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1 **The fate of cumulative applications of <sup>15</sup>N-labelled fertiliser in perennial**  
2 **and annual bioenergy crops**

3

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18 **Abstract**

19 The fate of nitrogen (N) fertiliser applied to bioenergy crops is a key issue to allow high  
20 biomass production while minimising environmental impacts due to N losses. The aim of this  
21 study was to follow the fate in the soil-plant system of N fertiliser applied to perennial  
22 (*Miscanthus × giganteus* and switchgrass), “semi-perennial” (fescue and alfalfa) and annual  
23 (sorghum and triticale) bioenergy crops. Crops received <sup>15</sup>N-labelled fertiliser (urea  
24 ammonium nitrate solution) during 4 or 5 successive years on the same subplots, at a rate  
25 varying from 24 to 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Biomass production, N and <sup>15</sup>N removal at harvest were  
26 measured each year. The <sup>15</sup>N recovery in crop residues, non-harvested crop parts and soil was  
27 measured at the end of the <sup>15</sup>N-labelling period. Perennial crops had higher biomass  
28 production but generally lower <sup>15</sup>N recovery in harvested biomass than other crops,  
29 particularly when harvested late (end of winter). At the end of the 4 or 5-year period, the  
30 proportion of <sup>15</sup>N recovered in harvested biomass was 13-34% for perennials, 23-38% for  
31 semi-perennials and 34-39% for annual crops. Perennial crops stored large amounts of N in  
32 their belowground organs; the mean <sup>15</sup>N recovery in these organs was 12%, corresponding to  
33 a N storage flux of 14 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The <sup>15</sup>N recovery in soil (including crop residues) was  
34 higher for perennials (average 36%) than semi-perennials (28%) and annual crops (19%),  
35 corresponding to a N immobilisation rate of 43, 15 and 12 kg N ha<sup>-1</sup> yr<sup>-1</sup> respectively. The  
36 mean overall <sup>15</sup>N recovery in the soil-plant system was 69% in perennials, 61% in semi-  
37 perennials to 56% in annual crops, suggesting that important fertiliser losses occurred through  
38 volatilisation and denitrification. Perennial bioenergy crops had the better efficiency by  
39 storing fertiliser-N in soil organic matter and living belowground biomass used as N reserves  
40 for succeeding years.

41 **Keywords**

42 bioenergy, nitrogen fertiliser,  $^{15}\text{N}$ , nitrogen use efficiency, miscanthus, switchgrass

43

44 **Highlights**

45 • The fate of  $^{15}\text{N}$ -labelled fertilizer was compared in bioenergy crops over 4-5 years

46 • Perennial crops exported the smallest amounts of  $^{15}\text{N}$  in harvested biomass

47 • They stored the highest amounts of  $^{15}\text{N}$  in their belowground organs and soil+litter

48 • The overall  $^{15}\text{N}$  recovery was greater in perennials than other crops

49 • The early harvested miscanthus gave the highest overall  $^{15}\text{N}$  recovery

## 50 **1. Introduction**

51 Bioenergy production from crops has been supported to contribute to the production of  
52 renewable energy in response to the challenges of climate change and depletion of fossil  
53 resources (Don *et al.*, 2011). However, the use of conventional food crops to produce biofuels  
54 has raised a lot of concerns about its environmental consequences (*e.g.* Galloway *et al.*, 2008;  
55 Smith and Searchinger, 2012). The large nitrogen (N) requirements of these first generation  
56 bioenergy crops may be harmful to the greenhouse gas balance of biofuels (Crutzen *et al.*,  
57 2008). The development of new conversion technologies and biorefineries allows considering  
58 a wide range of new bioenergy crops (Ragauskas *et al.*, 2006; Sanderson and Adler, 2008;  
59 Somerville *et al.*, 2010). Among them, perennial C4 crops such as miscanthus and switchgrass  
60 are considered as promising because of their high biomass production with low nutrient  
61 requirements and expected low greenhouse gas emissions (Don *et al.*, 2011; Jørgensen, 2011;  
62 Monti *et al.*, 2012; Cadoux *et al.*, 2014). However, even with these crops, N fertilisation may  
63 still be necessary to maintain high yields and soil fertility on the long term (Cadoux *et al.*,  
64 2012; Monti *et al.*, 2012; Cadoux *et al.*, 2014). Fertiliser-N use efficiency of bioenergy crops  
65 is therefore a key issue to allow high biomass production while minimising environmental  
66 impacts due to N losses.

67 There are various ways to define and measure fertiliser-N efficiency. Two different  
68 approaches are widely used in the literature: (1) the apparent recovery which is based on the  
69 difference in N uptake between a crop receiving N fertiliser and a reference plot without N  
70 applied (*e.g.* Cassman *et al.*, 2002) and (2) the actual recovery or <sup>15</sup>N recovery which is the  
71 fraction of labelled N that is taken up by a crop following application of <sup>15</sup>N-labelled fertiliser  
72 (Hauck and Bremner, 1976). Both methods can give similar or dissimilar results whether or  
73 not the uptake of inorganic soil N is different between fertilised and unfertilised treatments.  
74 "Pool substitution" between fertiliser-N and soil mineral N can lead to a higher apparent than

75 actual recovery (Jenkinson *et al.*, 1985). Nevertheless, only the  $^{15}\text{N}$  method allows to  
76 determine the fate of the fertiliser-N in the different compartments of the agroecosystem (*e.g.*  
77 crop and soil) and therefore the overall losses of fertiliser-N (Gardner and Drinkwater, 2009).  
78 Few studies have analysed the fate of  $^{15}\text{N}$ -labelled fertiliser applied to bioenergy crops.  
79 Christian *et al.* (2006) and Pedroso *et al.* (2014) analysed the effect of a single  $^{15}\text{N}$   
80 fertilisation pulse during 3 successive years on miscanthus and switchgrass respectively. They  
81 found a rather low  $^{15}\text{N}$  recovery in the harvested biomass (14-39%) and that belowground  
82 organs and soil represented important N sinks. Pedroso *et al.* (2014) also pointed out the  
83 effect of crop management, *i.e.* harvest date, on the  $^{15}\text{N}$  recovery and partitioning. However,  
84 no study has compared the  $^{15}\text{N}$  recovery of different bioenergy crops at the same site.  
85 Only a few experiments have followed the recovery of  $^{15}\text{N}$  in the soil-plant system on the long  
86 term. In arable cropping systems, a small proportion of the residual  $^{15}\text{N}$ , *i.e.* the labelled  
87 fertiliser-N remaining in soil (mainly in organic form) and crop residues after harvest, is re-  
88 mineralised each year from the soil organic matter pool and can be recovered by the following  
89 crops or lost through N leaching or gaseous losses (Glendining *et al.*, 2001; Macdonald *et al.*,  
90 2002; Sebilo *et al.*, 2013). The amount of  $^{15}\text{N}$  remaining in the soil-plant system after the year  
91 of application is likely to be greater with perennial bioenergy crops than with annual crops  
92 because of the presence of perennial organs. The  $^{15}\text{N}$  stored in perennial organs can be used  
93 by the crop in the subsequent years and partly recovered at harvest, as shown by Christian *et*  
94 *al.* (2006) for miscanthus. Using cumulative applications of  $^{15}\text{N}$  in the same plots over several  
95 growing seasons could allow to integrate part of the long-term fate of the residual  $^{15}\text{N}$  and to  
96 reduce the variability in plant N uptake and fertiliser-N losses due to climate conditions and  
97 agronomical context (*i.e.* age of the crop, other stresses, etc.). In this study, we used  
98 cumulative applications of  $^{15}\text{N}$ -labelled fertiliser for four or five years to determine (1) the  
99 fate of fertiliser applied to perennial, semi-perennial and annual bioenergy crops in the soil-

100 plant system, and (2) the interaction with crop management, *i.e.* harvest date of perennial  
101 crops and N fertiliser rate for all crops. We hypothesised that perennial crops would export  
102 smaller amounts of  $^{15}\text{N}$  through harvests than the other crops but would store larger amounts  
103 of  $^{15}\text{N}$  in perennial organs and soil organic matter, leading to an equal or improved overall  $^{15}\text{N}$   
104 recovery in the soil-plant system.

## 105 2. Materials and methods

### 106 2.1. Site and experimental design

107 The study is based on an ongoing long-term experiment established in 2006 at the INRA  
108 experimental station in Estrées-Mons, northern France (49.872 N, 3.013 E) called “Biomass  
109 & Environment” (B&E). The soil is a Haplic Luvisol (IUSS Working Group WRB, 2006).  
110 Soil characteristics are given in Table S1 (Appendix A). Over the period 2006-2011, the mean  
111 annual temperature was 10.6 °C, the mean rainfall and potential evapotranspiration were 673  
112 and 737 mm yr<sup>-1</sup> respectively. Before 2006, the field had been cultivated for many years with  
113 annual crops, winter wheat being the most common crop.

114 The experiment was initiated to study the production and the environmental impacts of a wide  
115 range of bioenergy crops. It compares eight “rotations”: four with C4 perennial crops  
116 (monocultures), two with C3 “semi-perennial” crops (destroyed every two or three years) and  
117 two with C3/C4 annual crops (Table 1). The perennial crops are miscanthus  
118 (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass  
119 (*Panicum virgatum* cv. Kanlow). They are harvested either early in October (E) or late in  
120 February (L). The semi-perennial crops are tall fescue (*Festuca arundinacea*) and alfalfa  
121 (*Medicago sativa*). Annual crops are fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133)  
122 and triticale (× *Triticosecale* Wittmack). The experiment also includes two nitrogen  
123 treatments (N- and N+) with fertiliser-N rates depending on the crops (Table 2). The rationale  
124 for defining the N rates was explained by Cadoux *et al.* (2014).

