for summer or autumn applications. The first authors also showed that ^{15}N recovery in summer was much smaller in case of water stress. In our experiment, the lower fescue yields observed in 2009 and 2010 and the equivalent repartition of the N applied between the spring, summer and autumn cuts may have reduced the ^{15}N recovery at harvest.

The ^{15}N recovery at harvest of annual crops ranged between 33.6 and 39.4% but sorghum had lower recovery (31.4%) than triticale (32.3-46.0%). Our results for sorghum fall in the lower range of results reported for maize (*Zea mays*) which vary between 32 and 71% (Balabane and Balesdent, 1992; Timmons and Baker, 1992; Reddy and Reddy, 1993; Jokela and Randall, 1997; Normand *et al.*, 1997; Sen Tran and Giroux, 1998; Stevens *et al.*, 2005; Seo *et al.*, 2006; Nannen *et al.*, 2011; Rimski-Korsakov *et al.*, 2012). This variability is not fully understood although ^{15}N recovery is lower for N applications at sowing than at later stages and for surface than injected applications (Jokela and Randall, 1997; Seo *et al.*, 2006). In our experiment, the timing of N application (at sowing) and the low growth of sorghum in May and June may explain the low ^{15}N recovery observed for this crop. The ^{15}N recovery measured for triticale in our study was also rather low (at least for N-) compared to previous results reported in the literature for winter wheat (*Triticum aestivum*), ranging from 33 to 68% (Recous *et al.*, 1988b; Macdonald *et al.*, 1989; Powlson *et al.*, 1992; Recous *et al.*, 1992; Karlen *et al.*, 1996; Macdonald *et al.*, 1997; Kumar and Goh, 2002; Thomsen and Christensen, 2007; Giacomini *et al.*, 2010). The ^{15}N recovery was lower for applications at tillering than at stem elongation. For example, Recous *et al.* (1988b) reported that ^{15}N

recovery increased from 36% for 50 kg N ha^{-1} applied at tillering to 55% for 100 kg N ha^{-1} applied at stem elongation. This may explain the difference observed in our study for triticale between N- and N+ treatments because N- received 60 kg N ha^{-1} at tillering whereas the 120 kg N ha^{-1} for N+ were split between tillering and stem elongation.

5.4.3.2 ^{15}N recovery in soil

Between 12.9 and 34.6% of the ^{15}N fertiliser applied was recovered in the soil. After N applications, the fertiliser inorganic N in soil is rapidly depleted due to plant uptake and immobilisation of N by the soil heterotrophic microflora (Bristow et al., 1987; Recous et al., 1988a; Recous and Machet, 1999). Microbial N is then incorporated into soil organic matter and slowly mineralised in subsequent years (Glendining *et al.*, 2001; Jenkinson *et al.*, 2004; Sebilo *et al.*, 2013). The ^{15}N recovered in soil could also derive from labelled crop residues returned to the soil after harvest (or crop destruction for fescue) and incorporated into the soil organic matter (Macdonald *et al.*, 2002). Almost certainly, the great majority of the ^{15}N recovered in soil in our experiment was in organic rather than inorganic forms since residual inorganic ^{15}N is negligible at harvest time for optimal or sub-optimal N rates (Recous *et al.*, 1988b; Macdonald *et al.*, 1989; Normand *et al.*, 1997).

For miscanthus, we measured a higher recovery in soil (28.7% over 0-60 cm) than that calculated from Christian *et al.* (2006), *i.e.* 20.6% over 0-50 cm. Pedroso *et al.* (2014) reported values for switchgrass (25 to 38% over 0-300 cm) closer to our results (34.6% over 0-60 cm). Surprisingly, they found that a large part of the soil ^{15}N was located in deep soil layers, whereas our results and other studies showed that the great majority of the ^{15}N recovered in soil was found in the topsoil (Glendining *et al.*, 1997; Christian *et al.*, 2006).

The ^{15}N recovery measured in soil for semi-perennial crops (23.3-33.9%) falls in the range of reported values for ryegrass or cut grassland (16 to 35%) (Whitehead and Dawson, 1984; Webster and Dowdell, 1985; Bristow *et al.*, 1987; Triboi, 1987; Ledgard *et al.*, 1988; Stevens and Laughlin, 1989; Kumar and Goh, 2002; Jenkinson *et al.*, 2004).

For annual crops, our results (12.9-24.4%) are consistent with those reported for wheat (between 9 and 36%) and maize (between 15 and 37%) in the studies cited earlier. We hypothesise that the gradient observed in our study between annual, semi-perennial and perennial crops is linked to the amount and composition of crop residues. The accumulation under perennial crops of undecomposed residues with a high C:N ratio probably created a high microbial demand for N. On the contrary, the small amount of residues returning to the

soil with annual crops results in a small microbial N immobilisation, explaining the lower ^{15}N recovery in soil.

5.4.3.3 Overall ^{15}N recovery

In our experiment, the overall ^{15}N recovery in the crop-soil system was rather low (average 60%), except for miscanthus E (82%). The recovery by Miscanthus L (66%) was smaller than that which can be calculated using data of Christian *et al.* (2006) (77%). The values reported by Pedroso *et al.* (2014) for switchgrass over three growing seasons (62-72%) were quite comparable to ours (64-66%). The overall recovery that we found in semi-perennial crops (average 61%) was lower than those reported in the previously cited studies for ryegrass and grassland (63-99% with an average of *ca.* 80%). Finally, the lowest recovery was found in annual crops (average 56%), falling in the lower range of values reported over one growing season for maize (47-100%) and wheat (52-96%) averaging *ca.* 75% for both crops. In a meta-analysis of published ^{15}N field experiments on temperate climate grain crops, Gardner and Drinkwater (2009) found a mean total ^{15}N recovery of 62%, with a large variability.

We believe that in a multi-annual study like ours, ^{15}N losses are likely to be higher than during a single growing season. The hypotheses provided earlier to explain the low ^{15}N recovery in fescue, sorghum and triticale can also apply to the overall recovery. Recous and Machet (1999) and Limaux *et al.* (1999) showed that any increase in plant ^{15}N uptake by winter wheat results in an increase in plant and soil ^{15}N recovery. This was confirmed by Gardner and Drinkwater (2009) who showed in their meta-analysis that practices increasing ^{15}N recovery in the crop, such as improved timing, sidedress or knifed-in applications, also increased the total ^{15}N recovery. Another factor that can explain the relative high ^{15}N losses measured in our study is the type of fertiliser, *i.e.* UAN containing 50% urea N. Urea is known to increase the risk of ammonia volatilisation compared to other forms of N fertiliser such as ammonium nitrate because of the temporary increase in soil pH during urea hydrolysis, particularly in a slightly alkaline soil like ours (Harrison and Webb, 2001). In a recent review, Cameron *et al.* (2013) reported measured ammonia volatilisation losses between 4 and 36% of the N applied as urea in various arable and grassland experiments. These losses are dependent on several factors such as soil type, weather conditions and application rates. For Harrison and Webb (2001), ammonia volatilisation losses from UAN solutions are intermediate between urea and ammonium nitrate, the latter form yielding losses generally less than 4% of the N applied. Fox *et al.* (1996) reported ammonia volatilisation

losses of 22% of the N fertiliser applied as UAN (134 kg N $ha^{-1}yr^{-1}$) on average for three years in a grain maize. Vaio *et al.* (2008) measured losses ranging from 6 to 33% of the 50 kg N ha^{-1} applied as UAN to a tall fescue in spring or fall. Similarly, losses of 23% of the N applied as UAN (40 kg N ha^{-1}) were reported by Suter *et al.* (2013) for a ryegrass fertilised in autumn. Surprisingly, according to the meta-analysis of Gardner and Drinkwater (2009), ^{15}N studies comparing urea to other N forms showed only a small impact on the overall ^{15}N recovery with a mean increase of 3% when urea is replaced by another fertiliser.

Losses of ^{15}N through nitrate leaching were probably low. Indeed, nitrate leaching was not favoured in our context with moderate winter rainfall, large soil water content and deep rooting depth (Chap. 2). Furthermore, at the timescale of our study, *i.e.* 4 to 5 years, experiments measuring the proportion of an initial application of ^{15}N which is lost as nitrate below the root zone report values lower than 5% (Webster and Dowdell, 1985; Triboi, 1987; Sebilo *et al.*, 2013). We therefore hypothesise that the ^{15}N not recovered in the soil-plant system mainly corresponded to gaseous losses through ammonia volatilisation and denitrification to a lesser extent.

5.5 Conclusions

This study provides an original evaluation of the fate of the N fertiliser applied to different perennial and annual bioenergy crops over 4-5 years. As expected, the N recovery measured in the harvested biomass was generally lower for perennial than other crops. However, the difference disappeared when taking into account non-harvested crop parts. Perennial crops were able to store high amounts of N in their belowground organs, particularly in late harvest. Furthermore, fertiliser-N immobilised in soil was greater under perennial than annual crops and consequently losses tended to be lower with perennial crops. Globally, N recovery was rather low for all crops compared to achievable efficiency with conventional crops. It could probably be increased by improvements in cultural practices (timing and form of fertiliser application). The effect of these cultural practices and the partition of the N losses between volatilisation, denitrification and leaching deserve further investigations.

6 Discussion générale, conclusions et perspectives

6.1 Synthèse des résultats obtenus

L'objectif général de la thèse était de quantifier les bilans d'eau, d'azote et de carbone pour différentes cultures candidates à la production de biocarburant de deuxième génération et différentes pratiques culturales. Ces cultures appartiennent à trois grandes catégories : cultures pérennes (miscanthus et switchgrass), pluriannuelles (fétuque et luzerne) et annuelles (sorgho et triticale). Les pratiques culturales étudiées sont le mode de récolte pour les cultures pérennes et la fertilisation azotée pour toutes les cultures. Le travail s'est appuyé sur un dispositif expérimental de long terme mis en place en 2006 à Estrées-Mons, dans la Somme : le dispositif « Biomasse & Environnement ». Des mesures variées ont été réalisées sur ce dispositif entre avril 2006 à mars 2014 afin de couvrir une large gamme d'impacts environnementaux relatifs à l'eau, l'azote et le carbone.

6.1.1 Bilan hydrique

Les stocks d'eau du sol du dispositif B&E ont été mesurés en continu sur 0-210 cm pendant sept ans (Chap. 2). En période estivale, ils montrent une forte variabilité entre années et entre espèces. Le déficit hydrique (différence entre le stock d'eau à la capacité au champ et le stock d'eau mesuré) maximal observé pendant la saison de croissance est en général plus élevé pour les cultures pluriannuelles que pour les pérennes et les annuelles, malgré une production de biomasse plus faible pour les pluriannuelles que pour les pérennes. Il est également d'autant plus important que la balance hydrique (différence entre pluie et ETP) est négative.

Si le prélèvement d'eau est en général plus important dans les couches de sol superficielles que dans les couches profondes, des différences apparaissent entre espèces. Ainsi, les cultures pérennes et pluriannuelles prélèvent en profondeur (150-210 cm) une plus grande proportion de l'eau disponible que les cultures annuelles. Inversement, elles consomment en moyenne moins d'eau dans la couche superficielle (0-30 cm) que les autres espèces. La variabilité des densités racinaires entre espèces explique en partie ces différences de prélèvements d'eau. En effet, nous avons observé une relation curvilinéaire asymptotique entre le prélèvement d'eau dans une couche de sol donnée et la densité racinaire dans cette couche. Pour décrire cette relation, nous avons utilisé un modèle dont les paramètres varient en fonction de l'espèce, car la capacité de prélèvement de la plante dépend non seulement de la densité racinaire mais aussi de l'espèce. Ainsi, pour les faibles densités racinaires (en profondeur) le prélèvement d'eau a tendance à être plus élevé pour la fétuque et la luzerne, à densité racinaire équivalente, que pour les autres espèces.

A l'automne (début novembre), le déficit hydrique du sol est plus marqué pour les cultures pérennes (au moins en N+) que pour les annuelles, ce qui s'explique par une plus grande consommation d'eau par les pérennes en fin de saison. Les mesures gravimétriques effectuées deux fois par an (début novembre et mi-mars) ont été utilisées pour quantifier le drainage avec le modèle STICS (Chap. 3). Ce drainage est particulièrement variable entre situations traitements × années (0 à 227 mm), les variations entre années étant en grande partie dues à la variabilité climatique. En moyenne sur 7 ans, le drainage est plus élevé pour les cultures annuelles (133 mm an^{-1}) que pour les pluriannuelles (64 mm an^{-1}), sans effet significatif de la fertilisation azotée. Il est intermédiaire pour les cultures pérennes (56 à 137 mm an^{-1}) et dépend de l'espèce et de la fertilisation azotée. Une forte corrélation négative a été observée sur la période 2007-2013 entre la production de biomasse et le drainage, pour les cultures pérennes et les cultures annuelles. Toutefois, le drainage sous les cultures pluriannuelles est bien plus faible que sous les annuelles malgré un niveau de production similaire. Ceci indique que les cultures pluriannuelles ont une plus faible efficience d'utilisation de l'eau (rapport entre la production de biomasse et l'évapotranspiration) que les autres cultures.

