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Abstract

The fate of 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. Crops received ^{15}N-labelled fertiliser (urea ammonium nitrate solution) during 4 or 5 successive years on the same subplots, at a rate varying from 24 to 120 kg N ha^{-1} yr^{-1}. 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, nitrogen use efficiency, miscanthus, switchgrass

Highlights

- The fate of ^{15}N-labelled fertilizer was compared in bioenergy crops over 4-5 years
- Perennial crops exported the smallest amounts of ^{15}N in harvested biomass
- They stored the highest amounts of ^{15}N in their belowground organs and soil+litter
- The overall ^{15}N recovery was greater in perennials than other crops
- The early harvested miscanthus gave the highest overall ^{15}N recovery

1. Introduction

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 nitrogen (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 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 may 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). Fertiliser-N 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 fertiliser-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 the uptake of inorganic soil N is different between fertilised and unfertilised treatments. "Pool substitution" between fertiliser-N and soil mineral N can 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 (*e.g.* crop and 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 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 (14-39%) and that belowground organs and soil represented important N sinks. 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.

Only a few experiments have followed the recovery of ^{15}N in the soil-plant system on the long term. In arable cropping systems, a small proportion of the residual ^{15}N, *i.e.* the labelled fertiliser-N remaining in soil (mainly in organic form) and crop residues after harvest, 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 than with annual 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 in the same plots over several growing seasons could allow to integrate part of the long-term fate of the residual ^{15}N and 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.). In this study, we used cumulative applications of ^{15}N-labelled fertiliser for four or five years to determine (1) the fate of fertiliser applied to perennial, semi-perennial and annual bioenergy crops in the soil-plant system, and (2) the interaction with crop management, *i.e.* harvest date of perennial crops and N fertiliser rate for all crops. We hypothesised that perennial crops would export smaller amounts of ^{15}N through harvests than the other crops but would store larger amounts of ^{15}N in perennial organs and soil organic matter, leading to an equal or improved overall ^{15}N recovery in the soil-plant system.

2. Materials and methods

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 S1 (Appendix A). Over the period 2006-2011, the mean annual temperature was 10.6 °C, 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" crops (destroyed every two or three years) 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 2). The rationale for defining the N rates was explained by Cadoux *et al.* (2014).

The 2.7 ha field was divided into two parts in order to facilitate cropping operations and limit competition between plants due to differences in canopy height: (1) a split-block design in the west part for perennial crops with "rotations" in the main plots (miscanthus early, miscanthus late, switchgrass early, switchgrass late) and N fertilisation rates in the subplots (N- and N+), and (2) 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+). Each of the two parts comprised three replicate blocks and 24 subplots of 360 m^2 (Fig. S1, Appendix A). 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 S1 in Appendix A).

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}). In 2006, perennial crops were not harvested because of the low biomass production during the first year of growth. Their aboveground biomass was cut and left on the soil surface. 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 2008 and mouldboard ploughed (*ca.* 22 cm deep) in 2010. These crops were harvested in two or three cuttings depending on years, with the last cut in October. Annual crops were tilled superficially (12-15 cm deep) without inversion ploughing. 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 surface-applied from 2007 onwards 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 between years. 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 2).

2.2. Sampling and analytical procedures

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 weighed 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).

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 total N 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 by Ferchaud *et al.* (2015b).

2.2.3. Dead and living crop biomass

In order to make a complete ^{15}N balance in the soil-plant system, crop residues and living crop biomass were sampled at the same time and location as in the final soil sampling. Crop residues included dead plant parts accumulated in soil or at soil surface whereas living crop 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 late were sampled in the whole micro-plot. Small stem fragments (2 to 10 mm) and leaf debris (for miscanthus late) 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 in each micro-plot before soil sampling in 2012. Triticale and catch crop (before sorghum) plants were pulled from the soil in each micro-plot in order to collect aboveground and part of the belowground biomass and washed to eliminate soil contamination. Roots of all crops (including remaining roots of triticale and catch crop) and perennial crop rhizomes collected in the six cores of each micro-plot were pooled for each layer and washed. 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. All plant samples were dried at 65 °C for 96 h, weighed and ground before analysis.

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).