125 The 2.7 ha field was divided into two parts in order to facilitate cropping operations and limit  
126 competition between plants due to differences in canopy height: (1) a split-block design in the  
127 west part for perennial crops with “rotations” in the main plots (miscanthus early, miscanthus  
128 late, switchgrass early, switchgrass late) and N fertilisation rates in the subplots (N- and N+),  
129 and (2) a split-plot design in the east part for the other crops with rotations in the main plots



130 (fescue-alfalfa, alfalfa-fescue, sorghum-triticale and triticale-sorghum) and N fertilisation  
131 rates in the subplots (N- and N+). Each of the two parts comprised three replicate blocks and  
132 24 subplots of 360 m<sup>2</sup> (Fig. S1, Appendix A). Soil analyses performed in 2006 revealed a  
133 slightly higher clay content in the west than in the east part ( $180 \pm 27$  vs.  $148 \pm 19$  g kg<sup>-1</sup> in  
134 the 0-30 cm layer, Table S1 in Appendix A).

135 At the start of the experiment, the field was mouldboard ploughed at a depth of *ca.* 25 cm.  
136 After seedbed preparation, miscanthus was planted in April 2006 (1.5 rhizome m<sup>-2</sup>) and  
137 switchgrass sown in June 2006 (seed rate = 15 kg ha<sup>-1</sup>). In 2006, perennial crops were not  
138 harvested because of the low biomass production during the first year of growth. Their  
139 aboveground biomass was cut and left on the soil surface. Semi-perennial crops were sown in  
140 2006, 2009 and 2011, usually in April. Before sowing, the previous crop (alfalfa or fescue)  
141 was destroyed in late autumn with a cultivator and a disc harrow (15 cm deep) in 2008 and  
142 mouldboard ploughed (*ca.* 22 cm deep) in 2010. These crops were harvested in two or three  
143 cuttings depending on years, with the last cut in October. Annual crops were tilled  
144 superficially (12-15 cm deep) without inversion ploughing. Sorghum was sown in late May  
145 and harvested in late September. Triticale was sown in mid-October and harvested in late July  
146 or early August. The N fertiliser was surface-applied from 2007 onwards as UAN solution  
147 (urea ammonium nitrate) containing 390 g N l<sup>-1</sup> (50% urea, 50% NH<sub>4</sub>NO<sub>3</sub>). Perennial crops  
148 received a single annual application in late April. Fescue received N fertiliser at the beginning  
149 of each cycle of regrowth and seedling crops were not fertilised before the first cut, so that the  
150 total N rate varied between years. Sorghum was fertilised just before sowing and triticale in  
151 March at mid-tillering for N- and N+ treatments and in late April at the beginning of stem  
152 elongation for N+. Further details about crop management are given by Cadoux *et al.* (2014).  
153 <sup>15</sup>N-labelled UAN fertiliser, uniformly labelled on urea, NH<sub>4</sub> and NO<sub>3</sub>, was applied to a  
154 subplot of 36 m<sup>2</sup> located north of each plot from 2007 to 2010 (perennial crops) or 2011

155 (other crops). Simultaneously, the unlabelled UAN was added at the same rate in the rest of  
156 the plot. The labelled UAN solution was applied with a CO<sub>2</sub>-pressurised hand sprayer to  
157 mimic the concentration and volume of liquid fertiliser applied in the rest of the plot. The  
158 isotopic excess of the labelled fertiliser varied between treatments in order to apply the same  
159 amounts of <sup>15</sup>N per surface area in all treatments (Table 2).

160

## 161 2.2. Sampling and analytical procedures

### 162 2.2.1. Aboveground biomass at harvest

163 Harvested crop production was measured every year from 2006 to 2011. On each harvest  
164 date, the aboveground biomass was collected manually in one micro-plot inside the <sup>15</sup>N-  
165 labelled subplot. The size of the micro-plot depended on the crops, according to the amount of  
166 biomass produced per unit area and stand homogeneity: 3.84 m<sup>2</sup> for miscanthus (six plants),  
167 2.5 m<sup>2</sup> for switchgrass, *ca.* 3.6 m<sup>2</sup> for sorghum and *ca.* 5 m<sup>2</sup> for fescue, alfalfa and triticale.  
168 The cutting height was 7 cm for all crops. The fresh biomass was weighed and a  
169 representative subsample was dried at 65 °C for 96 h to determine the dry matter content and  
170 ground before analysis. In order to better take into account canopy variability of miscanthus,  
171 the measured biomass was corrected by the number of stems determined in a wider  
172 undisturbed area of 25 m<sup>2</sup> according to Strullu *et al.* (2011). The N concentration and <sup>15</sup>N  
173 abundance were determined using an elemental analyser (FLASH EA 1112 series, Thermo  
174 Electron, Bremen, Germany) coupled to an isotope ratio mass spectrometer (DELTA V  
175 Advantage, Thermo Electron, Bremen, Germany).

176

### 177 2.2.2. Soil

178 The soil was sampled on two dates: at the beginning of the experiment in May 2006 to  
179 measure initial <sup>15</sup>N excess and at the end of the <sup>15</sup>N-labelling period, *i.e.* in March 2011 for

180 perennial crops (west part of the field trial) and March 2012 for other crops (east part of the  
181 field trial). Soil cores of 8 cm diameter were extracted with depth increments of 20 cm and  
182 inserted into plastic tubes using a powered soil corer (Humax soil sampler, Switzerland). In  
183 2006, two soil cores were taken in each plot down to 40 cm depth. In 2011 and 2012, six soil  
184 cores were taken in each plot down to 60 cm. All cores were located inside the labelled  
185 subplot in a 2.6 m<sup>2</sup> micro-plot and taken in intra-row and inter-row zones.

186 From 2006, the ploughing depth was reduced from ca. 30-35 cm to less than 25 cm in all  
187 treatments. The old ploughing depth (referred to below as *Y*) was identified in the soil cores  
188 on each sampling date by detecting changes in soil colour and structure. Soil cores removed  
189 from the plastic tubes in the laboratory were divided into five layers (0-5, 5-20, 20-*Y*, *Y*-40  
190 and 40-60 cm) in 2011 and 2012. Coarse residues (>2 mm), roots and rhizomes were then  
191 carefully removed from the soil by handpicking. Soil samples were dried at 38 °C for 96 h,  
192 crushed through a 2 mm sieve, subsampled and finely ground with a ball mill (PM 400,  
193 Retsch, Germany) before analysis. Soil samples were analysed for total N concentration and  
194 <sup>15</sup>N abundance using an elemental analyser (EURO EA, Eurovector, Italy) coupled to an  
195 isotope ratio mass spectrometer (Delta Plus Advantage, Thermo Electron, Germany). Bulk  
196 densities were also determined at each sampling date either with steel cylinders or a dual  
197 gamma probe (LPC-INRA, France). Full details of the methodology are given by Ferchaud *et*  
198 *al.* (2015b).

199

### 200 2.2.3. *Dead and living crop biomass*

201 In order to make a complete <sup>15</sup>N balance in the soil-plant system, crop residues and living  
202 crop biomass were sampled at the same time and location as in the final soil sampling. Crop  
203 residues included dead plant parts accumulated in soil or at soil surface whereas living crop

204 biomass was composed of living aboveground material in the case of alfalfa, fescue and  
205 triticale and living belowground material (roots and rhizomes) for all crops.

206 Crop residues from perennials present at soil surface were collected just before soil sampling  
207 in 2011. Stem bases and fragments (>10 mm) as well as fallen leaves (mulch) of miscanthus  
208 late were sampled in the whole micro-plot. Small stem fragments (2 to 10 mm) and leaf debris  
209 (for miscanthus late) present at soil surface were collected in six areas of 27 × 27 cm within  
210 each micro-plot, corresponding to the location of the soil cores. Stem fragments below soil  
211 surface (>2 mm) were collected in the 8 cm diameter cores. Aboveground residues from the  
212 six areas were pooled together, as well as belowground residues from the six soil cores. The  
213 residues from semi-perennial and annual crops, buried by soil tillage, were collected in the  
214 soil cores in 2012. All residues were dried at 65 °C for 96 h, weighed and ground before  
215 analysis.

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

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

230

## 231 2.3. Calculations

### 232 2.3.1. Biomass, N content and apparent N recovery

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

235 The annual crop production and harvested nitrogen measured in labelled subplots were  
236 compared to the measurements achieved in unlabelled subplots of the experiment and  
237 presented in an earlier paper (Cadoux *et al.*, 2014). We found a good relationship between the  
238 two estimates although the N content was slightly lower in labelled subplots ( $y = 0.98 x$ ,  $R^2 =$   
239  $0.93$  for biomass production;  $y = 0.94 x$ ,  $R^2 = 0.87$  for harvested N). This difference was  
240 considered acceptable. The apparent recovery of fertiliser-N ( $R_A$ , in %) was calculated as  
241 follows:

$$242 \quad R_A = \frac{T_N - T_0}{F}$$

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

247

### 248 2.3.2. Actual $^{15}\text{N}$ recovery

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

251 
$$Ndff = T \cdot \frac{(p - q)}{(f - q)}$$

252 where  $T$  is the amount of N in the labelled crop part or soil layer (kg N ha<sup>-1</sup>),  $p$  the <sup>15</sup>N excess  
253 atom fraction in the labelled crop part or soil layer,  $q$  the <sup>15</sup>N excess atom fraction in control  
254 crop or soil that did not receive labelled N and  $f$  the <sup>15</sup>N excess atom fraction of the labelled  
255 fertiliser. The <sup>15</sup>N recovery was calculated as the ratio between  $Ndff$  and the amount of N  
256 applied. The mean  $q$  value in aboveground biomass at harvest was derived from the analyses  
257 made in the unlabelled subplots in 2007 and 2009. For the final crop residues and living crop  
258 biomass,  $q$  was either obtained from corresponding unlabelled samples (N- treatments for  
259 perennial crops) or using the mean value calculated for aboveground biomass. The  $q$  values in  
260 soil samples were obtained with the measurements made in 2006 in the corresponding plots  
261 and soil layers.