6.1.2 Pertes de nitrate et devenir de l'azote de l'engrais

Les stocks d'azote minéral mesurés deux fois par an (début novembre et mi-mars) ont été utilisés pour évaluer les pertes en nitrate avec le modèle STICS (Chap. 3). La concentration en nitrate de l'eau drainée est généralement très faible (moins de 10 mg l^{-1} dans 80% des situations traitements × années). Ces faibles concentrations ont probablement été favorisées par le contexte pédoclimatique (sol profond et pluviométrie hivernale modérée). Les concentrations sont souvent plus faibles pour les cultures pérennes que pour les autres, sauf lors de la première année de mesure pour le miscanthus (en 2007-08, soit un an et demi après l'implantation) où elles atteignent 83 mg l^{-1}. La comparaison avec les teneurs mesurées dans le sous-sol en 2011 suggère cependant que la plus grande part de l'azote perdu après l'implantation du miscanthus a été par la suite prélevée par la plante en profondeur. En moyenne sur 7 ans, la concentration pondérée en nitrate varie entre 2 et 23 mg l^{-1} pour les pérennes, entre 3 et 17 mg l^{-1} pour les pluriannuelles et entre 6 et 16 mg l^{-1} pour les annuelles. La fertilisation azotée des cultures pérennes augmente la concentration nitrique mais pas celle des autres espèces. Ces concentrations correspondent à de très faibles quantités d'azote lixivié : 2 kg N ha^{-1} an^{-1} en moyenne pour tous les traitements (entre 1 et 5 kg N ha^{-1} an^{-1}).

De l'azote marqué ^{15}N a été apporté sur des sous-parcelles pendant 4 à 5 années consécutives. Nous avons mesuré le cumul d'azote marqué exporté par les récoltes pendant cette période, ainsi que l'azote restant dans le système sol-plante à la fin de la période : azote stocké dans les parties non récoltées des plantes, dans les résidus de cultures et dans le sol (Chap. 5). La proportion d'azote ^{15}N retrouvée dans les parties aériennes à la récolte (en cumulé sur la période de 4 à 5 ans) varie de 13 à 17% pour les cultures pérennes en coupe tardive, de 23 à 34% pour les pérennes en coupe précoce, de 23 à 38% pour les pluriannuelles et de 34 à 39% pour les annuelles. A l'issu de la période d'apport, les cultures pérennes avaient des quantités d'azote importantes dans leurs organes souterrains. L'azote ^{15}N retrouvé dans ces organes correspond à entre 8 et 17% de l'azote apporté (12% en moyenne). Une proportion significative de l'engrais marqué a également été retrouvée dans les résidus des cultures pérennes (4% de l'azote apporté en moyenne) alors que la quantité d'engrais marqué contenu dans les résidus est pratiquement négligeable pour les autres espèces. L'azote ^{15}N retrouvé dans le sol correspond en moyenne à 32% des apports pour les pérennes, 28% pour les pluriannuelles et 19% pour les annuelles. Le bilan complet de ^{15}N (fraction de l'engrais retrouvée dans le système sol-plante) était en moyenne de 69% pour les pérennes, 61% pour les pluriannuelles et 56% pour les annuelles, ce qui suggère des pertes d'azote importantes. Etant donné que les pertes d'azote par lixiviation ont été très faibles sur la période considérée, nous supposons que le défaut de bilan correspond essentiellement aux pertes gazeuses, par volatilisation (NH_3) et par dénitrification dans une moindre mesure. L'efficience de l'azote est globalement plus élevée pour les cultures pérennes qui stockent une fraction importante de l'azote de l'engrais dans leurs organes souterrains et dans la matière organique du sol.

6.1.3 Evolution des stocks de carbone du sol

Les stocks de carbone du sol ont été mesurés en avril 2006 avant l'installation du dispositif, puis en mars 2011 pour les cultures pérennes et mars 2012 pour les autres espèces (Chap. 4). Entre 2006 et 2011, les concentrations en C organique du sol sous les cultures pérennes ont augmenté dans la couche de surface (0-5 cm) et légèrement décliné en-dessous. Les variations de la composition isotopique du carbone ($\delta^{13}C$) montrent que les entrées de C sont majoritairement localisées dans la couche 0-18 cm. Au contraire, les concentrations en C ont augmenté au cours du temps sous les cultures pluriannuelles pour l'ensemble de l'ancienne couche labourée (environ 0-33 cm). Les stocks de C dans l'ancienne couche labourée ont augmenté de 0.93 ± 0.28 t C ha^{-1} an^{-1} sous les cultures pluriannuelles alors qu'ils n'ont pas significativement évolué pour les autres cultures. La fertilisation azotée n'a pas eu

d'effet significatif sur les stocks de C. L'accumulation de C « nouveau » est plus élevée sous les cultures pluriannuelles que sous les cultures pérennes (1.50 contre 0.58 t C ha^{-1} an^{-1} respectivement), alors que la vitesse de diminution du stock de C « ancien » est semblable dans les deux cas. Ceci montre que l'augmentation des stocks de C pour les cultures pluriannuelles est due à une augmentation des entrées de C plutôt qu'à une diminution de la vitesse de minéralisation du C organique du sol.

6.2 Discussion générale et conclusions

6.2.1 Intérêts et limites de la démarche expérimentale

Le principal intérêt de notre démarche est l'évaluation au champ, dans les mêmes conditions de production, d'une large gamme de cultures candidates à la production de biocarburant 2G. Cette prise en compte de la diversité des cultures candidates est particulièrement originale puisque la majorité des travaux existants portent sur une seule espèce à la fois. Ainsi, dans le cas de l'impact sur les stocks de C du sol, sur 21 publications recensées incluant miscanthus ou switchgrass, 15 ne s'intéressent qu'à une seule espèce. Toujours parmi ces 21 publications, une seule (Chimento *et al.*, 2014) compare différents types de cultures pérennes (miscanthus, switchgrass, TCR) ainsi qu'une culture annuelle (maïs). Concernant les pertes d'azote par lixiviation, une grande proportion des travaux sur les cultures lignocellulosiques sont également mono-spécifiques (Christian and Riche, 1998; Davis *et al.*, 2014; Lesur *et al.*, 2014). Seules les études de Pugesgaard *et al.* (2014) et Smith *et al.* (2013) prennent en compte une gamme de cultures comparable à la nôtre. La faible diversité des cultures prise en compte par chaque étude rend difficile la comparaison des cultures du fait de la variabilité des conditions de production entre études.

Nous avons également pris en compte différentes modalités liées aux pratiques culturales. Si la fertilisation azotée est un facteur assez souvent étudié dans les travaux sur les impacts relatifs à l'azote, il l'est en revanche très peu dans ceux portant sur les évolutions de stocks de C. L'étude de deux modalités de récolte pour les cultures pérennes est également très originale puisqu'à notre connaissance, l'effet du mode de récolte n'est jamais pris en compte pour le miscanthus (toutes les études sont faites pour des récoltes tardives) et très rarement pour le switchgrass (ex. : Pedroso *et al.*, 2014). Une récolte précoce a toutefois des conséquences importantes sur le fonctionnement de la culture, et en particulier sur le cycle de l'azote, comme le montrent nos résultats sur le suivi ^{15}N.

A l'inverse, une limite de notre travail est la prise en compte d'une seule situation pédoclimatique. Cet aspect a une importance particulière pour l'évaluation des impacts sur le

drainage et les pertes d'azote par lixiviation. En effet, on peut penser que dans notre situation avec un sol particulièrement profond et une réserve utile importante, les différences de potentiel d'enracinement et de consommation en eau entre espèces s'expriment pleinement. Cela favorise probablement les écarts importants observés sur le drainage. Par contre, ces conditions constituent une situation à faible risque pour les pertes de nitrate (Beaudoin *et al.*, 2005). Il serait donc intéressant d'effectuer la même comparaison dans un sol plus superficiel.

La durée de suivi des flux d'eau et de lixiviation (7 ans) apparaît plutôt longue au regard des travaux préexistants. Elle nous permet d'intégrer la variabilité climatique avec des années sèches comme 2009 et d'autres plus humides comme 2013, ainsi que les phases d'implantation et de destruction des cultures pluriannuelles. Le manque de données sur l'année d'implantation des cultures pérennes (2006) est par contre regrettable. Concernant le carbone, la durée prise en compte (5-6 ans) se situe plutôt dans la moyenne basse des travaux publiés. En effet, les évolutions de stocks de C du sol s'opèrent lentement et les changements sont souvent difficiles à détecter en-dessous d'une durée de 4 ou 5 ans.

Cette durée relativement courte pour le suivi des stocks de C a été compensée en partie par une méthodologie très rigoureuse sur la mesure des évolutions de stocks. En effet, nous avons réalisé un suivi synchronique avec un point initial détaillé et une mesure précise des stocks à masse de sol équivalente. Ces deux particularités méthodologiques, qui font souvent défaut dans les travaux publiés, nous permettent d'avoir un bon degré de confiance sur les évolutions de stocks mesurées. Elles contribuent aussi à la précision du bilan ^{15}N (part stockée dans le sol).

Enfin, l'évaluation concomitante des bilans d'eau, d'azote et carbone sur le même dispositif expérimental permet d'intégrer une large gamme d'impacts environnementaux et ainsi de contribuer à une évaluation globale des systèmes étudiés. Les travaux de ce type sont rares dans la littérature (ex. : Davis *et al.*, 2014). A ce titre, la principale lacune de notre travail concerne les émissions de N_2O, qui jouent un rôle important dans le bilan GES. En fait des séries de données continues ont été acquises pour certains traitements et seront valorisées prochainement.

6.2.2 Quelles cultures pour les biocarburants de deuxième génération ?

Dans notre situation expérimentale, les performances environnementales des différentes espèces candidates varient en fonction des critères étudiés. Le drainage le plus élevé est obtenu avec les cultures annuelles et les plus faibles concentrations en nitrate avec le switchgrass. D'après le bilan ^{15}N, les cultures pérennes et en particulier le miscanthus en

coupe précoce présentent les pertes gazeuses azotées les moins importantes. Par contre, seules les cultures pluriannuelles ont permis d'augmenter les stocks de carbone. Parmi les agrosystèmes étudiés, il n'y en a donc aucun qui puisse se démarquer pour l'ensemble des critères.

Cependant, si l'on tient compte également des critères de productivité et de besoins en intrants, on peut conclure que, dans notre contexte pédoclimatique, deux grands types de cultures candidates pourraient être particulièrement adaptés à la production de biocarburant 2G : les cultures pérennes d'un côté et la luzerne de l'autre.

En effet, les cultures pérennes, miscanthus et switchgrass, ont plusieurs atouts :

1) Elles allient forte production de biomasse (et potentiellement d'éthanol) et faible besoins en intrants (voir Chap. 1).
2) Elles permettent d'obtenir de faibles pertes d'azote par lixiviation, même si le risque est plus élevé lors des années suivant l'implantation. La capacité des cultures pérennes à limiter les pertes de nitrate par rapport aux cultures annuelles est confirmée par plusieurs autres travaux déjà publiés (McIsaac *et al.*, 2010; Smith *et al.*, 2013; Lesur *et al.*, 2014). Le risque de pertes à la suite de l'implantation semble plus important pour le miscanthus que pour le switchgrass, dont l'implantation est plus rapide. Il pourrait probablement être limité en réduisant la disponibilité en azote minéral à l'implantation (choix du précédent, mise en place d'une culture intermédiaire à l'automne précédant), en proscrivant la fertilisation azotée en première, voire en deuxième année, et en s'assurant de la réussite de l'implantation.
3) Elles présentent une bonne efficience d'utilisation de l'engrais azoté, si l'on prend en compte la part stockée dans les organes souterrains et dans la matière organique du sol. Ce stockage de l'azote permet sa réutilisation par la plante les années suivantes. Les pertes gazeuses azotées estimées par le bilan ^{15}N sont ainsi plus faibles pour ces cultures que pour les autres espèces étudiées.
4) Elles présentent une très bonne efficience d'utilisation de l'eau. La réduction de drainage qu'elles occasionnent est compensée par une forte production. Cependant il est clair qu'il faudra bien gérer ces cultures à l'échelle du bassin hydrologique, afin de trouver le meilleur compromis entre drainage et pertes de nitrate.

En revanche, contrairement à ce qui est souvent annoncé dans la littérature (ex. : Poeplau and Don, 2014), aucun stockage de carbone (ni déstockage) n'a été observé pour ces cultures sur notre dispositif expérimental, cinq années après leur implantation.