2.3. Calculations

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 biomass by 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.

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 receive 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 subplots in 2007 and 2009. For the final crop residues and living crop 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 by Ferchaud *et al.* (2015b) for soil organic carbon stocks and carbon isotopic composition. The same equations were applied here replacing carbon concentration by N concentration and $\delta^{13}C$ by ^{15}N excess atom fraction. In the following, soil layers on ESM basis are called L1 to L5.

2.6. 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. Rotation, nitrogen and their interaction were treated as fixed factors in both models. 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 detected using 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.

3. Results

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 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 (Table 3). The mean harvested biomass represented 19.0 t DM ha^{-1} yr^{-1} in perennial crops and 10.3 t DM ha^{-1} yr^{-1} in other crops. Rotation, fertiliser-N rate and their interaction had a significant effect on biomass production for both crop types (Table S2, Appendix A). 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 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. 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+). Annual data are given in Table S3 (Appendix A).

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 3). It was significantly affected by rotation, fertiliser-N rate and their interaction (Table S2, Appendix A). 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 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, fertilised annual crops (N+) exported more N than low fertilised ones (+50 kg ha^{-1} yr^{-1} on average). Annual data are given in Table S4 (Appendix A).

3.2. Dead and living crop 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 4). It did not change significantly with N fertilisation for perennials and other crops (Table S2, Appendix A). 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 crop 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 crop biomass of the other crops was only 0.4 to 6.7 t DM ha^{-1} in 2012.

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). 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%). The N content in the living biomass of the other crops 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+ (Table S2, Appendix A).

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 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 6). It was significantly affected by the rotation (Table S5, Appendix A). 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 independent 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.

The ^{15}N recovery was compared to the apparent recovery (R_A) calculated for perennial crops and sorghum (crops with an unfertilised control) (Fig. 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.

3.4. ^{15}N recovery in dead and living crop 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 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 (Ferchaud *et al.*, 2015a), 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 dead and living crop 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 dead and living biomass of perennial crops, and 1-15% for the other crops.

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 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 two upper 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 (Table S5, Appendix A). 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.

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 crop biomass, crop residues and soil at the end of the period, is presented in Fig. 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 crop 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).

4. Discussion

4.1. Crop production and nitrogen removal at harvest

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 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 fertilised, early harvested treatments were higher than those of fertilised, late harvested treatments of miscanthus because the aboveground biomass decreased in autumn and winter due to C translocation towards rhizomes and leaf fall (Strullu *et al.*, 2011).

4.2.2. 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; 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 (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).

4.2. N content of dead and living crop biomass

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 late: 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}).

4.2.2. Living crop 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.

4.3. ^{15}N recovery in the soil-plant system

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 total N and apparent recovery 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 late. 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 late) whereas the ^{15}N recovery in belowground organs was higher than ours (27.0 *vs.* 10.6%). The difference is mainly 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 late (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 late, 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*). 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; Stevens and Laughlin, 1989). However, smaller values have been observed by Dawson and Ryden (1985) (11 to 48%) who showed an effect of the date of application, the ^{15}N recovery at harvest being higher for spring than for summer or autumn applications. These 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; Normand *et al.*, 1997; Sen Tran and Giroux, 1998; Stevens *et al.*, 2005; 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 36 to 68% (Recous *et al.*, 1988b, 1992; Macdonald *et al.*, 1989, 1997; Powlson *et al.*, 1992; 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.

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 the values reported in the previously cited studies for ryegrass (16 to 32%).

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.

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 early (82%). The recovery by miscanthus late (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 (66-95% 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 (62-96%) 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.

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 or knifed-in applications, also increased the overall ^{15}N recovery.

The low overall ^{15}N recovery observed in our experiment suggests that important losses took place. We believe that in a multi-annual study like ours, ^{15}N losses are likely to be higher than during a single growing season because of the remineralisation of the ^{15}N previously immobilised in soil. The losses of ^{15}N could be due to nitrate leaching and gaseous losses, *i.e.* volatilization and denitrification.