262 The  $Ndff$  in soil samples were calculated in each treatment on an equivalent soil mass (ESM)  
263 basis (Ellert and Bettany, 1995). The “reference” soil masses used for calculations were those  
264 measured in 2006 (667, 2000, 2002, 884 and 3137 t ha<sup>-1</sup> for 0-5, 5-20, 20-Y, Y-40 and 40-60  
265 cm respectively). Detailed calculations are given by Ferchaud *et al.* (2015b) for soil organic  
266 carbon stocks and carbon isotopic composition. The same equations were applied here  
267 replacing carbon concentration by N concentration and  $\delta^{13}\text{C}$  by <sup>15</sup>N excess atom fraction. In  
268 the following, soil layers on ESM basis are called L1 to L5.

269

## 270 2.6. Statistical analyses

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

276 blocks and rotation  $\times$  blocks interaction as random factors), was used for the other crops.  
277 Rotation, nitrogen and their interaction were treated as fixed factors in both models. The *lme*  
278 function from the *nlme* package was used to fit the models (Pineiro *et al.*, 2014). Significant  
279 differences ( $p < 0.05$ ) between treatments were detected using the *lsmeans* function (Lenth,  
280 2014). The assumptions of ANOVA were checked by visual examination of the residuals  
281 against predicted values and using the Shapiro-Wilk and Levene tests. Log-transformed data  
282 or Box-Cox transformation were used if necessary to satisfy these assumptions.

### 283 3. Results

#### 284 3.1. Crop production and N removal at harvest

285 The mean harvested biomass was calculated from 2007 (first year with all crops present and  
286 beginning of N applications) to the end of the period during which <sup>15</sup>N-labelled fertiliser was  
287 applied, *i.e.* 2010 for perennial crops and 2011 for the other crops (Table 3). The mean  
288 harvested biomass represented 19.0 t DM ha<sup>-1</sup> yr<sup>-1</sup> in perennial crops and 10.3 t DM ha<sup>-1</sup> yr<sup>-1</sup>  
289 in other crops. Rotation, fertiliser-N rate and their interaction had a significant effect on  
290 biomass production for both crop types (Table S2, Appendix A). Among perennial crops, Mis  
291 E N+ was the most productive, yielding 26.6 ± 2.6 t ha<sup>-1</sup> yr<sup>-1</sup>. Miscanthus produced generally  
292 more than switchgrass, particularly the early harvest (E) treatments. An interaction between  
293 harvest date and N fertilisation was observed: biomass production was significantly higher in  
294 N+ than in N- for E treatments whereas N fertilisation had no significant effect for L  
295 treatments. Among semi-perennial and annuals crops, Tri-Sor N+ was the most productive  
296 treatment with 12.6 ± 1.3 t ha<sup>-1</sup> yr<sup>-1</sup>. The higher level of fertilisation (N+) significantly  
297 enhanced biomass production in annual crops compared to N-, but not in semi-perennials  
298 crops. Fescue alone had a small and significant response to N rate (+1.5 t ha<sup>-1</sup> yr<sup>-1</sup> in N+).  
299 Annual data are given in Table S3 (Appendix A).

300 The amount of N exported at harvest varied widely, from 38 ± 10 kg ha<sup>-1</sup> yr<sup>-1</sup> in Mis L N- in  
301 2007-2010 to 228 ± 15 kg ha<sup>-1</sup> yr<sup>-1</sup> in Alf-Fes N+ in 2007-2011 (Table 3). It was significantly  
302 affected by rotation, fertiliser-N rate and their interaction (Table S2, Appendix A). Fertilised  
303 perennial crops exported systematically higher amounts of N than unfertilised ones (+24 kg  
304 ha<sup>-1</sup> yr<sup>-1</sup> on average). The amount of N exported at harvest was greater in early than in late  
305 harvest treatments, particularly with miscanthus. It was high for semi-perennial crops (204 kg  
306 ha<sup>-1</sup> yr<sup>-1</sup> on average) but did not change significantly with N fertilisation. On the contrary,



307 fertilised annual crops (N+) exported more N than low fertilised ones (+50 kg ha<sup>-1</sup> yr<sup>-1</sup> on  
308 average). Annual data are given in Table S4 (Appendix A).

309

### 310 3.2. Dead and living crop biomass

311 The amount of crop residues found at soil surface or within soil at the end of the <sup>15</sup>N-labelling  
312 period was much higher in perennial than in other crops: 13.2 vs. 2.4 t DM ha<sup>-1</sup> on average  
313 respectively (Table 4). It did not change significantly with N fertilisation for perennials and  
314 other crops (Table S2, Appendix A). The amount of aboveground residues was particularly  
315 important in Mis L because of the presence of senescent leaves accumulated in mulch at soil  
316 surface. The total living crop biomass was also much higher in perennial crops: rhizomes and  
317 roots (0-60 cm) of perennial crops represented 12.9 to 24.7 t DM ha<sup>-1</sup> (18.5 t ha<sup>-1</sup> on average)  
318 in March 2011 whereas the total living crop biomass of the other crops was only 0.4 to 6.7 t  
319 DM ha<sup>-1</sup> in 2012.

320 The N content in crop residues was significantly higher in N+ than in N- for perennial crops  
321 but not for the other crops (Table 5). It was higher in Mis L than in the other perennial  
322 treatments (105 vs. 48 kg N ha<sup>-1</sup> respectively on average). Residues of semi-perennial and  
323 annual crops contained 5 to 30 kg N ha<sup>-1</sup>. Large amounts of nitrogen were stored in rhizomes  
324 and roots of perennial crops: 264 kg ha<sup>-1</sup> in Mis L and 148 kg ha<sup>-1</sup> in Mis E. A greater amount  
325 of N in the L treatment was also found for switchgrass but was not significant (p<0.05). N  
326 fertilisation increased N stocks in belowground organs of miscanthus and switchgrass by 65  
327 kg ha<sup>-1</sup> on average. Nitrogen was mainly stored in rhizomes for miscanthus (73%) and in roots  
328 for switchgrass (65%). The N content in the living biomass of the other crops ranged from 21  
329 kg ha<sup>-1</sup> (Sor-Tri: triticale sown in October 2011) to 158 kg ha<sup>-1</sup> (Fes-Alf: fescue sown in April  
330 2011) and did not differ significantly between N- and N+ (Table S2, Appendix A).

331

### 332 3.3. *Ndff* and <sup>15</sup>N recovery in the exported biomass

333 The amount of N derived from fertiliser exported at harvest was calculated each year from  
334 2007 to 2011 (Table 6). From 2008 onwards, the *Ndff* could derive either from the fertiliser  
335 applied during the same year or from preceding applications because the <sup>15</sup>N-labelled fertiliser  
336 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<sup>-1</sup>), except in 2011 for  
337 fescue and alfalfa N+ (5.6 kg ha<sup>-1</sup> on average), showing that the carry over effect of fertiliser  
338 was much smaller than its direct effect. The *Ndff* tended to increase with time for miscanthus  
339 but not for switchgrass. On average over the whole period, *Ndff* in the exported biomass  
340 represented 26, 18 and 28 kg N ha<sup>-1</sup> yr<sup>-1</sup> for perennial, semi-perennial and annual crops  
341 respectively. This corresponds to 28-30% of the exported N for perennial crops, 3-17% for  
342 semi-perennials and 11-37% for annual crops. The exported N derived from other sources  
343 (soil and atmosphere) was greater: 62, 186 and 65 kg ha<sup>-1</sup> yr<sup>-1</sup> for perennial, semi-perennial  
344 and annual crops respectively.

346 The actual <sup>15</sup>N recovery in the harvested biomass was on average 21.8% for perennial and  
347 33.5% for the other crops (Table 6). It was significantly affected by the rotation (Table S5,  
348 Appendix A). Perennial crops harvested late had a significantly lower recovery than the early  
349 harvested: 13.2 ± 1.4% for Mis L vs. 34.1 ± 8.5% for Mis E. The lower <sup>15</sup>N recovery in the  
350 Alf-Fes than in the Fes-Alf rotation (24.9 vs. 39.7%) could be due to the lower yields of  
351 fescue (6.8 vs. 11.7 t DM ha<sup>-1</sup> yr<sup>-1</sup>) in this rotation. The <sup>15</sup>N recovery was significantly higher  
352 in N+ than in N- treatments (mean difference of 4.4%). For each crop independent of the  
353 rotation, the mean <sup>15</sup>N recovery was 29.9 and 33.6% for fescue N- and N+ respectively,  
354 31.4% for sorghum N+ and 32.3 and 46.0% for triticale N- and N+ respectively.

355 The <sup>15</sup>N recovery was compared to the apparent recovery ( $R_A$ ) calculated for perennial crops  
356 and sorghum (crops with an unfertilised control) (Fig. 1). The two methods gave very similar

357 results: the regression equation was  $y = 0.95 R_A$  ( $R^2 = 0.69$ ). This good agreement confirmed  
358 the veracity of the low efficiency of fertiliser-N detected with the  $^{15}\text{N}$  data.