La luzerne présente également plusieurs avantages :

1) Grâce à sa capacité de fixation symbiotique de l'azote atmosphérique, elle est cultivée sans fertilisation azotée.
2) Sa production pourrait avoir une double finalité : protéines pour l'alimentation animale et lignocellulose pour l'énergie, dans le contexte émergeant des bioraffineries.
3) Cette culture pourrait s'insérer assez facilement dans les systèmes de grandes cultures actuels. Par rapport au système étudié sur le dispositif B&E, sa productivité pourrait être accrue en l'implantant en été (par exemple après une récolte de céréale) plutôt qu'en début de printemps.
4) La luzerne valorise bien l'azote du sol et a un système racinaire important, ce qui limite les pertes d'azote par lixiviation. Le risque de pertes de nitrate peut par contre augmenter après la destruction de la culture, du fait d'une minéralisation accrue. Cette minéralisation supplémentaire ne semble cependant pas conduire à des pertes de nitrate importantes et constitue de plus une source d'azote additionnelle pour les cultures suivantes (Justes *et al.*, 2001; Benoit *et al.*, 2014), pouvant permettre une réduction de la fertilisation azotée.
5) Elle peut contribuer à stocker du C dans le sol, par rapport à un système de grandes cultures annuelles. Notre dispositif expérimental ne nous permet pas de différencier la part du stockage provenant de la luzerne de celle provenant de la fétuque. Il est toutefois probable que les deux espèces y aient contribué, étant donnée la vitesse de stockage importante (0.93 t C ha^{-1} an^{-1}). Ce stockage de C peut constituer un atout important au niveau du bilan GES.

Cependant, la luzerne, en rotation avec la fétuque, conduit à un drainage réduit d'environ 70 mm an^{-1} par rapport aux cultures annuelles, à niveau de production équivalente.

6.2.3 Quel effet des pratiques culturales sur les bilans environnementaux ?

La récolte des cultures pérennes à l'automne (coupe précoce), en évitant une partie de la phase de mise en réserve ainsi que la chute des feuilles pour le miscanthus, permet de maximiser le potentiel de rendement (et donc de production d'éthanol), mais rend aussi ces cultures plus dépendantes de la fertilisation azotée qu'en coupe tardive. En effet, les exportations d'azote sont plus élevées en coupe précoce et les réserves d'azote dans les organes souterrains plus faibles : le recyclage de l'azote permis par les phénomènes de translocation est en partie tronqué. L'effet du mode de récolte sur les besoins en fertilisation azotée est particulièrement marqué pour le miscanthus. Ainsi, sur le dispositif B&E en 2013, soit 7 ans après l'implantation, le rendement en coupe tardive était encore équivalent avec ou

sans fertilisation azotée (environ 17 t MS ha^{-1}) alors qu'un écart de 10 tonnes était observé en coupe précoce (18 et 28 t MS ha^{-1}, respectivement en N- et N+). Les plus faibles pertes d'azote mesurées pour le miscanthus en coupe précoce avec le bilan ^{15}N, par rapport à la coupe tardive, pourraient être liées en partie à ce besoin en azote exogène plus élevé.

Un autre effet important du mode de récolte pour le miscanthus est lié à l'exportation des feuilles en coupe précoce, qui sinon, tombent pendant l'hiver. Ces feuilles qui ont une vitesse de décomposition assez lente forment un mulch épais et permanent à la surface du sol (Amougou *et al.*, 2012). Cinq ans après l'implantation, cet apport supplémentaire au sol en coupe tardive n'a pas permis d'obtenir un stockage de carbone dans le sol plus élevé, mais cela pourrait être différent à long terme. Ce mulch a de plus l'avantage d'empêcher pratiquement toute levée d'adventice. Par contre, il modifie les conditions du sol en surface, avec notamment une plus forte humidité au printemps, et pourrait ainsi favoriser la dénitrification.

Nous avons observé un faible effet de la fertilisation azotée sur les pertes d'azote par lixiviation, sauf pour le miscanthus lorsque des pertes élevées ont été mesurées en 2007-08. En dehors de cette situation, il semble que la fertilisation, y compris en N+, n'ait pas été fortement excédentaire par rapport aux besoins des cultures et n'a donc pas entraîné de pertes supplémentaires de nitrate. La lixiviation n'a pas pu être quantifiée directement pour le miscanthus en coupe tardive fertilisée, du fait de l'absence de mesure d'azote minéral du sol à l'automne. Cependant, les stocks d'azote minéral du sol mesurés pour ce traitement en fin d'hiver étaient équivalents à ceux observés en coupe précoce, ce qui laisse penser que les pertes par lixiviation devaient être similaires.

De plus, la fertilisation azotée a eu un effet sur le drainage, via son impact sur la production de biomasse et donc sur l'évapotranspiration. Par contre, elle n'a pas eu d'effet sur le stockage de carbone. Concernant les pertes d'azote issues de l'engrais, on peut comparer les deux niveaux de fertilisation azotée pour les cultures annuelles et pluriannuelles. Les pertes mesurées, en pourcentage de l'azote apporté, étaient équivalentes en N- et N+. On peut donc penser que les pertes gazeuses par volatilisation et dénitrification augmentent de manière à peu près proportionnelle avec la fertilisation.

6.2.4 La modélisation comme outil de généralisation

Comme décrit précédemment, la démarche expérimentale utilisée ici est limitée par la prise en compte d'un seul contexte pédoclimatique et par l'étude de situations types, pas forcément optimisées (fertilisation, rotations de cultures, etc.). Les réseaux expérimentaux

peuvent constituer un complément intéressant pour intégrer la diversité des conditions pédoclimatiques. Le réseau mis en place dans le cadre du projet ANR Regix (Cadoux *et al.*, 2010), et poursuivi pour les cultures pérennes et le sorgho dans le cadre du projet CasDar Lignoguide (RMT Biomasse Energie Environnement et Territoire, 2013), a permis d'obtenir des données sur la production de biomasse et les exportations d'éléments minéraux. Il est cependant difficile de réaliser des mesures fiables sur les impacts environnementaux dans de tels réseaux. Une approche complémentaire peut consister à étudier des situations agricoles réelles (ex. : Lesur *et al.*, 2014) mais celle-ci restent rares pour les cultures étudiées.

Au-delà de l'expérimentation, la modélisation sol-plante est un outil particulièrement intéressant pour généraliser la comparaison des cultures candidates à d'autres contextes pédoclimatiques et tester différents scénarios de production. Suite à la thèse de L. Strullu sur le fonctionnement du miscanthus (Strullu *et al.*, 2011; Strullu *et al.*, 2013), le modèle STICS a été adapté et paramétré pour simuler cette culture (Strullu *et al.*, 2014a). Dans le cadre du projet européen LogistEC, nous avons amélioré le modèle sur plusieurs aspects et nous l'avons testé sur les données du dispositif B&E, du réseau Lignoguide et d'un essai de long terme (20 ans) situé à Rothamsted, en Angleterre (Strullu *et al.*, 2015). Le modèle permet de reproduire de manière satisfaisante l'effet du pédoclimat, de la disponibilité en eau, de la fertilisation azotée et du mode de récolte sur la production et les exportations d'azote. Il est également capable de simuler correctement la phase d'implantation de la culture. D'autre part, les stocks d'eau et d'azote minéral du sol sont aussi correctement simulés. Ce modèle pourra donc être utilisé pour tester l'effet de différents scénarios de production sur le rendement et les impacts environnementaux de la culture. Un premier travail de comparaison de scénarios a ainsi été effectué dans le cadre du projet Futurol (Strullu *et al.*, 2014b).

6.3 Perspectives

Plusieurs perspectives peuvent être envisagées, tout d'abord au niveau expérimental, notamment à l'échelle du dispositif B&E, mais aussi au niveau de la modélisation.

L'évaluation complète de l'impact des systèmes étudiés sur les stocks de C du sol nécessite l'acquisition de données à long terme. Les mesures de stocks de C sur le dispositif B&E devront donc être renouvelées dans les années à venir. Cela permettra de tester l'apparition ou non d'effets à long terme, notamment sur l'impact du mode de récolte pour le miscanthus ou de la fertilisation azotée. Ces mesures pourront également être utilisées pour déterminer l'évolution des stocks d'azote total. En effet, les balances azotées (différence entre

les entrées et les exportations d'azote) très contrastées entre les différents traitements permettent de s'attendre à des évolutions variables des stocks d'azote à long terme.

Plus généralement, l'impact des cultures pérennes comme le miscanthus ou le switchgrass sur les stocks de carbone apparaît très incertain d'après les données disponibles dans la littérature, les évolutions de stocks mesurées étant extrêmement variables. Une méta-analyse des données publiées, prenant en compte des critères de qualité sur les méthodes de mesure, pourrait peut-être permettre de préciser des tendances.

D'autre part, les émissions de N_2O sont un point crucial pour préciser le bilan GES des agrosystèmes. Des mesures en continu avec des chambres automatiques ont été effectuées pendant plusieurs années pour certains traitements du dispositif B&E. Ces mesures pourraient être combinées avec l'évaluation des émissions de GES liées aux intrants (fertilisants, fioul, etc.) et aux évolutions de stocks de C pour réaliser un bilan GES complet à l'échelle de la parcelle.

Même si les cultures pérennes comme le miscanthus ou le switchgrass peuvent être productives pendant au moins une vingtaine d'années, leur destruction au bout d'un certain temps sera nécessaire, soit pour réimplanter une nouvelle culture pérenne, soit pour revenir à la production de cultures annuelles. Cette phase de destruction, avec le retour au sol et la décomposition d'importantes quantités de résidus (litière et organes souterrains) doit aussi faire l'objet d'une évaluation environnementale, afin de caractériser l'ensemble du cycle de la culture. Un premier travail de ce type a été réalisé dans le cadre du retournement d'une ancienne parcelle de miscanthus (20 ans) à Grignon (Dufossé *et al.*, 2014). La destruction prochaine du switchgrass (automne 2015) sur le site d'Estrées-Mons du SOERE ACBB[9] constitue également une opportunité pour en étudier les conséquences.

Le travail initié sur l'évaluation des cultures candidates par modélisation devra être poursuivi. Pour cela, il s'agira d'une part d'intégrer un plus grand nombre d'espèces, et d'autre part d'élargir la gamme des impacts simulés. Pour le premier point, un premier paramétrage de STICS a été réalisé sur le triticale et des projets sont en cours de montage pour le switchgrass et la luzerne. Pour le second point, les données du dispositif B&E pourront être utilisées pour tester le modèle sur les évolutions de stocks de C et les émissions de N_2O. Le modèle pourrait ainsi être utilisé pour évaluer les cultures candidates dans différents scénarios de production faisant varier le contexte pédoclimatique et les pratiques culturales.

[9] Système d'Observation et d'Expérimentation pour la Recherche en Environnement – Agroécosystèmes Cycles Biogéochimiques et Biodiversité.

Enfin, la durabilité de ces cultures sera également déterminée par les modalités de leur insertion dans les systèmes de culture actuels et dans les territoires. Les cultures pérennes ou la luzerne pourraient représenter une opportunité intéressante pour diversifier les systèmes de grande culture (effet de « coupure » sur le cycle des adventices et des maladies, fourniture d'azote, etc.), mais aussi pour répondre à certaines contraintes locales comme la protection de la ressource en eau. Leur insertion devra toutefois être raisonnée en fonction des systèmes de culture existants, des contraintes du territoire et des celles liées à leur valorisation. Il paraît donc important de poursuivre des travaux de recherche intégrant ces différentes échelles (culture, système de culture, bassin d'approvisionnement), en complément des évaluations à l'échelle des filières.

Références bibliographiques

Abraha, M., Chen, J., Chu, H., Zenone, T., John, R., Su, Y.-J., Hamilton, S.K., Robertson, G.P., 2015. Evapotranspiration of annual and perennial biofuel crops in a variable climate. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12239

ADEME, 2010. *Life cycle assessments applied to first generation biofuels used in France*. 229 p.

Amigues, J.P., Debaeke, B., Itier, B., Lemaire, G., Seguin, B., Tardieu, F., Thomas, A. (éditeurs), 2006. *Sécheresse et agriculture. Réduire la vulnérabilité de l'agriculture à un risque accru de manque d'eau*. Expertise scientifique collective, synthèse du rapport. INRA, Paris (France), 72 p.

Amougou, N., Bertrand, I., Cadoux, S., Recous, S., 2012. *Miscanthus* × *giganteus* leaf senescence, decomposition and C and N inputs to soil. *Global Change Biology Bioenergy* 4, 698-707.