Losses of ^{15}N through nitrate leaching were probably low. We evaluated nitrate leaching in the site during the same period as for the ^{15}N study (Ferchaud and Mary, 2016). The mean amount of total N leached (unlabelled + labelled) below 210 cm was 2, 1 and 3 kg N ha^{-1} yr^{-1} for perennial, semi-perennial and annual crops respectively, which represented 2, 2.5 and 5% of the fertiliser-N inputs. Indeed, nitrate leaching was not favoured in our context with moderate winter rainfall, large soil water content and deep rooting depth (Ferchaud *et al.*, 2015a). However, a small part of the added ^{15}N could have moved downwards in the soil and could be located between 60 and 210 cm at the time of soil sampling. This fraction was not measured and therefore not included in the ^{15}N recovery.

Regarding gaseous losses, ammonia volatilisation could have been favoured by the type of fertiliser used in our study, *i.e.* UAN containing 50% urea N. Urea is known to increase the risk of ammonia volatilisation compared to other forms of N fertiliser because of the temporary increase in soil pH during urea hydrolysis, particularly in a slightly alkaline soil like ours. In their review, Harrison and Webb (2001) found that volatilisation represented 0-4% and 6-47% of the N applied as ammonium nitrate and urea fertilisers respectively. They suggested that volatilisation losses from UAN applications were intermediate between the two other forms. Fox *et al.* (1996) reported ammonia volatilisation losses of 22% of the N fertiliser applied as UAN on average for three years in a grain maize. Vaio *et al.* (2008) measured losses ranging from 6 to 33% of the N applied as UAN to a tall fescue. Additional ^{15}N losses could have also occurred through denitrification but their importance in field conditions is largely unknown.

5. Conclusion

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. The fertiliser N recovery in the harvested biomass, determined using either the ^{15}N or the difference method, was generally lower for perennial than other crops. The difference between crops was lower when belowground organs of perennial crops were taken into account. Fertiliser-N immobilised in soil was greater under perennial than annual crops. The overall fertiliser-N recovery (exported + stored in living and dead biomass + stored in soil) tented to be greater with perennial than other crops, consistently with our initial hypothesis, but crop management also affected the overall recovery. Treatments ranked as follows: miscanthus harvested early > other perennial crops ≥ semi-perennial and annual crops. Globally, N recovery was rather low for all crops compared to achievable efficiency reported for conventional crops. It could probably be increased by improvements in cropping practices (rate, timing and form of fertiliser application). The effect of these practices and the partitioning of the N losses between leaching, volatilisation and denitrification deserve further investigations.

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Figures

Fig. 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.

