

Miscanthus Sinensis is as Efficient as Miscanthus × Giganteus for Nitrogen Recycling in Spite of Smaller N Fluxes

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1 *Miscanthus sinensis* is as efficient as *Miscanthus × giganteus* for nitrogen
2 *recycling in spite of smaller N fluxes*

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22 **Abstract**

23 Nitrogen (N) recycling is a key mechanism to ensure the sustainability of miscanthus production with no or small
24 fertiliser inputs, but little is known on the subject in miscanthus species other than the most cultivated *Miscanthus*
25 *× giganteus*. This field experiment on *Miscanthus × giganteus* and *Miscanthus sinensis* quantified plant biomass
26 and N stock dynamics during two years. Endogenous net N fluxes, calculated by the difference in plant N content
27 throughout time, were higher in *Miscanthus × giganteus* than in *Miscanthus sinensis*. Indeed, 79 kg N ha⁻¹ and 105
28 to 197 kg N ha⁻¹ were remobilized during spring and autumn respectively for *Miscanthus × giganteus*, as opposed
29 to 13 to 25 kg N ha⁻¹ and 46 to 128 kg N ha⁻¹ for *Miscanthus sinensis*. However, their N recycling efficiency,
30 defined as the ratio between N remobilisation fluxes and the maximum above-ground N content, did not differ
31 significantly. It ranged from 8 to 27% for spring remobilisation and from 63 to 74% and 24 to 38% for autumn
32 remobilization calculated on above-ground and below-ground N respectively. Exogenous N, the main source of N
33 to constitute maximum plant N content for all genotypes, was provided by fertilisation (22 to 24%) and organic
34 matter mineralisation or other sources (43 to 59%). During winter, 50 to 56% of plant N content was lost. Abscised
35 leaves constituted an additional loss of 6 to 12%. Our results show that *Miscanthus sinensis* is as efficient as
36 *Miscanthus × giganteus* and as performant as other perennial species concerning N functioning.

37

38 **Keywords**

39 Perennial crop, lignocellulosic biomass crop, nitrogen fluxes, nitrogen uptake, nitrogen losses, nitrogen use
40 efficiency

41

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47 The authors have no conflicts of interest to declare that are relevant to the content of this article.

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49 The datasets generated and analysed during the current study are available from the corresponding author on
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51 ***Code availability***

52 Not applicable

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58

59 **Abbreviations**

60 AP: Above-ground Parts

61 BP: Below-ground Parts

62 DM: Dry Matter

63 *M×g*: *Miscanthus × giganteus*

64 *Msin*: *Miscanthus sinensis*

65 *Msin* Goliath: *Miscanthus sinensis* Goliath

66 *Msin* Malepartus: *Miscanthus sinensis* Malepartus

67 N: Nitrogen

68 *N_{dff}*: Plant N content derived from fertiliser (kg N ha⁻¹)

69 *N_{other}*: N uptake by the plant not derived from fertiliser (kg N ha⁻¹)

- 70 N_{rec} : proportion of fertiliser-N recovered in the plant (%)
- 71 NUE : Nitrogen Use Efficiency ($\text{kg DM kg}^{-1} \text{ N}$)
- 72 NA : N content of the above-ground parts (kg N ha^{-1})
- 73 NA_1 : N content of the above-ground parts when N content in the below-ground parts is minimal (kg N ha^{-1})
- 74 NA_2 : Maximum N content of the above-ground parts (kg N ha^{-1})
- 75 NA_3 : N content of the above-ground parts when N content in the whole plant is maximal (kg N ha^{-1})
- 76 NA_4 : N content of the above-ground parts when N content in the below-ground parts is maximal (kg N ha^{-1})
- 77 NA_5 : N content of the above-ground parts in February of year n+1 (kg N ha^{-1})
- 78 NB : N content of the below-ground parts (kg N ha^{-1})
- 79 NB_0 : N content of the below-ground parts in February of year n (kg N ha^{-1})
- 80 NB_1 : Minimum N content of the below-ground parts (kg N ha^{-1})
- 81 NB_2 : N content of the below-ground parts when N content of the above-ground parts is maximal (kg N ha^{-1})
- 82 NB_3 : N content of the below-ground parts when N content in the whole plant is maximal (kg N ha^{-1})
- 83 NB_4 : Maximum N content of the below-ground parts (kg N ha^{-1})
- 84 NB_5 : N content of the below-ground parts in February of year n+1 (kg N ha^{-1})
- 85 NT_3 : Maximum N content of the whole plant (kg N ha^{-1})
- 86 NT_5 : N content of the whole plant in February of year n+1 (kg N ha^{-1})
- 87 WA : Biomass of above-ground parts (t DM ha^{-1})
- 88 WA_5 : Biomass of above-ground parts in February of year n+1 (t DM ha^{-1})
- 89 WA_{max} : Maximum biomass of above-ground parts (t DM ha^{-1})
- 90 WB : Biomass of below-ground parts (t DM ha^{-1})
- 91 WT : Biomass of the whole plant (t DM ha^{-1})