Amougou, N., Bertrand, I., Machet, J.M., Recous, S., 2011. Quality and decomposition in soil of rhizome, root and senescent leaf from *Miscanthus* × *giganteus*, as affected by harvest date and N fertilization. *Plant and Soil* 338, 83-97.

Anderson-Teixeira, K., Masters, M., Black, C., Zeri, M., Hussain, M., Bernacchi, C., DeLucia, E., 2013. Altered belowground carbon cycling following land-use change to perennial bioenergy crops. *Ecosystems* 16, 508-520.

Anderson-Teixeira, K.J., Davis, S.C., Masters, M.D., Delucia, E.H., 2009. Changes in soil organic carbon under biofuel crops. *Global Change Biology Bioenergy* 1, 75-96.

Andriulo, A., Guerif, J., Mary, B., 1999. Evolution of soil carbon with various cropping sequences on the rolling pampas. Determination of carbon origin using variations in natural C-13 abundance. *Agronomie* 19, 349-364.

Anglade, J., Billen, G., Garnier, J., 2015. Relationships for estimating N_2 fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. *Ecosphere* 6, art. 37.

Arrouays, D., Deslais, W., Badeau, V., 2001. The carbon content of topsoil and its geographical distribution in France. *Soil Use and Management* 17, 7-11.

Balabane, M., Balesdent, J., 1992. Input of fertilizer-derived labeled-N to soil organic-matter during a growing-season of maize in the field. *Soil Biology & Biochemistry* 24, 89-96.

Beale, C.V., Morison, J.I.L., Long, S.P., 1999. Water use efficiency of C 4 perennial grasses in a temperate climate. *Agricultural and Forest Meteorology* 96, 103-115.

Beaudoin, N., Denys, D., Muller, J.C., Monbrun, M.D., Ledain, C., 1992. Influence d'une culture de luzerne sur le lessivage du nitrate dans les sols de Champagne crayeuse. *Fourrages* 129, 45-57.

Beaudoin, N., Launay, M., Sauboua, E., Ponsardin, G., Mary, B., 2008. Evaluation of the soil crop model STICS over 8 years against the "on farm" database of Bruyeres catchment. *European Journal of Agronomy* 29, 46-57.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: effect of farming practices, soils and crop rotations. *Agriculture, Ecosystems & Environment*. 111, 292-310.

Benoit, M., Garnier, J., Anglade, J., Billen, G., 2014. Nitrate leaching from organic and conventional arable crop farms in the Seine Basin (France). *Nutrient Cycling in Agroecosystems* 100, 285-299.

Bentsen, N., Felby, C., 2012. Biomass for energy in the European Union - a review of bioenergy resource assessments. *Biotechnology for Biofuels* 5, 25.

Bessou, C., Ferchaud, F., Gabrielle, B., Mary, B., 2011. Biofuels, greenhouse gases and climate change. A review. *Agronomy for Sustainable Development* 31, 1-79.

Beudez, N., Doussan, C., Lefeuve-Mesgouez, G., Mesgouez, A., 2013. *Influence of three root spatial arrangement on soil water flow and uptake. Results from an explicit and an equivalent, upscaled, model*. In: Romano, N., Durso, G., Severino, G., Chirico, G.B., Palladino, M. (Eds.), Four decades of progress in monitoring and modeling of processes in the soil-plant-atmosphere system: applications and challenges. Elsevier Science Bv, Amsterdam, p. 37-46.

Bonin, C.L., Lal, R., 2014. Aboveground productivity and soil carbon storage of biofuel crops in Ohio. *Global Change Biology Bioenergy* 6, 67-75.

Brisson, N., Gary, C., Justes, E., Roche, R., Mary, B., Ripoche, D., Zimmer, D., Sierra, J., Bertuzzi, P., Burger, P., Bussiere, F., Cabidoche, Y.M., Cellier, P., Debaeke, P., Gaudillere, J.P., Henault, C., Maraux, F., Seguin, B., Sinoquet, H., 2003. An overview of the crop model STICS. *European Journal of Agronomy* 18, 309-332.

Brisson, N., Launay, M., Mary, B., Beaudoin, N., 2008. *Conceptual Basis, Formalisations and Parameterization of the Stics Crop Model*. Quae, Versailles, France, 297 p.

Bristow, A.W., Ryden, J.C., Whitehead, D.C., 1987. The fate at several time intervals of N-15-labeled ammonium-nitrate applied to an established grass sward. *Journal of Soil Science* 38, 245-254.

Butterbach-Bahl, K., Nemitz, E., Zaehle, S., Billen, G., Boeckx, P., Erisman, J.W., Garnier, J., Upstill-Goddard, R., Kreuzer, M., Oenema, O., Reis, S., Schaap, M., Simpson, D., de Vries, W., Winiwarter, W., Sutton, M., 2011. *Nitrogen as a threat to the European greehouse balance*. In: Sutton, M., Howard, C., Erisman, J.W., Billen, G., Bleeker, A., Grennfelt, P., Van Grinsven, H., Grizzetti, B. (Eds.), The European Nitrogen Assessment. Cambridge University Press, Cambridge, U.K., p. 434-462.

Cadoux, S., Briand, S., Chabbert, B., Besnard, A., Félix, I., Savouré, M.L., Marsac, S., Preudhomme, M., Labalette, F., Boizard, H., 2010. *Biomass productivity of different energy crops under French conditions*. Results of the « REGIX » experimental network. 18th European Biomass Conference, Lyon, France.

Cadoux, S., Ferchaud, F., Demay, C., Boizard, H., Machet, J.M., Fourdinier, E., Preudhomme, M., Chabbert, B., Gosse, G., Mary, B., 2014. Implications of productivity and nutrient requirements on greenhouse gas balance of annual and perennial bioenergy crops. *Global Change Biology Bioenergy* 6, 425-438.

Cadoux, S., Riche, A.B., Yates, N.E., Machet, J.-M., 2012. Nutrient requirements of *Miscanthus* × *giganteus*: Conclusions from a review of published studies. *Biomass and Bioenergy* 38, 14-22.

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology* 162, 145-173.

Campbell, C.A., Lafond, G.P., Zentner, R.P., Jame, Y.W., 1994. Nitrate leaching in a Udic Haploboroll as influenced by fertilization and legumes. *Journal of Environmental Quality* 23, 195-201.

Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio* 31, 132-140.

Cattaneo, F., Barbanti, L., Gioacchini, P., Ciavatta, C., Marzadori, C., 2014. C-13 abundance shows effective soil carbon sequestration in Miscanthus and giant reed compared to arable crops under Mediterranean climate. *Biology and Fertility of Soils* 50, 1121-1128.

Cherubini, F., 2010. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Conversion and Management* 51, 1412-1421.

Chimento, C., Almagro, M., Amaducci, S., 2014. Carbon sequestration potential in perennial bioenergy crops: the importance of organic matter inputs and its physical protection. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12232

Chopart, J.-L., Rodrigues, S., Carvalho de Azevedo, M., Conti Medina, C., 2008. Estimating sugarcane root length density through root mapping and orientation modelling. *Plant and Soil* 313, 101-112.

Chopart, J.L., Siband, P., 1999. Development and validation of a model to describe root length density of maize from root counts on soil profiles. *Plant and Soil* 214, 61-74.

Christian, D.G., Poulton, P.R., Riche, A.B., Yates, N.E., Todd, A.D., 2006. The recovery over several seasons of 15N-labelled fertilizer applied to *Miscanthus* × *giganteus* ranging from 1 to 3 years old. *Biomass and Bioenergy* 30, 125-133.

Christian, D.G., Riche, A.B., 1998. Nitrate leaching losses under Miscanthus grass planted on a silty clay loam soil. *Soil Use and Management* 14, 131-135.

Chum, H., Faaij, A., Moreira, J., Berndes, G., Dhamija, P., Dong, H., Gabrielle, B., Goss Eng, A., Lucht, W., Mapako, M., Masera Cerutti, O., McIntyre, T., Minowa, T., Pingoud, K., 2011. *Bioenergy*. In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Seyboth, K., Matschoss, P., Kadner, S., Zwickel, T., Eickemeier, P., Hansen, G., Schlömer, S., von Stechow, C. (Eds.), IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 209-332.

Collins, H.P., Smith, J.L., Fransen, S., Alva, A.K., Kruger, C.E., Granatstein, D.M., 2010. Carbon sequestration under irrigated switchgrass (*Panicum virgatum* L.) production. *Soil Science Society of America Journal* 74, 2049-2058.

Conant, R.T., Paustian, K., Elliott, E.T., 2001. Grassland management and conversion into grassland: effects on soil carbon. *Ecological Applications* 11, 343-355.

Constantin, J., Beaudoin, N., Launay, M., Duval, J., Mary, B., 2012. Long-term nitrogen dynamics in various catch crop scenarios: test and simulations with STICS model in a temperate climate. *Agriculture, Ecosystems & Environment* 147, 36-46.

Constantin, J., Mary, B., Laurent, F., Aubrion, G., Fontaine, A., Kerveillant, P., Beaudoin, N., 2010. Effects of catch crops, no till and reduced nitrogen fertilization on nitrogen

leaching and balance in three long-term experiments. *Agriculture, Ecosystems & Environment* 135, 268-278.

Coucheney, E., Buis, S., Launay, M., Constantin, J., Mary, B., García de Cortázar-Atauri, I., Ripoche, D., Beaudoin, N., Ruget, F., Andrianarisoa, K.S., Le Bas, C., Justes, E., Léonard, J., 2015. Accuracy, robustness and behavior of the STICS soil–crop model for plant, water and nitrogen outputs: evaluation over a wide range of agro-environmental conditions in France. *Environmental Modelling & Software* 64, 177-190.

Crutzen, P.J., Mosier, A.R., Smith, K.A., Winiwarter, W., 2008. N_2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmospheric Chemistry and Physics* 8, 389-395.

Da Silva Perez, D., Briand, S., Leygue, J., Laboubée, C., Chabbert, B., Labalette, F., Cadoux, S., 2010. *Comparison of agricultural and forest biomass with the regard to biological processes for bioethanol production of second generation*. 18th European Biomass Conference, Lyon, France, p. 506-510.

Dardanelli, J.L., Bachmeier, O.A., Sereno, R., Gil, R., 1997. Rooting depth and soil water extraction patterns of different crops in a silty loam Haplustoll. *Field Crops Research* 54, 29-38.

Dardanelli, J.L., Ritchie, J.T., Calmon, M., Andriani, J.M., Collino, D.J., 2004. An empirical model for root water uptake. *Field Crops Research* 87, 59-71.

Davidson, E.A., 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience* 2, 659-662.

Davis-Knight, H.R., Weightman, R.M., 2007. *The potential of triticale as low input cereal for bioethanol production*. Project Report n° 434. Home-Grown Cereals Authority, Cambridge, U.K., 41 p.

Davis, M.P., David, M.B., Voigt, T.B., Mitchell, C.A., 2014. Effect of nitrogen addition on *Miscanthus* × *giganteus* yield, nitrogen losses, and soil organic matter across five sites. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12217

Dawson, K.P., Ryden, J.C., 1985. Uptake of fertilizer and soil-nitrogen by ryegrass swards during spring and mid-season. *Fertilizer Research* 6, 177-188.

de Cara, S., Goussebaïle, A., Grateau, R., Levert, F., Quemener, J., Vermont, B., 2012. *Revue critique des études évaluant l'effet des changements d'affectation des sols sur les bilans environnementaux des biocarburants*. INRA, ADEME, Angers, France, 96 p.

Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piraux, F., Cohan, J.-P., 2014. Long-term effect of contrasted tillage and crop management on soil carbon dynamics during 41 years. *Agriculture, Ecosystems & Environment* 188, 134-146.

Dimitriou, I., Mola-Yudego, B., Aronsson, P., 2012. Impact of willow short rotation coppice on water quality. *BioEnergy Research* 5, 537-545.

Dohleman, F.G., Heaton, E.A., Arundale, R.A., Long, S.P., 2012. Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus* × *giganteus* and Panicum virgatum across three growing seasons. *Global Change Biology Bioenergy* 4, 534-544.

Don, A., Osborne, B., Hastings, A., Skiba, U., Carter, M.S., Drewer, J., Flessa, H., Freibauer, A., Hyvonen, N., Jones, M.B., Lanigan, G.J., Mander, U., Monti, A., Djomo, S.N.,

Valentine, J., Walter, K., Zegada-Lizarazu, W., Zenone, T., 2011. Land-use change to bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon. *Global Change Biology Bioenergy* 4, 372-391.

Dondini, M., Hastings, A., Saiz, G., Jones, M.B., Smith, P., 2009. The potential of Miscanthus to sequester carbon in soils: comparing field measurements in Carlow, Ireland to model predictions. *Global Change Biology Bioenergy* 1, 413-425.