359

#### 360 3.4. $^{15}\text{N}$ recovery in dead and living crop biomass

361 A significant share of the  $^{15}\text{N}$  fertiliser was found in residues of perennial crops (4.2% on  
362 average) whereas it was almost negligible for the other crops (0.3%, Table 7). As expected,  
363 the  $^{15}\text{N}$  recovery in crop residues was higher in Mis L (6.6%) than in the other perennial  
364 crops. The  $^{15}\text{N}$  recovery in the living biomass of perennial crops (belowground organs) was  
365 also important. It was higher in Mis L (17.5%) than in other perennials (9.8% on average).  
366 The *Ndff* was mainly located in rhizomes for miscanthus and in roots for switchgrass. In spite  
367 of their deep rooting system (Ferchaud *et al.*, 2015a), miscanthus and switchgrass allocated a  
368 very small fraction of fertiliser in roots below 40 cm (0.2% on average). Finally the  $^{15}\text{N}$   
369 recovery in the living biomass of semi-perennial and annual crops (above and belowground)  
370 was low: 0.3 to 1.2%. The whole  $^{15}\text{N}$  recovery in dead and living crop biomass ranged from  
371 11.2 to 24.1% in perennial crops and 0.5 to 1.3% in the other crops. The *Ndff* represented 27-  
372 32% of the total N content in dead and living biomass of perennial crops, and 1-15% for the  
373 other crops.

374

#### 375 3.5. $^{15}\text{N}$ recovery in soil

376 The average  $^{15}\text{N}$  recovery of labelled fertiliser in all soil layers (L1-5, *ca.* 0-58 cm depth) was  
377 31.7% for perennial crops and 23.1% for the other crops (Table 8), corresponding to 38 and  
378 13 kg N ha<sup>-1</sup> yr<sup>-1</sup> respectively. Under perennial crops, the  $^{15}\text{N}$  recovery did not differ between  
379 treatments whatever the soil layer and was mainly located (85%) in the two upper layers (*ca.*  
380 0-18 cm). Under semi-perennial and annual crops, the  $^{15}\text{N}$  recovery in soil was significantly  
381 affected by the rotation, the fertiliser-N rate and their interaction (Table S5, Appendix A). It

382 was higher under semi-perennial than annual crops (27.7 vs. 18.6% respectively on average in  
383 L1-5) and higher in N- than in N+ (+5.6% on average) although the difference was only  
384 significant for Sor-Tri. Similarly to perennial crops, 83% of the fertiliser recovered under  
385 annual crops was found in the upper soil layers (*ca.* 0-19 cm). It was only 62% under semi-  
386 perennial crops due to the soil ploughing event in 2011 which incorporated a part of the  
387 labelled N below 19 cm.

388

### 389 3.6. Overall <sup>15</sup>N recovery

390 The overall <sup>15</sup>N recovery in the soil-plant system at the end of the <sup>15</sup>N-labelling period, *i.e.* the  
391 sum of the labelled N exported in the harvested biomass during the four or five year period  
392 and stored in living crop biomass, crop residues and soil at the end of the period, is presented  
393 in Fig. 2. It ranged from 51.6 ± 4.4% (Sor-Tri N+) to 82.1 ± 6.5% (Mis E N+). It was  
394 significantly higher for Mis E N+ than for the other perennial crops (82.1 vs. 65.2%  
395 respectively on average). Overall <sup>15</sup>N recovery in semi-perennial and annual crops did not  
396 differ between treatments and was 58.3% on average. The unrecovered <sup>15</sup>N is attributed to  
397 losses towards the groundwater and the atmosphere, *i.e.* leaching, volatilization and  
398 denitrification. It represented a large part of the fertiliser: 17.9% for Mis E N+, 34.8% for  
399 other perennial crops and 41.7% for semi-perennial and annual crops.

400 The *Ndff* exported at harvest represented 20% (Mis L N+) to 74% (Sor-Tri N+) of the overall  
401 recovery (50% on average for all treatments). The *Ndff* stored in living crop biomass in 2011  
402 or 2012 was 17% of the overall recovery for perennial crops and only 1% for the other crops.

403 The *Ndff* stored in crop residues was 6% and <1% of the overall recovery for perennials and  
404 other crops respectively. Finally, the *Ndff* stored in soil in 2011 or 2012 ranged between 25%  
405 (Sor-Tri N+) and 59% (Alf-Fes N-) of the overall recovery (42% on average for all  
406 treatments).

407 **4. Discussion**

408 4.1. Crop production and nitrogen removal at harvest

409 *4.1.1. Crop production*

410 Perennial C4 crops were the most productive crops in this experiment. The crop yields were  
411 in the range of those reviewed in the literature by Gabrielle *et al.* (2014), except for fibre  
412 sorghum which had a lower production in our experiment compared to literature data  
413 originating from southern Europe.

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

421

422 *4.2.2. N removal at harvest*

423 As already shown previously (Cadoux *et al.*, 2014), N exported by late harvested perennial  
424 crops was particularly low because N concentration in the aboveground biomass was very low  
425 at the end of winter. This is due to N translocation in autumn (Garten *et al.*, 2010; Strullu *et*  
426 *al.*, 2011; Pedroso *et al.*, 2014). N exported by perennial crops harvested early was closer to  
427 that observed for annual crops due to the higher N concentrations and yields than in late  
428 harvest, as a result of incomplete N and C translocation. Semi-perennial crops showed the  
429 highest N removal with high N concentrations, particularly for alfalfa. This result is in  
430 accordance with previous studies showing high N concentrations in the harvested biomass of  
431 these crops (Da Silva Perez *et al.*, 2010; Kanapeckas *et al.*, 2011). However, a large part of

432 the N removed by alfalfa probably originated from the atmosphere through symbiotic N  
433 fixation (Anglade *et al.*, 2015).

434

#### 435 4.2. N content of dead and living crop biomass

##### 436 4.2.1. *Crop residues*

437 Although the biomass of crop residues was much higher in perennial than other crops, the  
438 difference was smaller for their N content because residues of perennial crops had a higher  
439 C:N ratio than other crops (85 vs. 21). The greatest amount of crop residues was found in  
440 miscanthus late: 19.9 t DM ha<sup>-1</sup> and 105 kg N ha<sup>-1</sup> (average of N- and N+). About half of this  
441 amount was contained in senescent leaves accumulated in a thick mulch at soil surface (8.1 t  
442 DM ha<sup>-1</sup> and 50 kg N ha<sup>-1</sup>) and the rest was located in stem residues. The values obtained in  
443 our study for the leaf mulch were almost identical to those measured by Amougou *et al.*  
444 (2012) one year earlier in the same experiment and close to the measurements of Christian *et*  
445 *al.* (2006) on a 4-year-old miscanthus in late harvest (6.9 t DM ha<sup>-1</sup> and 57 kg N ha<sup>-1</sup>). The  
446 biomass and amount of N in switchgrass residues (10.9 t DM ha<sup>-1</sup> and 49 kg N ha<sup>-1</sup>) were very  
447 close to the measurements reported by Garten *et al.* (2010) for a 4-year-old switchgrass (10.7 t  
448 DM ha<sup>-1</sup> and 52 kg N ha<sup>-1</sup>).

449

##### 450 4.2.2. *Living crop biomass*

451 Perennial crops were also characterized by a large amount of N stored in living belowground  
452 organs. The biomass and N content of the miscanthus rhizomes observed in our experiment  
453 were comparable to those reported by Himken *et al.* (1997) (16 t DM ha<sup>-1</sup> and 179-227 kg N  
454 ha<sup>-1</sup>) and higher than the values reported by Christian *et al.* (2006) (9.9 t DM ha<sup>-1</sup> and 140 kg  
455 N ha<sup>-1</sup>) also in a 4-year-old plantation with late harvests. The root biomass and N content  
456 found in our experiment were intermediate between those reported by Christian *et al.* (2006)

457 and Neukirchen *et al.* (1999). For switchgrass, the amount of N stored in the rhizome was  
458 higher than that reported by Garten *et al.* (2010) but the root N content over 0-60 cm was  
459 similar. To our knowledge, the combined effects of N fertilisation and harvest date on the  
460 belowground N content of switchgrass have not been studied in other experiments. Pedroso *et*  
461 *al.* (2014) compared a two-harvest system to a single post-anthesis harvest system and found  
462 that the two-harvest system increased the N removal at harvest by 51 kg N ha<sup>-1</sup> yr<sup>-1</sup> and  
463 reduced belowground N stock over 0-100 cm by 36%, in accordance with our results.  
464 The belowground N content of semi-perennial crops measured in 2012 over 0-60 cm (47 and  
465 38 kg N ha<sup>-1</sup> for fescue and alfalfa respectively) was smaller than that of perennial crops.  
466 However, these crops were re-sown in spring 2011 and the dry spring of that year caused  
467 difficulties in alfalfa establishment. Indeed, the root biomass measured in 2012 was twice  
468 lower than that reported by Thiébeau *et al.* (2011) at the end of the first year of growth.