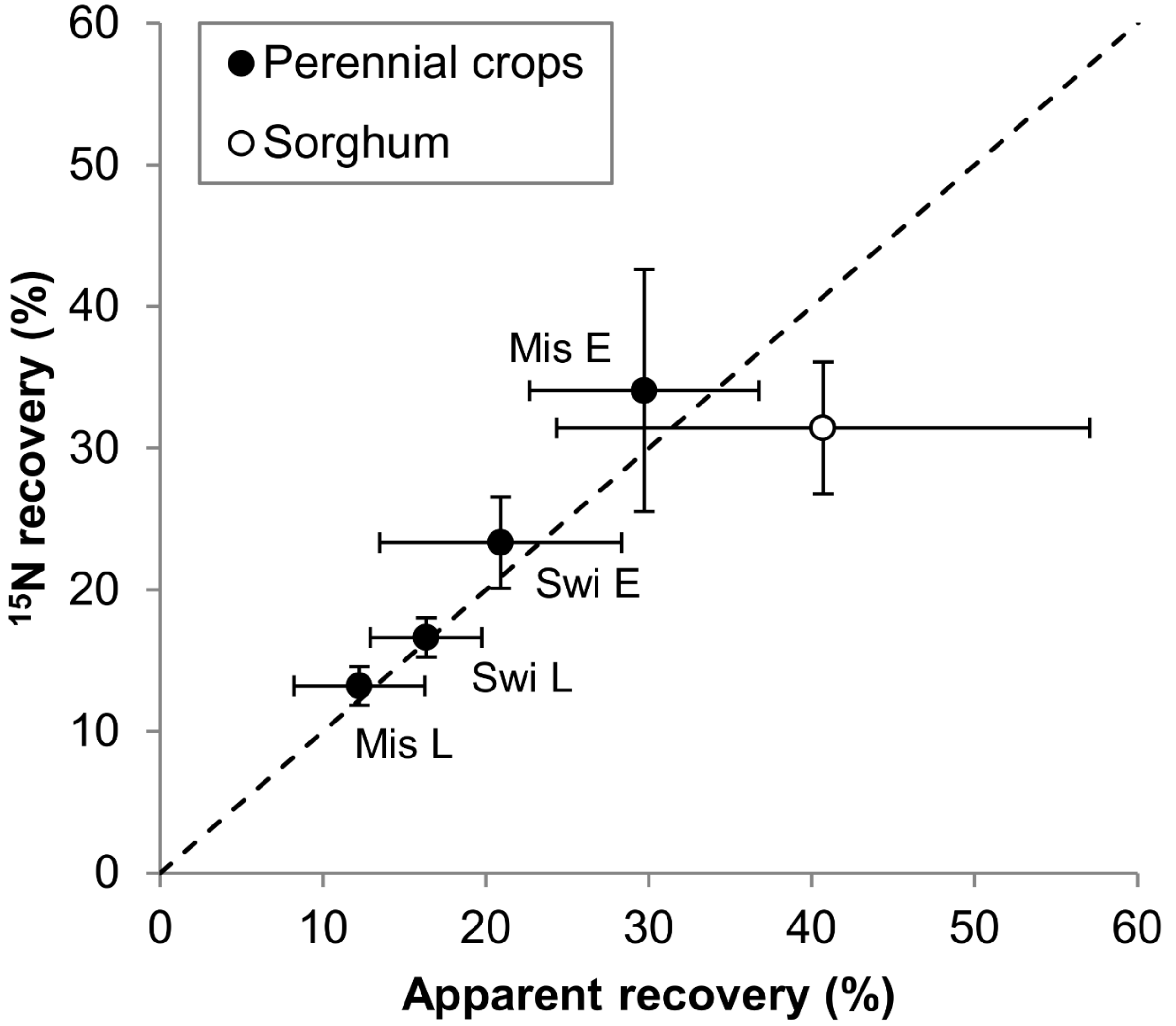
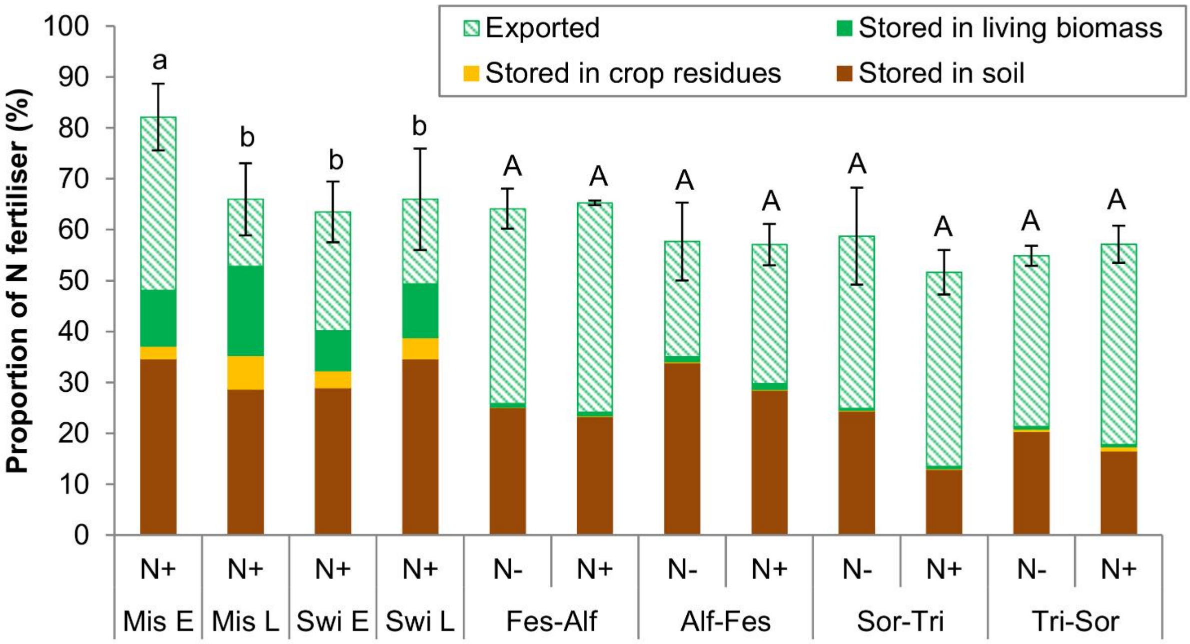