Dou, F.G., Hons, F.M., Ocumpaugh, W.R., Read, J.C., Hussey, M.A., Muir, J.P., 2013. Soil organic carbon pools under switchgrass grown as a bioenergy crop compared to other conventional crops. *Pedosphere* 23, 409-416.

Ducharne, A., Baubion, C., Beaudoin, N., Benoit, M., Billen, G., Brisson, N., Garnier, J., Kieken, H., Lebonvallet, S., Ledoux, E., Mary, B., Mignolet, C., Poux, X., Sauboua, E., Schott, C., Thery, S., Viennot, P., 2007. Long term prospective of the Seine River system: confronting climatic and direct anthropogenic changes. *Science of the Total Environment* 375, 292-311.

Dufossé, K., Drewer, J., Gabrielle, B., Drouet, J.L., 2014. Effects of a 20-year old *Miscanthus* × *giganteus* stand and its removal on soil characteristics and greenhouse gas emissions. *Biomass and Bioenergy* 69, 198-210.

Dusserre, J., Audebert, A., Radanielson, A., Chopart, J.L., 2009. Towards a simple generic model for upland rice root length density estimation from root intersections on soil profile. *Plant and Soil* 325, 277-288.

Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Canadian Journal of Soil Science* 75, 529-538.

Entz, M.W., Bullied, W.J., Forster, D.A., Gulden, R., Vessey, J.K., 2001. Extraction of subsoil nitrogen by alfalfa, alfalfa-wheat, and perennial grass systems. *Agronomy Journal* 93, 495-503.

EurObserv'ER, 2011. *Etat des énergies renouvelables en Europe*. Edition 2011. Observ'ER, Paris, France, 248 p.

EurObserv'ER, 2014. *Baromètre biocarburants*. Paris, France, p. 40-53.

European Parliament, 2015. *Parliament supports shift towards advanced biofuels*. (http://www.europarl.europa.eu/news/en/news-room/content/20150424IPR45730 /html/Parliament-supports-shift-towards-advanced-biofuels), vérifié le 03/05/15

FAO, 2008. *La situation mondiale de l'alimentation et de l'agriculture. Les biocarburants : perspectives, risques et opportunités*. FAO, Rome, Italie, 144 p.

Fargione, J., Hill, J., Tilman, D., Polasky, S., Hawthorne, P., 2008. Land clearing and the biofuel carbon debt. *Science* 319, 1235-1238.

Felten, D., Emmerling, C., 2012. Accumulation of Miscanthus-derived carbon in soils in relation to soil depth and duration of land use under commercial farming conditions. *Journal of Plant Nutrition and Soil Science* 175, 661-670.

Ferchaud, F., Vitte, G., Mary, B., 2012. *Belowground biomass and root distribution of two perennial biomass crops in a deep loamy soil*. 4th International Congress EUROSOIL 2012. ECSSS, Bari, Italy, p. 1983.

Finch, J.W., Riche, A., 2008. Soil water deficits and evaporation rates associated with Miscanthus in England. *Aspects of Applied Biology* 90, 295-302.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J., 2012. Soil Carbon Sequestration by Switchgrass and No-Till Maize Grown for Bioenergy. *BioEnergy Research* 5, 866-875.

Fox, R.H., Piekielek, W.P., Macneal, K.E., 1996. Estimating ammonia volatilization losses from urea fertilizers using a simplified micrometeorological sampler. *Soil Science Society of America Journal* 60, 596-601.

Gabrielle, B., Bamière, L., Caldes, N., De Cara, S., Decocq, G., Ferchaud, F., Loyce, C., Pelzer, E., Perez, Y., Wohlfahrt, J., Richard, G., 2014. Paving the way for sustainable bioenergy in Europe: technological options and research avenues for large-scale biomass feedstock supply. *Renewable and Sustainable Energy Reviews* 33, 11-25.

Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. *Bioscience* 53, 341-356.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889-892.

Gardner, J.B., Drinkwater, L.E., 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of N-15 field experiments. *Ecological Applications* 19, 2167-2184.

Garrigues, E., Doussan, C., Pierret, A., 2006. Water uptake by plant roots: I - Formation and propagation of a water extraction front in mature root systems as evidenced by 2D light transmission imaging. *Plant and Soil* 283, 83-98.

Garten, C.T., Smith, J.L., Tyler, D.D., Amonette, J.E., Bailey, V.L., Brice, D.J., Castro, H.F., Graham, R.L., Gunderson, C.A., Izaurralde, R.C., Jardine, P.M., Jastrow, J.D., Kerley, M.K., Matamala, R., Mayes, M.A., Metting, F.B., Miller, R.M., Moran, K.K., Post, W.M., Sands, R.D., Schadt, C.W., Phillips, J.R., Thomson, A.M., Vugteveen, T., West, T.O., Wullschleger, S.D., 2010. Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA. *Agriculture, Ecosystems & Environment* 136, 177-184.

Giacomini, S.J., Machet, J.M., Boizard, H., Recous, S., 2010. Dynamics and recovery of fertilizer N-15 in soil and winter wheat crop under minimum versus conventional tillage. *Soil & Tillage Research* 108, 51-58.

GIEC, 2013. *Résumé à l'intention des décideurs, Changements climatiques 2013 : Les éléments scientifiques*. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Contribution du Groupe de travail I au cinquième rapport d'évaluation du Groupe d'experts intergouvernemental sur l'évolution du climat. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1-24.

Glendining, M.J., Poulton, P.R., Powlson, D.S., Jenkinson, D.S., 1997. Fate of N-15-labelled fertilizer applied to spring barley grown on soils of contrasting nutrient. *Plant and Soil* 195, 83-98.

Glendining, M.J., Poulton, P.R., Powlson, D.S., Macdonald, A.J., Jenkinson, D.S., 2001. Availability of the residual nitrogen from a single application of N-15-labelled fertilizer to subsequent crops in a long-term continuous barley experiment. *Plant and Soil* 233, 231-239.

Gregory, P.J., 2006. Roots, rhizosphere and soil: the route to a better understanding of soil science? *European Journal of Soil Science* 57, 2-12.

Grizzetti, B., Bouraoui, F., Billen, G., Van Grinsven, H., Cardoso, A.C., Thieu, V., Garnier, J., Curtis, C., Howarth, R., Johnes, P., 2011. *Nitrogen as a threat to European water quality*. In: Sutton, M., Howard, C., Erisman, J.W., Billen, G., Bleeker, A., Grennfelt, P., Van Grinsven, H., Grizzetti, B. (Eds.), The European Nitrogen Assessment. Cambridge University Press, Cambridge, U.K., p. 379-404.

Hansen, E.M., Christensen, B.T., Jensen, L.S., Kristensen, K., 2004. Carbon sequestration in soil beneath long-term Miscanthus plantations as determined by ^{13}C abundance. *Biomass and Bioenergy* 26, 97-105.

Harrison, R., Webb, J., 2001. A review of the effect of N fertilizer type on gaseous emissions. *Advances in Agronomy* 73, 65-108.

Hauck, R.D., Bremner, J.M., 1976. Use of tracers for soil and fertilizer nitrogen research. *Advances in Agronomy* 28, 219-266.

Heaton, E.A., Dohleman, F.G., Miguez, A.F., Juvik, J.A., Lozovaya, V., Widholm, J., Zabotina, O.A., McIsaac, G.F., David, M.B., Voigt, T.B., Boersma, N.N., Long, S.P., 2010. *Miscanthus: A Promising Biomass Crop*. In: Kader, J.C., Delseny, M. (Eds.), Advances in Botanical Research, Vol 56. Academic Press Ltd-Elsevier Science Ltd, London, U.K., p. 75-137.

Hickman, G.C., Vanloocke, A., Dohleman, F.G., Bernacchi, C.J., 2010. A comparison of canopy evapotranspiration for maize and two perennial grasses identified as potential bioenergy crops. *Global Change Biology Bioenergy* 2, 157-168.

Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D., 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences* 103, 11206-11210.

Himken, M., Lammel, J., Neukirchen, D., CzypionkaKrause, U., Olfs, H.W., 1997. Cultivation of Miscanthus under west European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant and Soil* 189, 117-126.

Hoad, S.P., Russell, G., Lucas, M.E., Bingham, I.J., 2001. The management of wheat, barley, and oat root systems. *Advances in Agronomy* 74, 193-246.

International Energy Agency, 2011. *Technology roadmap: biofuels for transport*. Paris, France, 52 p.

IPCC, 2011. *Summary for Policymakers*. In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Seyboth, K., Matschoss, P., Kadner, S., Zwickel, T., Eickemeier, P., Hansen, G., Schlömer, S., von Stechow, C. (Eds.), IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1-24.

IPCC, 2014. *Climate Change 2014: Synthesis Report*. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, 151 p.

IUSS Working Group WRB, 2006. *World reference base for soil resources 2006*. World Soil Resources Reports 103. FAO, Rome, 145 p.

Jackson, R.B., Sperry, J.S., Dawson, T.E., 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* 5, 482-488.

Jenkinson, D.S., Fox, R.H., Rayner, J.H., 1985. Interactions between fertilizer nitrogen and soil-nitrogen - the so-called priming effect. *Journal of Soil Science* 36, 425-444.

Jenkinson, D.S., Poulton, P.R., Johnston, A.E., Powlson, D.S., 2004. Turnover of nitrogen-15-labeled fertilizer in old grassland. *Soil Science Society of America Journal* 68, 865-875.

Jokela, W.E., Randall, G.W., 1997. Fate of fertilizer nitrogen as affected by time and rate of application on corn. *Soil Science Society of America Journal* 61, 1695-1703.

Joly, D., Brossard, T., Cardot, H., Cavailhes, J., Hilal, M., Wavresky, P., 2010. Les types de climats en France, une construction spatiale. Cybergeo : *European Journal of Geography* 501. http://cybergeo.revues.org/23155

Jørgensen, U., 2011. Benefits versus risks of growing biofuel crops: the case of Miscanthus. *Current Opinion in Environmental Sustainability* 3, 24-30.

Justes, E., Thiébeau, P., Cattin, G., Larbre, D., Nicolardot, B., 2001. Libération d'azote après retournement de luzerne : un effet sur deux campagnes. *Perspectives Agricoles* 264, 22-28.

Kanapeckas, J., Lemeziene, N., Butkute, B., Stukonis, V., 2011. Evaluation of tall fescue (*Festuca arundinacea* Schreb.) varieties and wild ecotypes as feedstock for biogas production. *Zemdirbyste (Agriculture)* 98, 149-156.

Karlen, D.L., Hunt, P.G., Matheny, T.A., 1996. Fertilizer (15)nitrogen recovery by corn, wheat, and cotton grown with and without pre-plant tillage on Norfolk loamy sand. *Crop Science* 36, 975-981.

Karp, A., Richter, G.M., 2011. Meeting the challenge of food and energy security. *Journal of Experimental Botany* 62, 3263-3271.

Karp, A., Shield, I., 2008. Bioenergy from plants and the sustainable yield challenge. *New Phytologist* 179, 15-32.

Kauter, D., Lewandowski, I., Claupein, W., 2003. Quantity and quality of harvestable biomass from Populus short rotation coppice for solid fuel use - a review of the physiological basis and management influences. *Biomass and Bioenergy* 24, 411-427.

King, J., Gay, A., Sylvester-Bradley, R., Bingham, I., Foulkes, J., Gregory, P., Robinson, D., 2003. Modelling cereal root systems for water and nitrogen capture: Towards an economic optimum. *Annals of Botany* 91, 383-390.

Kumar, K., Goh, K.M., 2002. Recovery of N-15-labelled fertilizer applied to winter wheat and perennial ryegrass crops and residual N-15 recovery by succeeding wheat crops under different crop residue management practices. *Nutrient Cycling in Agroecosystems* 62, 123-130.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623-1627.

Lal, R., 2008. Crop residues as soil amendments and feedstock for bioethanol production. *Waste Management* 28, 747-758.

Laurent, A., Pelzer, E., Loyce, C., Makowski, D., 2015. Ranking yields of energy crops: A meta-analysis using direct and indirect comparisons. *Renewable and Sustainable Energy Reviews* 46, 41-50.

Le, P.V.V., Kumar, P., Drewry, D.T., 2011. Implications for the hydrologic cycle under climate change due to the expansion of bioenergy crops in the Midwestern United States. *Proceedings of the National Academy of Sciences* 108, 15085-15090.

Ledgard, S.F., Steele, K.W., Feyter, C., 1988. Influence of time of application on the fate of N-15-labelled urea applied to dairy pasture. *New Zealand Journal of Agricultural Research* 31, 87-91.

Lee, D.K., Owens, V.N., Doolittle, J.J., 2007. Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. *Agronomy Journal* 99, 462-468.

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. *Agriculture, Ecosystems & Environment* 134, 251-256.