469

#### 470 4.3. <sup>15</sup>N recovery in the soil-plant system

##### 471 4.3.1. <sup>15</sup>N recovery in crops

472 Perennial crops harvested late were characterized both by a low <sup>15</sup>N recovery in the harvested  
473 biomass and a high <sup>15</sup>N recovery in living belowground organs. This is consistent with the  
474 observations made for total N and apparent recovery and attributed to the important N  
475 remobilisation from aboveground to belowground organs occurring in autumn. Christian *et al.*  
476 (2006) also observed that the greatest part of the labelled fertiliser taken up by miscanthus  
477 was located in the belowground biomass at the end of winter. Using their results, we could  
478 calculate that the <sup>15</sup>N recovery in the cumulative harvested biomass over 3 years was 28.4%.  
479 This is much higher than the 13.2% observed in our experiment for miscanthus late. A similar  
480 difference between the two studies was observed for the total N removed at harvest (73 vs. 38  
481 kg N ha<sup>-1</sup> yr<sup>-1</sup>) which suggests a greater N remobilisation in autumn in our experimental

482 conditions. Pedroso *et al.* (2014) found a similar effect of the harvest mode on a 2-year-old  
483 switchgrass:  $^{15}\text{N}$  recovery at harvest increased from 18.4 to 39.1% with a two-harvest system  
484 compared to a single post-anthesis harvest system, and simultaneously the  $^{15}\text{N}$  recovery in  
485 belowground organs decreased from 27.0 to 10.4%. The  $^{15}\text{N}$  recovery in harvested biomass in  
486 the single harvest treatment was consistent with the 16.6% observed in our study (switchgrass  
487 late) whereas the  $^{15}\text{N}$  recovery in belowground organs was higher than ours (27.0 vs. 10.6%).  
488 The difference is mainly due to the high  $^{15}\text{N}$  recovery in deep roots (*ca.* 10% below 60 cm).  
489 Finally, the  $^{15}\text{N}$  recovery in the whole plant including crop residues measured in our study for  
490 miscanthus late (37.3%) was smaller than the 55.8% which can be calculated using the data  
491 reported by Christian *et al.* (2006) in a 4-year-old crop. For switchgrass late, there was also a  
492 large gap between our result (31.3%) and the value reported by Pedroso *et al.* (2014) (47.8%)  
493 that could be partly due to the difference in root sampling depth.

494 Concerning semi-perennial crops, the  $^{15}\text{N}$  recovery in the harvested biomass ranged between  
495 22.6 and 41.1%, with a significant difference between the two rotations. In the literature, the  
496  $^{15}\text{N}$  recovery by forage crops has been mainly studied in perennial ryegrass (*Lolium perenne*).  
497 Reported values of  $^{15}\text{N}$  recovery at harvest range generally between 50 and 60% (Whitehead  
498 and Dawson, 1984; Webster and Dowdell, 1985; Bristow *et al.*, 1987; Stevens and Laughlin,  
499 1989). However, smaller values have been observed by Dawson and Ryden (1985) (11 to  
500 48%) who showed an effect of the date of application, the  $^{15}\text{N}$  recovery at harvest being  
501 higher for spring than for summer or autumn applications. These authors also showed that  $^{15}\text{N}$   
502 recovery in summer was much smaller in case of water stress. In our experiment, the lower  
503 fescue yields observed in 2009 and 2010 and the equivalent repartition of the N applied  
504 between the spring, summer and autumn cuts may have reduced the  $^{15}\text{N}$  recovery at harvest.

505 The  $^{15}\text{N}$  recovery at harvest of annual crops ranged between 33.6 and 39.4% but sorghum had  
506 lower recovery (31.4%) than triticale (32.3-46.0%). Our results for sorghum fall in the lower



507 range of results reported for maize (*Zea mays*) which vary between 32 and 71% (Balabane  
508 and Balesdent, 1992; Timmons and Baker, 1992; Reddy and Reddy, 1993; Normand *et al.*,  
509 1997; Sen Tran and Giroux, 1998; Stevens *et al.*, 2005; Rimski-Korsakov *et al.*, 2012). This  
510 variability is not fully understood although <sup>15</sup>N recovery is lower for N applications at sowing  
511 than at later stages and for surface than injected applications (Jokela and Randall, 1997; Seo  
512 *et al.*, 2006). In our experiment, the timing of N application (at sowing) and the low growth of  
513 sorghum in May and June may explain the low <sup>15</sup>N recovery observed for this crop. The <sup>15</sup>N  
514 recovery measured for triticale in our study was also rather low (at least for N-) compared to  
515 previous results reported in the literature for winter wheat (*Triticum aestivum*), ranging from  
516 36 to 68% (Recous *et al.*, 1988b, 1992; Macdonald *et al.*, 1989, 1997; Powlson *et al.*, 1992;  
517 Thomsen and Christensen, 2007; Giacomini *et al.*, 2010). The <sup>15</sup>N recovery was lower for  
518 applications at tillering than at stem elongation. For example, Recous *et al.* (1988b) reported  
519 that <sup>15</sup>N recovery increased from 36% for 50 kg N ha<sup>-1</sup> applied at tillering to 55% for 100 kg  
520 N ha<sup>-1</sup> applied at stem elongation. This may explain the difference observed in our study for  
521 triticale between N- and N+ treatments because N- received 60 kg N ha<sup>-1</sup> at tillering whereas  
522 the 120 kg N ha<sup>-1</sup> for N+ were split between tillering and stem elongation.

523

#### 524 4.3.2. <sup>15</sup>N recovery in soil

525 Between 12.9 and 34.6% of the <sup>15</sup>N fertiliser applied was recovered in the soil. After N  
526 applications, the fertiliser inorganic N in soil is rapidly depleted due to plant uptake and  
527 immobilisation of N by the soil heterotrophic microflora (Bristow *et al.*, 1987; Recous *et al.*,  
528 1988a; Recous and Mchet, 1999). Microbial N is then incorporated into soil organic matter  
529 and slowly mineralised in subsequent years (Glendining *et al.*, 2001; Jenkinson *et al.*, 2004;  
530 Sebilo *et al.*, 2013). The <sup>15</sup>N recovered in soil could also derive from labelled crop residues  
531 returned to the soil after harvest (or crop destruction for fescue) and incorporated into the soil

532 organic matter (Macdonald *et al.*, 2002). Almost certainly, the great majority of the <sup>15</sup>N  
533 recovered in soil in our experiment was in organic rather than inorganic forms since residual  
534 inorganic <sup>15</sup>N is negligible at harvest time for optimal or sub-optimal N rates (Recous *et al.*,  
535 1988b; Macdonald *et al.*, 1989; Normand *et al.*, 1997).

536 For miscanthus, we measured a higher recovery in soil (28.7% over 0-60 cm) than that  
537 calculated from Christian *et al.* (2006), *i.e.* 20.6% over 0-50 cm. Pedroso *et al.* (2014)  
538 reported values for switchgrass (25 to 38% over 0-300 cm) closer to our results (34.6% over  
539 0-60 cm). Surprisingly, they found that a large part of the soil <sup>15</sup>N was located in deep soil  
540 layers, whereas our results and other studies showed that the great majority of the <sup>15</sup>N  
541 recovered in soil was found in the topsoil (Glendining *et al.*, 1997; Christian *et al.*, 2006).

542 The <sup>15</sup>N recovery measured in soil for semi-perennial crops (23.3-33.9%) falls in the range of  
543 the values reported in the previously cited studies for ryegrass (16 to 32%).

544 For annual crops, our results (12.9-24.4%) are consistent with those reported for wheat  
545 (between 9 and 36%) and maize (between 15 and 37%) in the studies cited earlier. We  
546 hypothesise that the gradient observed in our study between annual, semi-perennial and  
547 perennial crops is linked to the amount and composition of crop residues. The accumulation  
548 under perennial crops of undecomposed residues with a high C:N ratio probably created a  
549 high microbial demand for N. On the contrary, the small amount of residues returning to the  
550 soil with annual crops results in a small microbial N immobilisation, explaining the lower <sup>15</sup>N  
551 recovery in soil.

552

#### 553 4.3.3. Overall <sup>15</sup>N recovery

554 In our experiment, the overall <sup>15</sup>N recovery in the crop-soil system was rather low (average  
555 60%), except for miscanthus early (82%). The recovery by miscanthus late (66%) was smaller  
556 than that which can be calculated using data of Christian *et al.* (2006) (77%). The values

557 reported by Pedroso *et al.* (2014) for switchgrass over three growing seasons (62-72%) were  
558 quite comparable to ours (64-66%). The overall recovery that we found in semi-perennial  
559 crops (average 61%) was lower than those reported in the previously cited studies for ryegrass  
560 (66-95% with an average of *ca.* 80%). Finally, the lowest recovery was found in annual crops  
561 (average 56%), falling in the lower range of values reported over one growing season for  
562 maize (47-100%) and wheat (62-96%) averaging *ca.* 75% for both crops. In a meta-analysis  
563 of published <sup>15</sup>N field experiments on temperate climate grain crops, Gardner and Drinkwater  
564 (2009) found a mean total <sup>15</sup>N recovery of 62%, with a large variability.

565 The hypotheses provided earlier to explain the low <sup>15</sup>N recovery in fescue, sorghum and  
566 triticale can also apply to the overall recovery. Recous and Machet (1999) and Limaux *et al.*  
567 (1999) showed that any increase in plant <sup>15</sup>N uptake by winter wheat results in an increase in  
568 plant and soil <sup>15</sup>N recovery. This was confirmed by Gardner and Drinkwater (2009) who  
569 showed in their meta-analysis that practices increasing <sup>15</sup>N recovery in the crop, such as  
570 improved timing or knifed-in applications, also increased the overall <sup>15</sup>N recovery.

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

576 Losses of <sup>15</sup>N through nitrate leaching were probably low. We evaluated nitrate leaching in  
577 the site during the same period as for the <sup>15</sup>N study (Ferchaud and Mary, 2016). The mean  
578 amount of total N leached (unlabelled + labelled) below 210 cm was 2, 1 and 3 kg N ha<sup>-1</sup> yr<sup>-1</sup>  
579 for perennial, semi-perennial and annual crops respectively, which represented 2, 2.5 and 5%  
580 of the fertiliser-N inputs. Indeed, nitrate leaching was not favoured in our context with  
581 moderate winter rainfall, large soil water content and deep rooting depth (Ferchaud *et al.*,

582 2015a). However, a small part of the added  $^{15}\text{N}$  could have moved downwards in the soil and  
583 could be located between 60 and 210 cm at the time of soil sampling. This fraction was not  
584 measured and therefore not included in the  $^{15}\text{N}$  recovery.