Fig. 2. Overall ^{15}N recovery (%) measured for perennial (2007-2010) and semi-perennial/annual crops (2007-2011). See Table 1 for abbreviations and Table 2 for fertiliser-N rates. Bars represent the standard deviations. Different letters indicate significant differences ($p<0.05$) between treatments (lower case: perennial crops; upper case: semi-perennial and annual crops).



Tables

Table 1. Rotations of the B&E long term experiment (Mis = miscanthus, Swi = switchgrass, Fes = fescue, Alf = alfalfa, Sor = fiber 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 (oats 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 2. Nitrogen fertilisation rates applied to the B&E long term experiment using ^{15}N-labelled UAN. See Table 1 for abbreviations.

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

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

Table 4. Dead (crop residues) and living crop biomass (t DM ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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)	4.1 (2.3)	5.7 (2.2)	4.5 (1.5)	3.4 (0.1)	4.0 (1.3)	5.8 (3.9)	4.6 (2.4)
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 crop 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)	2.8 (0.3)	1.5 (1.2)	1.3 (0.6)				
b) Total living crop 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. N content in dead (crop residues) and living crop biomass (kg ha^{-1}) measured in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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)		18 (7)		24 (9)		30 (11)		13 (1)		19 (4)		23 (16)		28 (18)	
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 crop 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)		50 (8)		43 (37)		34 (20)									
b) Total living crop 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*

Table 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. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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})					Total N exported (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+

Table 7. ^{15}N recovery (%) measured in dead (crop residues) and living crop biomass in March 2011 for perennial crops and March 2012 for semi-perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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)		1.9 (0.7)		1.2 (0.2)		1.8 (1.4)
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 crop 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)	0.5 (0.2)	0.4 (0.1)	0.4 (0.2)				
Belowground living biomass	0.2 (0.0)	0.2 (0.0)	0.6 (0.4)	0.8 (0.5)				
b) Total living crop 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)*	*0.8 (0.2)*	*1.2 (0.6)*	*1.3 (0.8)*	*0.5 (0.1)*	*0.5 (0.1)*	*1.0 (0.3)*	*1.2 (0.6)*

Table 8. ^{15}N recovery (%) measured in soil layers in 2011 for perennial crops and in 2012 for semi-perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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) a*	*28.7 (6.0) a*	*28.9 (5.8) a*	*34.6 (3.0) a*	*25.1 (1.8) BC*	*23.3 (1.8) BC*	*33.9 (2.0) A*	*28.5 (3.8) AB*	*24.4 (4.6) BC*	*12.9 (2.5) E*	*20.4 (2.1) CD*	*16.6 (1.3) DE*