Lenth, R.V., 2014. *lsmeans: Least-Squares Means*. http://CRAN.R-project.org/package=lsmeans

Lesur, C., Bazot, M., Bio-Beri, F., Mary, B., Jeuffroy, M.H., Loyce, C., 2014. Assessing nitrate leaching during the three-first years of *Miscanthus* × *giganteus* from on-farm measurements and modeling. *Global Change Biology Bioenergy* 6, 439-449.

Lewandowski, I., Schmidt, U., 2006. Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. *Agriculture, Ecosystems & Environment* 112, 335-346.

Lewandowski, I., Scurlock, J.M.O., Lindvall, E., Christou, M., 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy* 25, 335-361.

Liebig, M.A., Schmer, M.R., Vogel, K.P., Mitchell, R.B., 2008. Soil carbon storage by switchgrass grown for bioenergy. *BioEnergy Research* 1, 215-222.

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

Lloveras, J., Ferran, J., Boixadera, J., Bonet, J., 2001. Potassium fertilization effects on alfalfa in a Mediterranean climate. *Agronomy Journal* 93, 139-143.

Ma, Z., Wood, C.W., Bransby, D.I., 2000. Impacts of soil management on root characteristics of switchgrass. *Biomass and Bioenergy* 18, 105-112.

Macdonald, A.J., Poulton, P.R., Powlson, D.S., Jenkinson, D.S., 1997. Effects of season, soil type and cropping on recoveries, residues and losses of N-15-labelled fertilizer applied to arable crops in spring. *Journal of Agricultural Science* 129, 125-154.

Macdonald, A.J., Poulton, P.R., Stockdale, E.A., Powlson, D.S., Jenkinson, D.S., 2002. The fate of residual N-15-labelled fertilizer in arable soils: its availability to subsequent crops and retention in soil. *Plant and Soil* 246, 123-137.

Macdonald, A.J., Powlson, D.S., Poulton, P.R., Jenkinson, D.S., 1989. Unused fertiliser nitrogen in arable soils - its contribution to nitrate leaching. *Journal of the Science of Food and Agriculture* 46, 407-419.

Mary, B., Saffih-Hdadi, K., Duparque, A., Tomis, V., 2009. Préserver le stock de carbone du sol. *Perspectives Agricoles* 354, 6-9.

McGlade, C., Ekins, P., 2015. The geographical distribution of fossil fuels unused when limiting global warming to 2 °C. *Nature* 517, 187-190.

McIsaac, G.F., David, M.B., Mitchell, C.A., 2010. Miscanthus and switchgrass production in Central Illinois: impacts on hydrology and inorganic nitrogen leaching. *Journal of Environmental Quality* 39, 1790-1799.

Monti, A., Barbanti, L., Zatta, A., Zegada-Lizarazu, W., 2012. The contribution of switchgrass in reducing GHG emissions. *Global Change Biology Bioenergy* 4, 420-434.

Monti, A., Zatta, A., 2009. Root distribution and soil moisture retrieval in perennial and annual energy crops in Northern Italy. *Agriculture, Ecosystems & Environment* 132, 252-259.

Mortensen, J., Nielsen, K.H., Jorgensen, U., 1998. Nitrate leaching during establishment of willow (*Salix viminalis*) on two soil types and at two fertilization levels. *Biomass and Bioenergy* 15, 457-466.

Nannen, D.U., Herrmann, A., Loges, R., Dittert, K., Taube, F., 2011. Recovery of mineral fertiliser N and slurry N in continuous silage maize using the N-15 and difference methods. *Nutrient Cycling in Agroecosystems* 89, 269-280.

Neukirchen, D., Himken, M., Lammel, J., Czyionka-Krause, U., Olfs, H.W., 1999. Spatial and temporal distribution of the root system and root nutrient content of an established Miscanthus crop. *European Journal of Agronomy* 11, 301-309.

Ng, T.L., Eheart, J.W., Cai, X.M., Miguez, F., 2010. Modeling miscanthus in the Soil and Water Assessment Tool (SWAT) to simulate its water quality effects as a bioenergy crop. *Environmental Science & Technology* 44, 7138-7144.

Nieder, R., Kersebaum, K.C., Richter, J., 1995. Significance of nitrate leaching and long-term N immobilization after deepening the plough layers for the N regime of arable soils in N.W. Germany. *Plant and Soil* 173, 167-175.

Nippert, J.B., Wieme, R.A., Ocheltree, T.W., Craine, J.M., 2012. Root characteristics of C-4 grasses limit reliance on deep soil water in tallgrass prairie. *Plant and Soil* 355, 385-394.

Normand, B., Recous, S., Vachaud, G., Kengni, L., Garino, B., 1997. Nitrogen-15 tracers combined with tensio-neutronic method to estimate the nitrogen balance of irrigated maize. *Soil Science Society of America Journal* 61, 1508-1518.

Olson, S.N., Ritter, K., Rooney, W., Kemanian, A., McCarl, B.A., Zhang, Y., Hall, S., Packer, D., Mullet, J., 2012. High biomass yield energy sorghum: developing a genetic model for C4 grass bioenergy crops. *Biofuels, Bioproducts and Biorefining* 6, 640-655

Pedroso, G.M., van Kessel, C., Six, J., Putnam, D.H., Linquist, B.A., 2014. Productivity, 15N dynamics and water use efficiency in low- and high-input switchgrass systems. *Global Change Biology Bioenergy* 6, 704-716.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2014. *nlme: Linear and Nonlinear Mixed Effects Models*. http://CRAN.R-project.org/package=nlme

Pires, L.F., Pereira, A.B., 2014. Gamma-Ray Attenuation to Evaluate Soil Porosity: An Analysis of Methods. *Scientific World Journal* vol. 2014. doi: 10.1155/2014/723041

Poeplau, C., Don, A., 2014. Soil carbon changes under Miscanthus driven by C-4 accumulation and C-3 decompostion - toward a default sequestration function. *Global Change Biology Bioenergy* 6, 327-338.

Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology* 6, 317-327.

Powlson, D.S., Glendining, M.J., Coleman, K., Whitmore, A.P., 2011. Implications for soil properties of removing cereal straw: results from long-term studies. *Agronomy Journal* 103, 279-287.

Powlson, D.S., Hart, P.B.S., Poulton, P.R., Johnston, A.E., Jenkinson, D.S., 1992. Influence of soil type, crop management and weather on the recovery of N-15-labeled fertilizer applied to winter-wheat in spring. *Journal of Agricultural Science* 118, 83-100.

Powlson, D.S., Riche, A.B., Shield, I., 2005. Biofuels and other approaches for decreasing fossil fuel emissions from agriculture. *Annals of Applied Biology* 146, 193-201.

Powlson, D.S., Stirling, C.M., Jat, M.L., Gerard, B.G., Palm, C.A., Sanchez, P.A., Cassman, K.G., 2014. Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change* 4, 678-683.

Pugesgaard, S., Schelde, K., Larsen, S.U., Lærke, P.E., Jørgensen, U., 2014. Comparing annual and perennial crops for bioenergy production – influence on nitrate leaching and energy balance. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12215

R Core Team, 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick, W.J., Jr., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. *Science* 311, 484-489.

Reay, D.S., Davidson, E.A., Smith, K.A., Smith, P., Melillo, J.M., Dentener, F., Crutzen, P.J., 2012. Global agriculture and nitrous oxide emissions. *Nature Climate Change* 2, 410-416.

Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988a. The fate of labelled15N urea and ammonium nitrate applied to a winter wheat crop .1. Nitrogen transformations in the soil. *Plant and Soil* 112, 205-214.

Recous, S., Machet, J.-M., 1999. Short-term immobilisation and crop uptake of fertiliser nitrogen applied to winter wheat: Effect of date of application in spring. *Plant and Soil* 206, 137-149.

Recous, S., Machet, J.M., Mary, B., 1988b. The fate of labeled N-15 urea and ammonium-nitrate applied to a winter-wheat crop.2. Plant uptake and N-efficiency. *Plant and Soil* 112, 215-224.

Recous, S., Machet, J.M., Mary, B., 1992. The partitioning of fertilizer-N between soil and crop - comparison of ammonium and nitrate applications. *Plant and Soil* 144, 101-111.

Reddy, G.B., Reddy, K.R., 1993. Fate of N-15 enriched ammonium-nitrate applied to corn. *Soil Science Society of America Journal* 57, 111-115.

Riche, A., Christian, D.G., 2001. Estimates of rhizome weight of Miscanthus with time and rooting depth compared to switchgrass. *Aspects of Applied Biology* 65.

Richter, G.M., Agostini, F., Redmile-Gordon, M., White, R., Goulding, K.W.T., 2015. Sequestration of C in soils under Miscanthus can be marginal and is affected by genotype-specific root distribution. *Agriculture, Ecosystems & Environment* 200, 169-177.

Rimski-Korsakov, H., Rubio, G., Lavado, R.S., 2012. Fate of the nitrogen from fertilizers in field-grown maize. *Nutrient Cycling in Agroecosystems* 93, 253-263.

RMT Biomasse Energie Environnement et Territoire, 2013. *LIGNOGUIDE : Guide d'aide au choix des cultures lignocellulosiques*. Besnard, A., Ferchaud, F., Levrault, F., Marsac, S., Nguyen, E., Savouré, M.L. (Eds.), 215 p.

Robertson, M.J., Fukai, S., Ludlow, M.M., Hammer, G.L., 1993. Water extraction by grain-sorghum in a subhumid environment .1. Analysis of the water extraction pattern. *Field Crops Research* 33, 81-97.

Rowe, R.L., Street, N.R., Taylor, G., 2009. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. *Renewable and Sustainable Energy Reviews* 13, 260-279.

Rudiger, C., Western, A.W., Walker, J.P., Smith, A.B., Kalma, J.D., Willgoose, G.R., 2010. Towards a general equation for frequency domain reflectometers. *Journal of Hydrology* 383, 319-329.

Saffih-Hdadi, K., Mary, B., 2008. Modeling consequences of straw residues export on soil organic carbon. *Soil Biology & Biochemistry* 40, 594-607.

Sanderson, M.A., Adler, P.R., 2008. Perennial forages as second generation bioenergy crops. International *Journal of Molecular Sciences* 9, 768-788.

Scarlat, N., Martinov, M., Dallemand, J.-F., 2010. Assessment of the availability of agricultural crop residues in the European Union: potential and limitations for bioenergy use. *Waste Management* 30, 1889-1897.

Scharlemann, J.P.W., Laurance, W.F., 2008. Environmental science - How green are biofuels? *Science* 319, 43-44.

Schmer, M.R., Liebig, M.A., Vogel, K.P., Mitchell, R.B., 2011. Field-scale soil property changes under switchgrass managed for bioenergy. *Global Change Biology Bioenergy* 3, 439-448.

Schneckenberger, K., Kuzyakov, Y., 2007. Carbon sequestration under Miscanthus in sandy and loamy soils estimated by natural ^{13}C abundance. *Journal of Plant Nutrition and Soil Science* 170, 538-542.

Searchinger, T., Heimlich, R., Houghton, R.A., Dong, F.X., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., Yu, T.H., 2008. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science* 319, 1238-1240.

Sebilo, M., Mayer, B., Nicolardot, B., Pinay, G., Mariotti, A., 2013. Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences* 110, 18185-18189.

Sen Tran, T., Giroux, M., 1998. Fate of N-15-labelled fertilizer applied to corn grown on different soil types. Canadian Journal of Soil Science 78, 597-605.

Seo, J.H., Meisinger, I.J., Lee, H.J., 2006. Recovery of nitrogen-15-labeled hairy vetch and fertilizer applied to corn. *Agronomy Journal* 98, 245-254.

Simmelsgaard, S.E., 1998. The effect of crop, N-level, soil type and drainage on nitrate leaching from Danish soil. *Soil Use and Management* 14, 30-36.

Smith, C.M., David, M.B., Mitchell, C.A., Masters, M.D., Anderson-Teixeira, K.J., Bernacchi, C.J., DeLucia, E.H., 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. *Journal of Environmental Quality* 42, 219-228.

Smith, K.A., Searchinger, T.D., 2012. Crop-based biofuels and associated environmental concerns. *Global Change Biology Bioenergy* 4, 479-484.

Somerville, C., Youngs, H., Taylor, C., Davis, S.C., Long, S.P., 2010. Feedstocks for lignocellulosic biofuels. *Science* 329, 790-792.

Soussana, J.F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., Raschi, A., Baronti, S., Rees, R.M., Skiba, U., Stefani, P., Manca, G., Sutton, M., Tuba, Z., Valentini, R., 2007. Full accounting of the greenhouse gas (CO_2, N_2O, CH_4) budget of nine European grassland sites. *Agriculture, Ecosystems & Environment* 121, 121-134.

Soussana, J.F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays, D., 2004. Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219-230.