585 Regarding gaseous losses, ammonia volatilisation could have been favoured by the type of  
586 fertiliser used in our study, *i.e.* UAN containing 50% urea N. Urea is known to increase the  
587 risk of ammonia volatilisation compared to other forms of N fertiliser because of the  
588 temporary increase in soil pH during urea hydrolysis, particularly in a slightly alkaline soil  
589 like ours. In their review, Harrison and Webb (2001) found that volatilisation represented 0-  
590 4% and 6-47% of the N applied as ammonium nitrate and urea fertilisers respectively. They  
591 suggested that volatilisation losses from UAN applications were intermediate between the two  
592 other forms. Fox *et al.* (1996) reported ammonia volatilisation losses of 22% of the N  
593 fertiliser applied as UAN on average for three years in a grain maize. Vaio *et al.* (2008)  
594 measured losses ranging from 6 to 33% of the N applied as UAN to a tall fescue. Additional  
595  $^{15}\text{N}$  losses could have also occurred through denitrification but their importance in field  
596 conditions is largely unknown.

597 **5. Conclusion**

598 This study provides an original evaluation of the fate of the N fertiliser applied to different  
599 perennial and annual bioenergy crops over 4-5 years. The fertiliser N recovery in the  
600 harvested biomass, determined using either the  $^{15}\text{N}$  or the difference method, was generally  
601 lower for perennial than other crops. The difference between crops was lower when  
602 belowground organs of perennial crops were taken into account. Fertiliser-N immobilised in  
603 soil was greater under perennial than annual crops. The overall fertiliser-N recovery (exported  
604 + stored in living and dead biomass + stored in soil) tended to be greater with perennial than  
605 other crops, consistently with our initial hypothesis, but crop management also affected the  
606 overall recovery. Treatments ranked as follows: miscanthus harvested early > other perennial  
607 crops  $\geq$  semi-perennial and annual crops. Globally, N recovery was rather low for all crops  
608 compared to achievable efficiency reported for conventional crops. It could probably be  
609 increased by improvements in cropping practices (rate, timing and form of fertiliser  
610 application). The effect of these practices and the partitioning of the N losses between  
611 leaching, volatilisation and denitrification deserve further investigations.

612

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620 **References**

621 Amougou, N., Bertrand, I., Cadoux, S., Recous, S., 2012. *Miscanthus x giganteus* leaf  
622 senescence, decomposition and C and N inputs to soil. *Global Change Biology Bioenergy* 4,  
623 698-707.

624 Anglade, J., Billen, G., Garnier, J., 2015. Relationships for estimating N<sub>2</sub> fixation in  
625 legumes: incidence for N balance of legume-based cropping systems in Europe. *Ecosphere* 6,  
626 art. 37.

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

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

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

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

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

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

644           Crutzen, P.J., Mosier, A.R., Smith, K.A., Winiwarter, W., 2008. N<sub>2</sub>O release from  
645 agro-biofuel production negates global warming reduction by replacing fossil fuels.  
646 *Atmospheric Chemistry and Physics* 8, 389-395.

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

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

653           Don, A., Osborne, B., Hastings, A., Skiba, U., Carter, M.S., Drewer, J., Flessa, H.,  
654 Freibauer, A., Hyvonen, N., Jones, M.B., Lanigan, G.J., Mander, U., Monti, A., Djomo, S.N.,  
655 Valentine, J., Walter, K., Zegada-Lizarazu, W., Zenone, T., 2011. Land-use change to  
656 bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon.  
657 *Global Change Biology Bioenergy* 4, 372-391.

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

660           Ferchaud, F., Vitte, G., Bornet, F., Strullu, L., Mary, B., 2015a. Soil water uptake and  
661 root distribution of different perennial and annual bioenergy crops. *Plant and Soil* 388, 307-  
662 322.

663           Ferchaud, F., Vitte, G., Mary, B., 2015b. Changes in soil carbon stocks under  
664 perennial and annual bioenergy crops. *Global Change Biology Bioenergy*, DOI:  
665 10.1111/gcbb.12249

666           Ferchaud, F., Mary, B., 2016. Drainage and nitrate leaching assessed during seven  
667 years under perennial and annual bioenergy crops. *BioEnergy Research*, DOI:  
668 10.1007/s12155-015-9710-2

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

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

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

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

681 Garten, C.T., Smith, J.L., Tyler, D.D., Amonette, J.E., Bailey, V.L., Brice, D.J.,  
682 Castro, H.F., Graham, R.L., Gunderson, C.A., Izaurrealde, R.C., Jardine, P.M., Jastrow, J.D.,  
683 Kerley, M.K., Matamala, R., Mayes, M.A., Metting, F.B., Miller, R.M., Moran, K.K., Post,  
684 W.M., Sands, R.D., Schadt, C.W., Phillips, J.R., Thomson, A.M., Vugteveen, T., West, T.O.,  
685 Wullschleger, S.D., 2010. Intra-annual changes in biomass, carbon, and nitrogen dynamics at  
686 4-year old switchgrass field trials in west Tennessee, USA. *Agric. Ecosyst. Environ.* 136, 177-  
687 184.

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

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



694           Glendining, M.J., Poulton, P.R., Powlson, D.S., Macdonald, A.J., Jenkinson, D.S.,  
695 2001. Availability of the residual nitrogen from a single application of N-15-labelled fertilizer  
696 to subsequent crops in a long-term continuous barley experiment. *Plant and Soil* 233, 231-  
697 239.

698           Harrison, R., Webb, J., 2001. A review of the effect of N fertilizer type on gaseous  
699 emissions. In: Sparks, D.L. (Ed.), *Advances in Agronomy*, Vol 73. Elsevier Academic Press  
700 Inc, San Diego, pp. 65-108.

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

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

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

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

710           Jenkinson, D.S., Poulton, P.R., Johnston, A.E., Powlson, D.S., 2004. Turnover of  
711 nitrogen-15-labeled fertilizer in old grassland. *Soil Science Society of America Journal* 68,  
712 865-875.

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

715           Jørgensen, U., 2011. Benefits versus risks of growing biofuel crops: the case of  
716 *Miscanthus*. *Curr. Opin. Environ. Sustain.* 3, 24-30.

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

720 Lenth, R.V., 2014. lsmeans: Least-Squares Means. [http://CRAN.R-](http://CRAN.R-project.org/package=lsmeans)  
721 [project.org/package=lsmeans](http://CRAN.R-project.org/package=lsmeans)

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

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

728 Macdonald, A.J., Poulton, P.R., Stockdale, E.A., Powlson, D.S., Jenkinson, D.S.,  
729 2002. The fate of residual N-15-labelled fertilizer in arable soils: its availability to subsequent  
730 crops and retention in soil. *Plant and Soil* 246, 123-137.

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

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

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

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

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

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

747 Powlson, D.S., Hart, P.B.S., Poulton, P.R., Johnston, A.E., Jenkinson, D.S., 1992.  
748 Influence of soil type, crop management and weather on the recovery of N-<sup>15</sup>-labeled  
749 fertilizer applied to winter-wheat in spring. *J. Agric. Sci.* 118, 83-100.

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

752 Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert,  
753 C.A., Frederick, W.J., Jr., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R.,  
754 Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. *Science*  
755 311, 484-489.

756 Recous, S., Fresneau, C., Faurie, G., Mary, B., 1988a. The fate of labelled <sup>15</sup>N urea  
757 and ammonium nitrate applied to a winter wheat crop .1. Nitrogen transformations in the soil.  
758 *Plant and Soil* 112, 205-214.

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

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

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

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

769 Rimski-Korsakov, H., Rubio, G., Lavado, R.S., 2012. Fate of the nitrogen from  
770 fertilizers in field-grown maize. Nutr. Cycl. Agroecosyst. 93, 253-263.

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

773 Sebito, M., Mayer, B., Nicolardot, B., Pinay, G., Mariotti, A., 2013. Long-term fate of  
774 nitrate fertilizer in agricultural soils. Proceedings of the National Academy of Sciences 110,  
775 18185-18189.

776 Sen Tran, T., Giroux, M., 1998. Fate of N-15-labelled fertilizer applied to corn grown  
777 on different soil types. Can. J. Soil Sci. 78, 597-605.

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

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

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

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

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

788 Strullu, L., Cadoux, S., Preudhomme, M., Jeuffroy, M.H., Beudoin, N., 2011.  
789 Biomass production and nitrogen accumulation and remobilisation by *Miscanthus x giganteus*  
790 as influenced by nitrogen stocks in belowground organs. Field Crop. Res. 121, 381-391.

791 Thiébeau, P., Beaudoin, N., Justes, E., Allirand, J.-M., Lemaire, G., 2011. Radiation  
792 use efficiency and shoot:root dry matter partitioning in seedling growths and regrowth crops  
793 of lucerne (*Medicago sativa* L.) after spring and autumn sowings. *European Journal of*  
794 *Agronomy* 35, 255-268.

795 Thomsen, I.K., Christensen, B.T., 2007. Fertilizer N-15 recovery in cereal crops and  
796 soil under shallow tillage. *Soil Tillage Res.* 97, 117-121.

797 Timmons, D.R., Baker, J.L., 1992. Fertilizer management effect on recovery of  
798 labeled nitrogen by continuous no-till. *Agronomy Journal* 84, 490-496.