Appendix A. Supplementary data

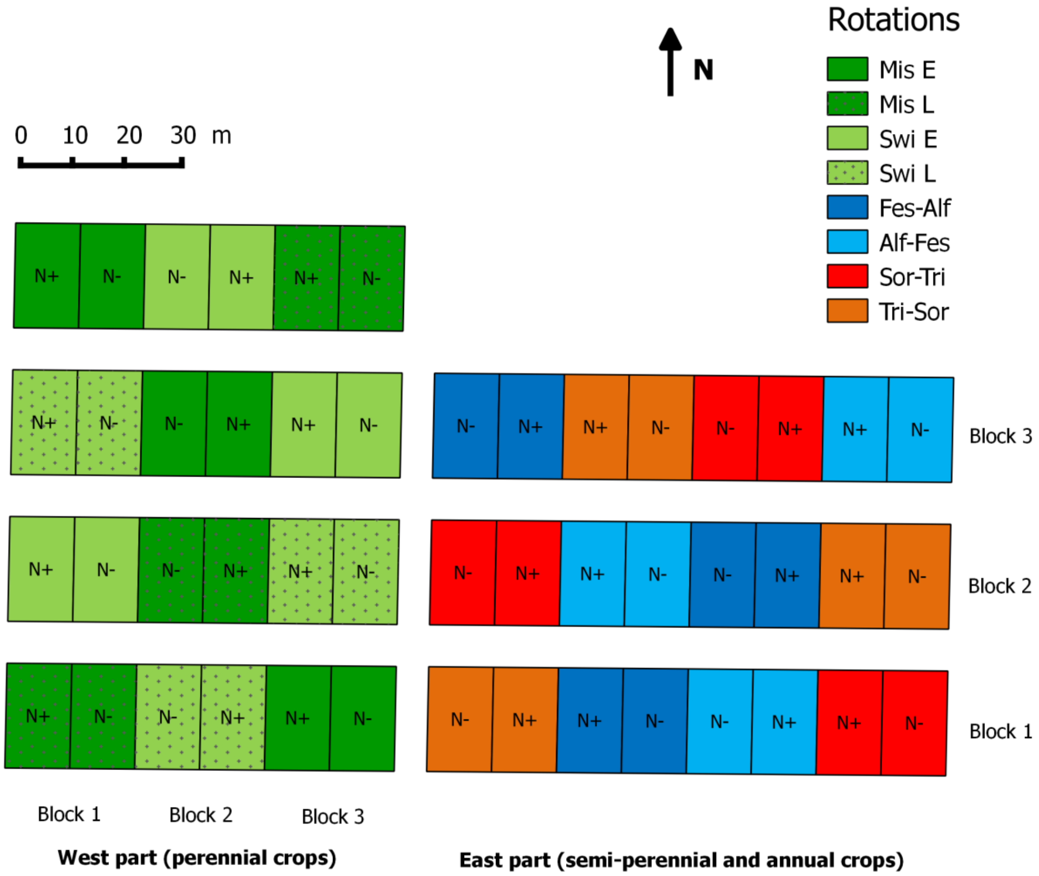


Fig. S1. Map representing the experimental design of the B&E long-term experiment (see Table 1 for abbreviations and Table 2 for fertiliser-N rates). All plots are 12 × 30 m (360 m^2) and the whole field is 2.7 ha

Table S1. Physical and chemical soil characteristics measured in 2006 for the two parts of the field trial. 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)

Table S2. Statistical analysis of the effects of rotation, nitrogen fertilisation and their interaction on mean harvested biomass and mean N exported for perennial crops and annual/semi-perennial crops. Asterisks indicate probability levels: * p<0.05; ** p<0.01; *** p<0.001; NS = not significant

		Biomass		Nitrogen content	
Factor or interaction		Perennials	Other crops	Perennials	Other crops
Harvested biomass					
Rotation	1	***	***	***	***
Nitrogen	2	***	***	***	***
	1 x 2	**	**	*	**
Total crop residues					
Rotation	1	***	**	***	**
Nitrogen	2	NS	NS	*	NS
	1 x 2	NS	NS	NS	NS
Total living biomass					
Rotation	1	*	***	***	**
Nitrogen	2	NS	NS	*	NS
	1 x 2	NS	NS	NS	NS

Table S3. Biomass harvested (t DM ha^{-1}) from 2006 to 2011. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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 S4. Nitrogen exported (kg ha^{-1}) from 2006 to 2011. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. 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)

Table S5. Statistical analysis of the effects of rotation, nitrogen fertilisation and their interaction on ^{15}N recovery for perennial crops and annual/semi-perennial crops. Asterisks indicate probability levels: * p<0.05; ** p<0.01; *** p<0.001; NS = not significant

		Perennials	Other crops
Harvested biomass			
Rotation	1	**	*
Nitrogen	2		*
	1 x 2		NS
Total crop residues			
Rotation	1	**	***
Nitrogen	2		NS
	1 x 2		NS
Total living biomass			
Rotation	1	*	*
Nitrogen	2		NS
	1 x 2		NS
Soil			
Rotation	1	NS	***
Nitrogen	2		***
	1 x 2		*
Overall recovery			
Rotation	1	**	NS
Nitrogen	2		NS
	1 x 2		NS