Stevens, R.J., Laughlin, R.J., 1989. A microplot study of the fate of N-15-labelled ammonium-nitrate and urea applied at 2 rates to ryegrass in spring. *Fertilizer Research* 20, 33-39.

Stevens, W.B., Hoeft, R.G., Mulvaney, R.L., 2005. Fate of nitirogen-15 in a long-term nitrogen rate study: II. Nitrogen uptake efficiency. *Agronomy Journal* 97, 1046-1053.

Strullu, L., Beaudoin, N., Atauri, I.G.d.C., Mary, B., 2014a. Simulation of biomass and nitrogen dynamics in perennial organs and shoots of *Miscanthus* × *giganteus* using the STICS model. *BioEnergy Research* 7, 1253-1269.

Strullu, L., Cadoux, S., Beaudoin, N., Jeuffroy, M.H., 2013. Influence of belowground nitrogen stocks on light interception and conversion of *Miscanthus* × *giganteus*. *European Journal of Agronomy* 47, 1-10.

Strullu, L., Cadoux, S., Preudhomme, M., Jeuffroy, M.H., Beaudoin, N., 2011. Biomass production and nitrogen accumulation and remobilisation by *Miscanthus* × *giganteus* as influenced by nitrogen stocks in belowground organs. *Field Crops Research* 121, 381-391.

Strullu, L., Didier, A., Beaudoin, N., Mary, B., Ferchaud, F., 2014b. *Comparing nitrogen fluxes in different cropping systems dedicated to lignocellulosic biomass production*. In: Cordovil, C.M.d.S. (Ed.), 18th Nitrogen Workshop - The nitrogen challenge: building a blueprint for nitrogen use efficiency and food security, Lisbon, Portugal, p. 210-211.

Strullu, L., Ferchaud, F., Yates, N.E., Shield, I.F., Beaudoin, N., García de Cortázar-Atauri, I., Besnard, A., Mary, B., 2015. Multisite yield gap analysis of Miscanthus x giganteus using the STICS model. *BioEnergy Research*. doi: 10.1007/s12155-015-9625-y

Suter, H., Sultana, H., Turner, D., Davies, R., Walker, C., Chen, D.L., 2013. Influence of urea fertiliser formulation, urease inhibitor and season on ammonia loss from ryegrass. *Nutrient Cycling in Agroecosystems* 95, 175-185.

Sutton, M.A., Oenema, O., Erisman, J.W., Leip, A., van Grinsven, H., Winiwarter, W., 2011. Too much of a good thing. *Nature* 472, 159-161.

Sweeney, D.W., Moyer, J.L., Havlin, J.L., 1996. Multinutrient fertilization and placement to improve yield and nutrient concentration of tall fescue. *Agronomy Journal* 88, 982-986.

Tardieu, F., 1988. Analysis of the spatial variability of maize root density - I. Effect of wheel compaction on the spatial arrangement of roots. *Plant and Soil* 107, 259-266.

Thiébeau, P., Beaudoin, N., Justes, E., Allirand, J.-M., Lemaire, G., 2011. Radiation use efficiency and shoot:root dry matter partitioning in seedling growths and regrowth crops of lucerne (*Medicago sativa* L.) after spring and autumn sowings. *European Journal of Agronomy* 35, 255-268.

Thieu, V., Billen, G., Garnier, J., Benoit, M., 2011. Nitrogen cycling in a hypothetical scenario of generalised organic agriculture in the Seine, Somme and Scheldt watersheds. *Regional Environmental Change* 11, 359-370.

Thomsen, I.K., Christensen, B.T., 2007. Fertilizer N-15 recovery in cereal crops and soil under shallow tillage. *Soil & Tillage Research* 97, 117-121.

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

Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T., Somerville, C., Williams, R., 2009. Beneficial biofuels - the food, energy, and environment trilemma. *Science* 325, 270-271.

Timmons, D.R., Baker, J.L., 1992. Fertilizer management effect on recovery of labeled nitrogen by continuous no-till. *Agronomy Journal* 84, 490-496.

Triboi, E., 1987. Recovery of mineral-N fertilizer in herbage and soil organic-matter in grasslands of the Massif-Central, France. *Fertilizer Research* 13, 99-116.

Vaio, N., Cabrera, M.L., Kissel, D.E., Rema, J.A., Newsome, J.F., Calvert, V.H., 2008. Ammonia Volatilization from Urea-Based Fertilizers Applied to Tall Fescue Pastures in Georgia, USA. *Soil Science Society of America Journal* 72, 1665-1671.

van der Weijde, T., Kamei, C.L.A., Torres, A.F., Vermerris, W., Dolstra, O., Visser, R.G.F., Trindade, L.M., 2013. The potential of C4 grasses for cellulosic biofuel production. *Frontiers in Plant Science* 4, 107.

Vanloocke, A., Bernacchi, C.J., Twine, T.E., 2010. The impacts of Miscanthus x giganteus production on the Midwest US hydrologic cycle. *Global Change Biology Bioenergy* 2, 180-191.

Webster, C.P., Dowdell, R.J., 1985. A lysimeter study of the fate of nitrogen applied to perennial ryegrass swards - soil analyses and the final balance-sheet. *Journal of Soil Science* 36, 605-611.

Whitaker, J., Ludley, K.E., Rowe, R., Taylor, G., Howard, D.C., 2010. Sources of variability in greenhouse gas and energy balances for biofuel production: a systematic review. *Global Change Biology Bioenergy* 2, 99-112.

Whitehead, D.C., Dawson, K.P., 1984. Nitrogen, including N-15-labelled fertilizer nitrogen, in components of a grass sward. *Journal of Applied Ecology* 21, 983-989.

Yimam, Y.T., Ochsner, T.E., Kakani, V.G., Warren, J.G., 2014. Soil Water Dynamics and Evapotranspiration under Annual and Perennial Bioenergy Crops. *Soil Science Society of America Journal* 78, 1584-1593.

Zatta, A., Clifton-Brown, J., Robson, P., Hastings, A., Monti, A., 2014. Land use change from C3 grassland to C4 Miscanthus: effects on soil carbon content and estimated mitigation benefit after six years. *Global Change Biology Bioenergy* 6, 360-370.

Zegada-Lizarazu, W., Monti, A., 2011. Energy crops in rotation. A review. *Biomass and Bioenergy* 35, 12-25.

Zegada-Lizarazu, W., Parrish, D., Berti, M., Monti, A., 2013. Dedicated crops for advanced biofuels: Consistent and diverging agronomic points of view between the USA and the EU-27. *Biofuels Bioproducts & Biorefining* 7, 715-731.

Zhang, X., Pei, D., Chen, S., 2004. Root growth and soil water utilization of winter wheat in the North China Plain. *Hydrological Processes* 18, 2275-2287.

Zimmermann, J., Dauber, J., Jones, M.B., 2012. Soil carbon sequestration during the establishment phase of *Miscanthus* × *giganteus*: a regional-scale study on commercial farms using ^{13}C natural abundance. *Global Change Biology Bioenergy* 4, 453-461.

Annexes

Annexe 1……1
Annexe 2……5
Annexe 3……6
Annexe 4……9

Annexe 1 : stocks d'eau et d'azote minéral mesurés sur le dispositif B&E

Table S1. Soil water content (mm) over 0-150 cm measured in early November between 2006 and 2013.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013
Mis E	N-		426	420	409	407	429	444	503
	N+		418	427	392	390	361	415	462
Swi E	N-		479	507	429	470	465	475	509
	N+		473	490	406	405	411	440	493
Fes-Alf	N-	447	396	489	344	309	374	408	474
	N+	449	407	476	330	303	380	420	491
Alf-Fes	N-	356	430	475	358	335	453	416	473
	N+	366	429	483	358	347	448	410	469
Sor-Tri	N-	452	424	514	407	443	472	506	498
	N+	447	417	511	398	414	463	490	494
Tri-Sor	N-	402	502	492	458	427	402	461	508
	N+	415	514	500	454	433	409	460	512

Table S2. Soil nitrate (kg N ha^{-1}) over 0-150 cm measured in early November between 2006 and 2013.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013
Mis E	N-		37	15	29	11	10	19	21
	N+		63	26	49	20	20	26	41
Swi E	N-		8	11	15	11	7	11	14
	N+		18	15	29	14	11	22	25
Fes-Alf	N-	125	9	10	27	31	15	17	27
	N+	113	7	11	30	30	13	14	21
Alf-Fes	N-	26	41	33	28	14	54	29	57
	N+	35	30	46	27	18	33	29	61
Sor-Tri	N-	96	21	18	32	27	43	60	27
	N+	110	51	18	37	29	118	46	38
Tri-Sor	N-	43	13	26	34	25	10	74	30
	N+	46	13	23	40	26	10	68	22

Table S3. Soil ammonium (kg N ha^{-1}) over 0-150 cm measured in early November between 2006 and 2013.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013
Mis E	N-		13	11	12	6	11	9	10
	N+		17	13	18	12	17	14	14
Swi E	N-		15	11	9	11	10	9	15
	N+		17	13	12	15	16	17	19
Fes-Alf	N-	10	11	11	36	17	16	14	27
	N+	10	10	10	30	19	15	10	28
Alf-Fes	N-	16	15	17	14	11	19	11	15
	N+	22	17	15	15	16	14	11	19
Sor-Tri	N-	11	9	9	8	7	8	7	6
	N+	10	10	9	8	8	15	8	21
Tri-Sor	N-	7	18	11	14	11	10	7	10
	N+	8	17	11	16	7	11	8	10

Table S4. Soil water content (mm) over 0-150 cm measured in mid-March (mid-April in 2006) between 2006 and 2014.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013	2014
Mis E	N-	488	536	533	529	509	509	518	511	501
	N+	487	537	530	524	507	508	519	516	503
Mis L	N-	491	532	533	521	506	511	519	519	518
	N+	492	535	526	529	504	509	502	516	519
Swi E	N-	488	530	538	528	512	520	529	516	520
	N+	486	531	537	532	512	507	524	514	511
Swi L	N-	490	530	540	531	513	516	531	527	521
	N+	491	532	539	533	508	509	513	519	506
Fes-Alf	N-	478	522	517	523	504	489	520	505	482
	N+	479	521	509	521	502	492	518	515	496
Alf-Fes	N-	478	502	506	518	498	492	517	500	488
	N+	477	513	517	521	504	504	521	497	493
Sor-Tri	N-	483	523	524	516	494	492	515	494	496
	N+	474	522	518	516	486	491	513	486	484
Tri-Sor	N-	484	517	531	509	491	491	517	502	486
	N+	490	533	533	523	506	496	525	503	494

Table S5. Soil nitrate (kg N ha^{-1}) over 0-150 cm measured in mid-March (mid-April in 2006) between 2006 and 2014.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013	2014
Mis E	N-	184	143	21	11	11	14	17	12	28
	N+	190	145	51	16	25	29	25	32	50
Mis L	N-	175	162	19	12	12	14	15	16	29
	N+	173	158	46	18	15	37	28	33	54
Swi E	N-	180	98	7	8	10	16	12	6	19
	N+	178	68	18	17	19	24	19	13	35
Swi L	N-	169	91	10	11	11	17	12	10	25
	N+	188	104	25	21	20	29	29	19	57
Fes-Alf	N-	133	60	8	9	22	115	15	16	62
	N+	138	47	7	9	26	115	16	23	48
Alf-Fes	N-	117	26	54	47	15	42	66	51	116
	N+	112	29	61	46	14	51	61	63	113
Sor-Tri	N-	123	64	21	13	24	19	28	62	26
	N+	130	87	35	14	26	21	89	48	31
Tri-Sor	N-	132	11	7	44	23	25	26	70	68
	N+	135	17	7	33	27	26	30	73	70

Table S6. Soil ammonium (kg N ha^{-1}) over 0-150 cm measured in mid-March (mid-April in 2006) between 2006 and 2014.