799 Vaio, N., Cabrera, M.L., Kissel, D.E., Rema, J.A., Newsome, J.F., Calvert, V.H.,  
800 2008. Ammonia Volatilization from Urea-Based Fertilizers Applied to Tall Fescue Pastures in  
801 Georgia, USA. *Soil Science Society of America Journal* 72, 1665-1671.

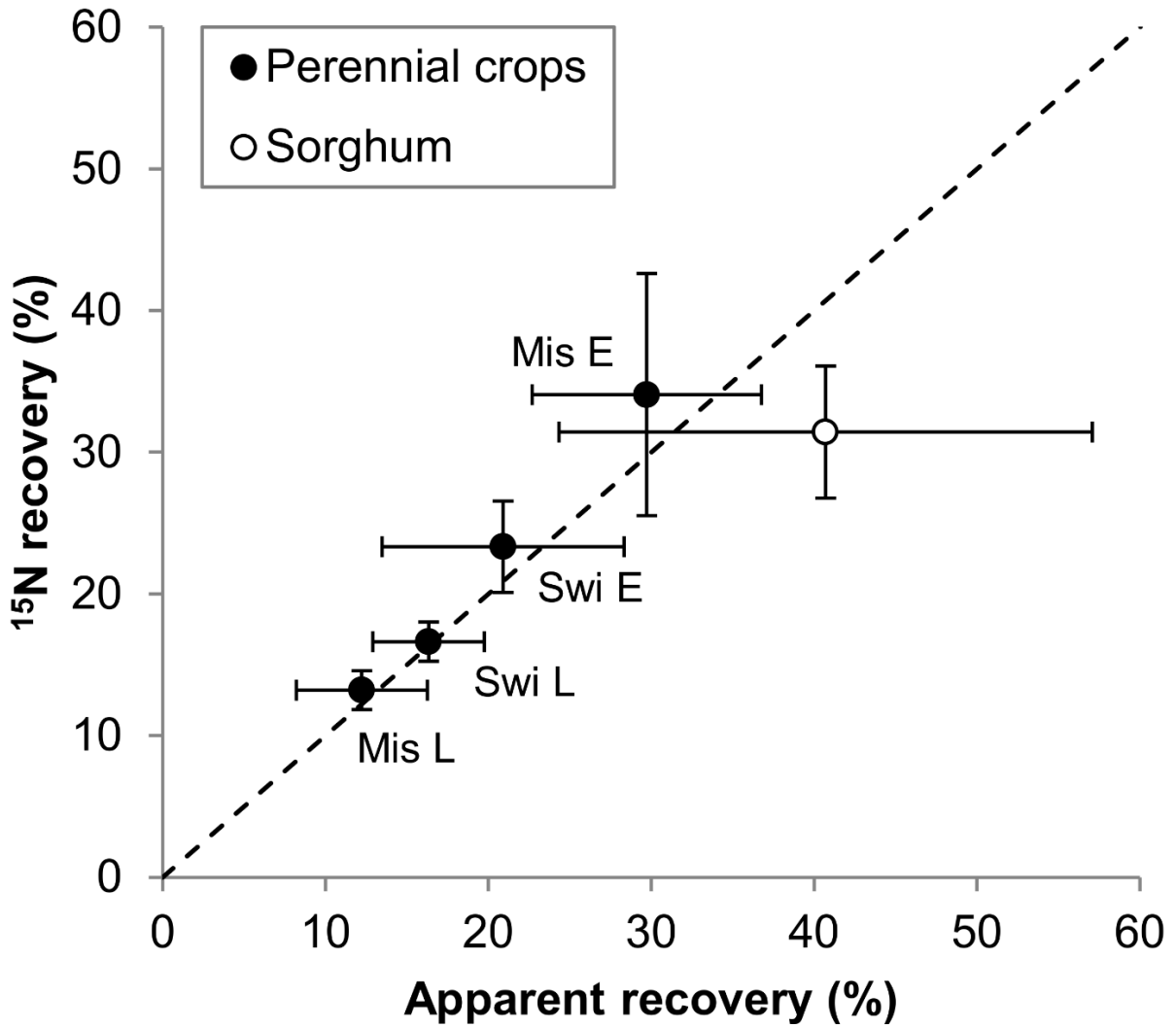
802 Webster, C.P., Dowdell, R.J., 1985. A lysimeter study of the fate of nitrogen applied  
803 to perennial ryegrass swards - soil analyses and the final balance-sheet. *Journal of Soil*  
804 *Science* 36, 605-611.

805 Whitehead, D.C., Dawson, K.P., 1984. Nitrogen, including N-15-labelled fertilizer  
806 nitrogen, in components of a grass sward. *J. Appl. Ecol.* 21, 983-989.

807 **Figures**

808

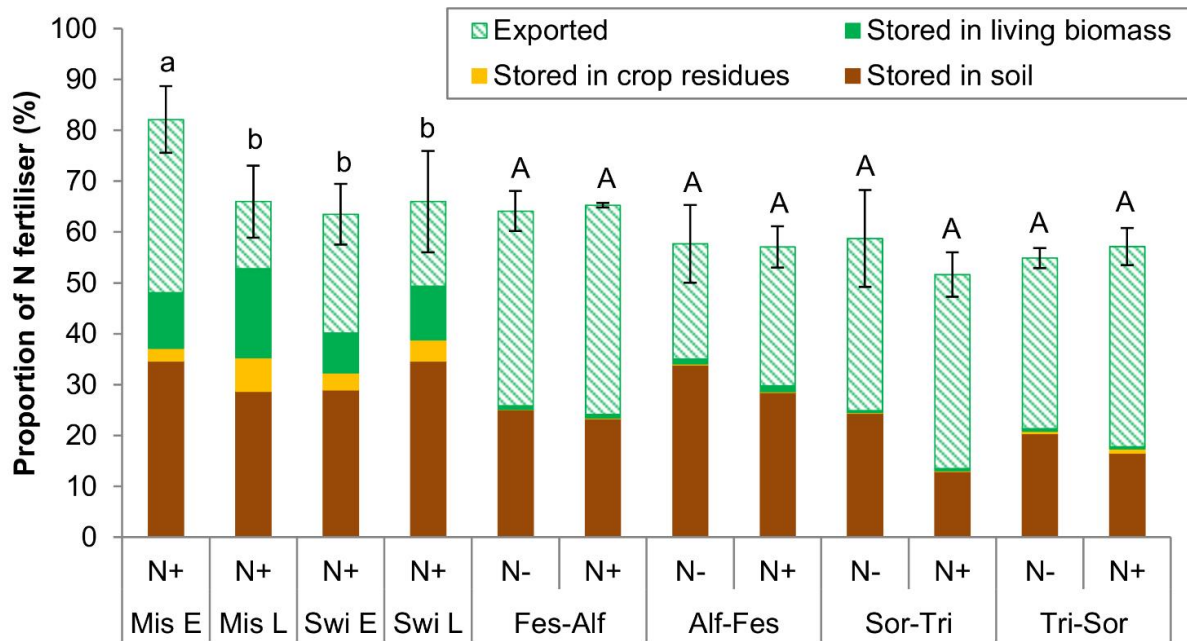
809 Fig. 1. Relationship between the  $^{15}\text{N}$  recovery and the apparent recovery (%) in the exported  
810 biomass of perennial crops (2007-2010) and sorghum N+ (2007-2011). The dashed line  
811 represents the 1:1 line. Bars represent the standard deviations.



812

813

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



819

820 **Tables**

821

822 Table 1. Rotations of the B&E long term experiment (Mis = miscanthus, Swi = switchgrass,  
 823 Fes = fescue, Alf = alfalfa, Sor = fiber sorghum, Tri = triticale, CC = catch crop; E = early  
 824 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

825 \*Rotations with catch crops (oats in 2006, rye in 2007, mustard in 2008, oat-vetch mixture in  
 826 2009 and mustard-clover mixture from 2010 to 2011) which were sown every year in late  
 827 August or early September between triticale and sorghum.

828



829 Table 2. Nitrogen fertilisation rates applied to the B&E long term experiment using <sup>15</sup>N-  
 830 labelled UAN. See Table 1 for abbreviations.

Rotation	N	N fertiliser rate (kg ha <sup>-1</sup> )						Total N applied (kg ha <sup>-1</sup> )	<sup>15</sup> 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

831

832

833 Table 3. Mean harvested biomass (t DM ha<sup>-1</sup> yr<sup>-1</sup>) and nitrogen exported (kg ha<sup>-1</sup> yr<sup>-1</sup>) from  
834 2007 to 2010 (perennial crops) or 2011 (other crops). See Table 1 for abbreviations and Table  
835 2 for fertiliser-N rates. Values in brackets are standard deviations. Different letters indicate  
836 significant differences (p<0.05) between treatments (lower case: perennial crops; upper case:  
837 other crops).

Rotation	Mean harvested biomass (t DM ha <sup>-1</sup> yr <sup>-1</sup> )				Mean N exported (kg N ha <sup>-1</sup> yr <sup>-1</sup> )			
	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

838



840 Table 4. Dead (crop residues) and living crop biomass (t DM ha<sup>-1</sup>) measured in March 2011 for perennial crops and March 2012 for semi-  
 841 perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. Values in brackets are standard deviations. Different  
 842 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)
<i>a) Total crop residues</i>	<i>11.9 (3.5) b</i>	<i>10.6 (2.6) b</i>	<i>21.9 (1.6) a</i>	<i>17.8 (2.7) a</i>	<i>8.9 (2.4) b</i>	<i>10.8 (1.2) b</i>	<i>12.5 (4.7) b</i>	<i>11.4 (2.5) b</i>
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
<i>b) Total living crop biomass</i>	<i>22.9 (6.2) ab</i>	<i>18.1 (0.5) ab</i>	<i>24.7 (3.0) a</i>	<i>21.2 (3.3) a</i>	<i>16.0 (3.2) b</i>	<i>14.2 (6.1) b</i>	<i>18.2 (6.4) b</i>	<i>12.9 (6.3) b</i>
<i>Total (a+b)</i>	<i>34.8 (9.7) b</i>	<i>28.8 (2.1) b</i>	<i>46.6 (3.0) a</i>	<i>39.0 (4.0) a</i>	<i>24.9 (5.7) b</i>	<i>25.0 (5.5) b</i>	<i>30.7 (11.1) b</i>	<i>24.3 (8.9) b</i>
	Fes-Alf		Alf-Fes		Sor-Tri		Tri-Sor	
	N-	N+	N-	N+	N-	N+	N-	N+
<i>a) Total crop residues</i>	<i>3.5 (1.2) A</i>	<i>4.0 (1.7) A</i>	<i>0.8 (0.2) B</i>	<i>0.6 (0.2) B</i>	<i>1.6 (0.3) B</i>	<i>1.6 (0.6) B</i>	<i>3.3 (0.8) A</i>	<i>3.7 (0.7) A</i>
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)				
<i>b) Total living crop biomass</i>	<i>6.0 (1.2) A</i>	<i>6.7 (0.9) A</i>	<i>2.6 (1.7) B</i>	<i>2.4 (1.2) B</i>	<i>0.4 (0.0) C</i>	<i>0.4 (0.1) C</i>	<i>1.2 (0.5) BC</i>	<i>1.2 (0.2) BC</i>
<i>Total (a+b)</i>	<i>9.5 (2.3) A</i>	<i>10.6 (0.9) A</i>	<i>3.4 (1.7) BC</i>	<i>3.1 (1.4) BC</i>	<i>2.0 (0.3) C</i>	<i>2.0 (0.5) C</i>	<i>4.5 (1.1) B</i>	<i>5.0 (0.9) B</i>

843 Table 5. N content in dead (crop residues) and living crop biomass (kg ha<sup>-1</sup>) measured in March 2011 for perennial crops and March 2012 for  
 844 semi-perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. Values in brackets are standard deviations. Different  
 845 letters indicate significant differences (p<0.05) between treatments (lower case: perennial crops; upper case: other crops). The signs - and +  
 846 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)	
<i>a) Total crop residues</i>	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
<i>b) Total living crop biomass</i>	106 (27)	b-	190 (37)	b+	216 (11)	a-	313 (71)	a+	79 (19)	b-	119 (33)	b+	120 (39)	b-	159 (77)	b+
<i>Total (a+b)</i>	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+	
<i>a) Total crop residues</i>	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)									
<i>b) Total living crop biomass</i>	126 (16)	A	140 (39)	A	61 (46)	B	55 (32)	B	15 (0)	B	15 (4)	B	26 (7)	B	27 (7)	B
<i>Total (a+b)</i>	147 (24)	A	170 (25)	A	68 (48)	B	60 (33)	B	20 (0)	C	22 (3)	C	37 (8)	BC	50 (16)	BC

847

848 Table 6. *Ndff* in the exported biomass (kg N ha<sup>-1</sup>) during each year, N derived from fertiliser  
849 and other sources and <sup>15</sup>N recovery (%) in the exported biomass over the whole period. See  
850 Table 1 for abbreviations and Table 2 for fertiliser-N rates. Values in brackets are standard  
851 deviations. Different letters indicate significant differences (p<0.05) between treatments  
852 (lower case: perennial crops; upper case: other crops). The signs - and + indicate a significant  
853 mean effect of N fertilisation (without interaction with rotations).

Rotation	N	<i>Ndff</i> in the exported biomass (kg N ha <sup>-1</sup> )					Total N exported (kg N ha <sup>-1</sup> yr <sup>-1</sup> )		<sup>15</sup> 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+

854

855 Table 7. <sup>15</sup>N recovery (%) measured in dead (crop residues) and living crop biomass in March 2011 for perennial crops and March 2012 for semi-  
856 perennial/annual crops. See Table 1 for abbreviations and Table 2 for fertiliser-N rates. Values in brackets are standard deviations. Different  
857 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)
<i>a) Total crop residues</i>		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
<i>b) Total living crop biomass</i>		10.9 (2.8) b		17.5 (2.5) a		7.9 (2.2) b		10.6 (5.4) b
<i>Total (a+b)</i>		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+
<i>a) Total crop residues</i>	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)				
<i>b) Total living crop biomass</i>	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
<i>Total (a+b)</i>	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)

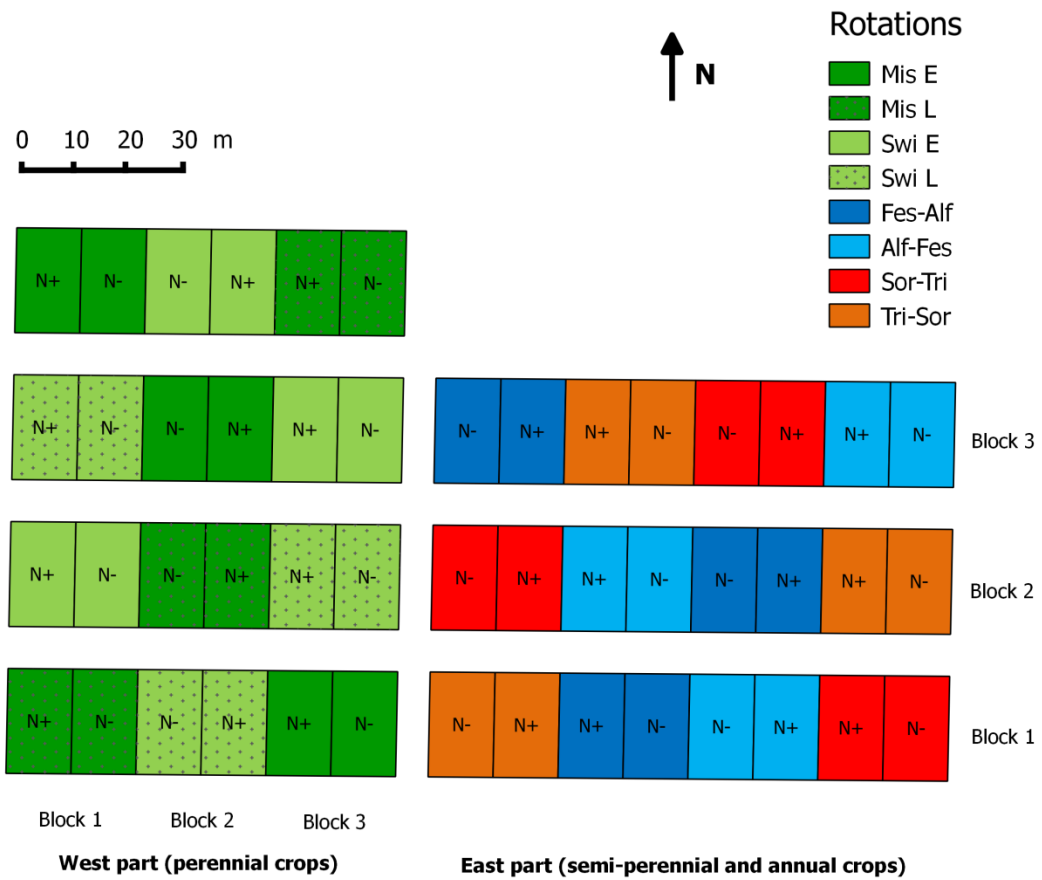
858

859 Table 8. <sup>15</sup>N recovery (%) measured in soil layers in 2011 for perennial crops and in 2012 for semi-perennial/annual crops. See Table 1 for  
 860 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  
 861 respectively. Values in brackets are standard deviations. Different letters indicate significant differences ( $p < 0.05$ ) between treatments (lower  
 862 case: perennial crops; upper case: semi-perennial and annual crops).

Layer	Soil mass (t ha <sup>-1</sup> )	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)
		<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>BC</i>	<i>BC</i>	<i>A</i>	<i>AB</i>	<i>BC</i>	<i>E</i>	<i>CD</i>	<i>DE</i>

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866

867 Fig. S1. Map representing the experimental design of the B&E long-term experiment (see

868 Table 1 for abbreviations and Table 2 for fertiliser-N rates). All plots are  $12 \times 30$  m ( $360 \text{ m}^2$ )

869 and the whole field is 2.7 ha

870

871 Table S1. Physical and chemical soil characteristics measured in 2006 for the two parts of the  
 872 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 $\mu\text{m}$ (g kg <sup>-1</sup> )	Fine silt 2-20 $\mu\text{m}$ (g kg <sup>-1</sup> )	Coarse silt 20-50 $\mu\text{m}$ (g kg <sup>-1</sup> )	Fine sand 50-200 $\mu\text{m}$ (g kg <sup>-1</sup> )	Coarse sand 200-2000 $\mu\text{m}$ (g kg <sup>-1</sup> )	CaCO <sub>3</sub> (g kg <sup>-1</sup> )	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)

873

874

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

Factor or interaction		Biomass		Nitrogen content	
		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

879

880

881 Table S3. Biomass harvested (t DM ha<sup>-1</sup>) from 2006 to 2011. See Table 1 for abbreviations  
 882 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)

883

884

885 Table S4. Nitrogen exported ( $\text{kg ha}^{-1}$ ) from 2006 to 2011. See Table 1 for abbreviations and  
 886 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)

887

888

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

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

892

893