Rotation	N	2006	2007	2008	2009	2010	2011	2012	2013	2014
Mis E	N-	4	16	25	17	5	3	10	20	14
	N+	3	14	18	23	6	5	14	35	16
Mis L	N-	4	17	32	19	7	6	19	24	27
	N+	5	20	28	28	8	8	24	29	30
Swi E	N-	3	22	22	18	7	5	11	22	22
	N+	3	31	19	22	8	4	20	29	23
Swi L	N-	3	23	24	18	6	4	20	28	27
	N+	3	25	25	25	6	6	17	36	19
Fes-Alf	N-	5	18	14	14	9	9	18	31	21
	N+	5	14	12	13	6	13	22	23	22
Alf-Fes	N-	6	23	13	15	9	5	15	23	12
	N+	6	26	17	21	10	5	13	17	9
Sor-Tri	N-	6	18	10	9	5	4	7	11	18
	N+	5	21	10	10	7	5	9	14	13
Tri-Sor	N-	6	16	17	8	8	3	9	12	6
	N+	7	19	14	8	8	4	9	10	8

Annexe 2 : Méthode de prélèvement utilisée sur miscanthus

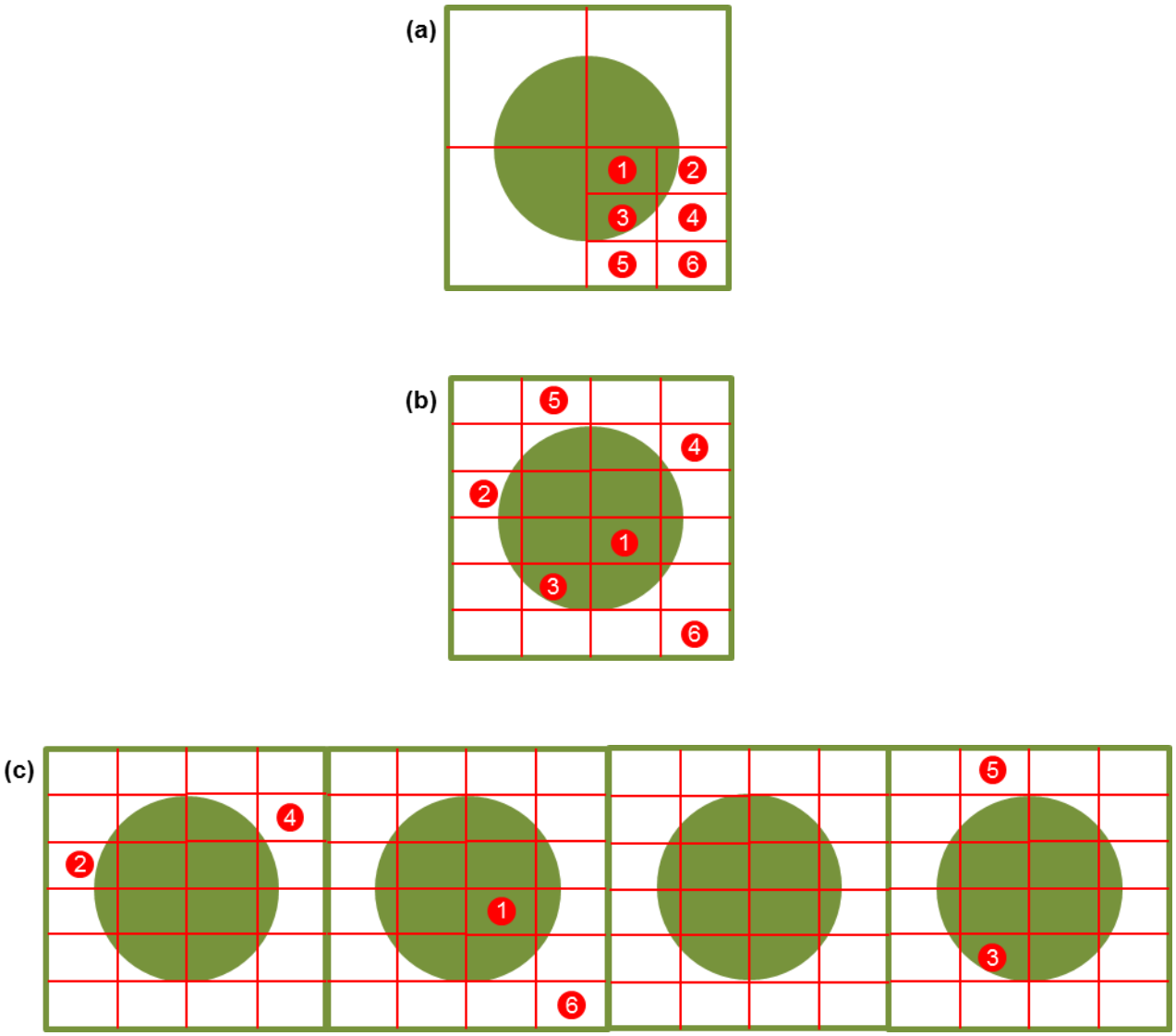


Diagram showing the three steps defining the sampling strategy for soil organic carbon measurements in miscanthus plots. A green circle represents one plant (rhizome area) and a green square the soil surface available for one plant (0.8 × 0.8 m). Red circles represent soil cores. (a) Step 1: elementary cells representing one plant; (b) step 2: random sampling representing one plant; (c) step 3: random sampling representing the micro-plot with four plants, one of them being extracted for rhizome analysis.

Annexe 3 : Tableaux d'analyses statistiques sur le carbone du sol

Table S1. Statistical analysis of soil organic carbon (SOC) concentrations and $\delta^{13}C$ on each date of measurement for perennial crops and annual/semi-perennial crops. Asterisks indicate probability levels: * p<0.05; ** p<0.01; *** p<0.001; NS = not significant

		Perennial crops		Annuals and semi-perennials	
Factor or interaction		2006	2011	2006	2012
SOC concentrations					
Rotation	1	NS	NS	NS	NS
Nitrogen	2	NS	NS	NS	NS
Soil layer	3	***	***	***	***
	1 x 2	NS	NS	NS	NS
	1 x 3	NS	NS	NS	***
	2 x 3	NS	NS	NS	NS
	1 x 2 x 3	NS	NS	NS	NS
$\delta^{13}C$					
Rotation	1	NS	NS	NS	*
Nitrogen	2	NS	NS	NS	NS
Soil layer	3	***	***	***	***
	1 x 2	NS	**	NS	*
	1 x 3	NS	NS	NS	***
	2 x 3	NS	NS	NS	NS
	1 x 2 x 3	NS	NS	NS	NS

Table S2. Statistical analysis of cumulative soil organic carbon (SOC) stocks and mean $\delta^{13}C$ in 2006 in each soil layer for perennial crops and for annual and semi-perennial crops. Asterisks indicate probability levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant

		Perennial crops 2006				Semi-perennials and annuals 2006			
Factor or interaction		L1	L1-2	L1-3	L1-4	L1	L1-2	L1-3	L1-4
SOC stocks									
Rotation	1	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen	2	NS	NS	NS	NS	***	***	***	***
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS
Mean $\delta^{13}C$									
Rotation	1	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen	2	NS	NS	NS	NS	NS	NS	NS	NS
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS

Table S3. Statistical analysis of cumulative soil organic carbon (SOC) stocks and mean $\delta^{13}C$ on an equivalent soil mass basis in each soil layer in 2011 for perennial crops and 2012 for annual/semi-perennial crops. Asterisks indicate probability levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant

		Perennial crops 2011					Semi-perennials and annuals 2012				
Factor or interaction		L1	L1-2	L1-3	L1-4	L1-5	L1	L1-2	L1-3	L1-4	L1-5
SOC stocks											
Rotation	1	NS	NS	NS	NS	NS	NS	*	**	**	*
Nitrogen	2	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean $\delta^{13}C$											
Rotation	1	**	NS	NS	NS	NS	**	*	*	*	*
Nitrogen	2	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table S4. Statistical analysis of cumulative soil organic carbon (SOC) stocks and mean $\delta^{13}C$ on an equivalent soil mass basis in each soil layer for perennial and annual/semi-perennial crops, testing the effects of rotation, nitrogen rate and year. Asterisks indicate probability levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS = not significant

		Perennial crops				Semi-perennials and annuals			
Factor or interaction		L1	L1-2	L1-3	L1-4	L1	L1-2	L1-3	L1-4
SOC stocks									
Rotation	1	NS	NS	NS	NS	NS	NS	*	**
Nitrogen	2	NS	NS	NS	NS	NS	NS	NS	NS
Year	3	***	***	NS	NS	***	***	***	***
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS
	1 x 3	NS	NS	NS	NS	NS	***	***	***
	2 x 3	NS	NS	NS	NS	NS	NS	NS	NS
	1 x 2 x 3	NS	NS	NS	NS	NS	NS	NS	NS
Mean $\delta^{13}C$									
Rotation	1	NS	NS	NS	NS	*	NS	NS	NS
Nitrogen	2	NS	NS	NS	NS	NS	NS	NS	NS
Year	3	***	***	***	***	*	*	***	***
	1 x 2	NS	NS	NS	NS	NS	NS	NS	NS
	1 x 3	***	**	***	***	***	***	***	***
	2 x 3	NS	NS	NS	NS	NS	NS	NS	NS
	1 x 2 x 3	NS	NS	NS	NS	NS	NS	NS	NS

Annexe 4 : Production de biomasse et exportations d'azote mesurées sur les placettes ^{15}N

Table S1. Biomass harvested (t DM ha^{-1}) from 2006 to 2011. Values in brackets are standard deviations.

Rotation	N	2006	2007	2008	2009	2010	2011
Mis E	N-	0 (0)	23.0 (4.9)	23.6 (2.4)	24.0 (3.3)	26.1 (1.5)	
	N+	0 (0)	21.7 (4.5)	25.2 (2.6)	28.8 (3.3)	30.6 (2.0)	
Mis L	N-	0 (0)	14.3 (4.5)	18.5 (1.7)	20.9 (1.9)	22.2 (2.2)	
	N+	0 (0)	13.9 (2.3)	18.7 (2.4)	19.6 (1.4)	22.4 (1.7)	
Swi E	N-	0 (0)	19.6 (2.4)	18.9 (0.2)	14.9 (1.2)	9.2 (0.7)	
	N+	0 (0)	21.5 (2.9)	16.7 (5.9)	19.2 (1.2)	15.2 (4.2)	
Swi L	N-	0 (0)	15.9 (1.0)	16.7 (0.8)	13.8 (0.6)	12.6 (1.2)	
	N+	0 (0)	15.2 (1.4)	15.9 (2.0)	15.2 (2.3)	14.0 (0.7)	
Fes-Alf	N-	0 (0)	16.1 (0.6)	7.7 (0.7)	3.5 (0.4)	12.4 (1.0)	9.2 (1.9)
	N+	0 (0)	17.3 (0.4)	12.1 (0.6)	2.7 (0.0)	11.8 (0.8)	8.1 (0.9)
Alf-Fes	N-	8.0 (0.6)	14.6 (0.9)	15.8 (0.2)	5.8 (1.1)	6.3 (2.0)	2.0 (1.8)
	N+	7.5 (1.2)	14.6 (0.4)	16.0 (0.3)	6.4 (0.9)	8.6 (0.6)	2.4 (2.1)
Sor-Tri	N-	0 (0)	14.0 (2.3)	9.7 (0.6)	12.3 (1.8)	9.3 (0.3)	3.6 (0.6)
	N+	0 (0)	12.8 (1.8)	14.8 (1.5)	14.8 (2.1)	12.7 (0.8)	4.5 (0.6)
Tri-Sor	N-	15.2 (0.6)	11.5 (1.2)	11.1 (2.2)	8.3 (1.1)	7.8 (1.2)	7.7 (0.4)
	N+	15.2 (0.5)	13.5 (0.2)	14.2 (1.6)	12.3 (0.6)	13.4 (2.6)	9.9 (2.0)

Table S2. Nitrogen exported (kg ha^{-1}) from 2006 to 2011. Values in brackets are standard deviations.

Rotation	N	2006	2007	2008	2009	2010	2011
Mis E	N-	0 (0)	108 (15)	90 (10)	75 (19)	126 (11)	
	N+	0 (0)	99 (25)	108 (18)	137 (25)	197 (12)	
Mis L	N-	0 (0)	22 (8)	33 (12)	33 (9)	61 (17)	
	N+	0 (0)	24 (5)	44 (9)	55 (2)	86 (14)	
Swi E	N-	0 (0)	105 (14)	89 (8)	50 (9)	37 (1)	
	N+	0 (0)	149 (20)	83 (18)	71 (5)	78 (21)	
Swi L	N-	0 (0)	80 (12)	60 (8)	29 (2)	37 (5)	
	N+	0 (0)	95 (10)	86 (8)	48 (7)	54 (3)	
Fes-Alf	N-	0 (0)	218 (15)	66 (9)	86 (8)	320 (33)	210 (47)
	N+	0 (0)	275 (35)	118 (10)	68 (5)	315 (25)	175 (25)
Alf-Fes	N-	212 (13)	408 (15)	417 (32)	86 (6)	99 (29)	76 (67)
	N+	198 (31)	409 (7)	441 (6)	80 (15)	136 (16)	75 (65)
Sor-Tri	N-	0 (0)	134 (23)	53 (7)	67 (6)	54 (3)	49 (10)
	N+	0 (0)	149 (52)	108 (26)	140 (18)	104 (11)	110 (26)
Tri-Sor	N-	170 (7)	92 (10)	89 (21)	56 (11)	37 (8)	55 (7)
	N+	170 (6)	134 (7)	124 (15)	107 (7)	97 (34)	109 (13)