92 **1. Introduction**

93 Nowadays, environmental issues are major concerns because anthropogenic activities have accentuated global
94 warming and the degradation of ecosystems. The extraction and use of fossil resources contribute to the increase
95 in greenhouse gas emissions, and their non-renewable stocks are rapidly decreasing. In the last decades, sustainable
96 resources have been developed [1-2] and biomass is the most studied [3]. To be acceptable, lignocellulosic biomass
97 crops must satisfy several criteria: producing a lot of biomass per unit area, generating low environmental impacts
98 and avoiding competition with food crops as much as possible.

99 Some perennial crops appear to be promising, as they combine high biomass production and low environmental
100 impacts, with particularly low nitrogen (N) fertiliser requirements [4], which are known to be a major source of
101 pollution in agriculture [5-7]. The perennial rhizomatous grass *Miscanthus × giganteus* (*M×g*) is a good candidate
102 [4, 8-9]: it reaches its maximum biomass production after three to six years depending on locations [10] and
103 produces between 17 and 49 t DM ha⁻¹ at autumn harvest and 10 to 30 t DM ha⁻¹ at winter harvest [11]. It is
104 important to note that this later type of harvest requires less exogenous N thanks to nutrient recycling by the plant
105 [12-14] and through leaf fall in winter which constitutes organic mulching. In contrast, the autumn harvest of non-
106 totally senesced plants in October does not allow plants to entirely recycle nutrients [14-15] and prevents the
107 accumulation of senescent leaves on the soil surface (mulch) which otherwise limits competition with weeds [15].

108 Although there are several species within the *Miscanthus* genus [16], European miscanthus cultivation mainly
109 focuses on interspecific hybrid *M×g* cultivars derived from a single genotype both for research and biomass
110 production, resulting in low genetic variability [17-18]. This is risky in terms of production security, limits the
111 production area and restricts the possible end-uses due to a unique biomass quality. Varietal offer has to be
112 expanded to overcome these disadvantages.

113 The *Miscanthus sinensis* (*Msin*) species is interesting with regard the expansion of the varietal offer: it presents
114 huge genetic variability [19], better abiotic stress tolerance than *M×g* [20], phytoremediation activity [21] and
115 intraspecific variability concerning the occurrence dates of developmental stages and the growing season length
116 [22]. All these characteristics make it possible to enlarge the production area while maintaining decent yields, from
117 Mediterranean Europe such as Turkey to northern regions like Sweden [20, 23]. *Msin* could potentially be
118 cultivated on marginal lands with higher yields and under more stressful conditions than *M×g* [20]. *Msin* genotypes
119 also present contrasted biomass compositions which can be better adapted to different end-uses [10, 24-25].
120 Finally, sowing non-invasive triploids *Msin* seeds in comparison with planting rhizomes or plantlets of the sterile

121 *M×g* [20] can reduce crop establishment costs. Furthermore, *M×g* and *Msin* show differences in their growth
122 dynamic: while *M×g* provides a unique cohort of functional shoots at the beginning of the growing season, *Msin*
123 multiplies periods of shoot emission throughout the growing season [26-27] and a part of the latest shoots emitted
124 stays green during winter whereas all *M×g* shoots are senesced.

125 Concerning N requirements to produce biomass, Zapater *et al.* [28] showed that the critical N dilution curve, *i.e.*
126 the minimum N concentration required in shoots at a given time to maximize above-ground biomass production,
127 was the same between *M×g* and *Msin*. However, although endogenous N recycling is globally well characterized
128 for *M×g* [12-14], to our knowledge, no study dealing with *Msin* has been published. At the beginning of the
129 growing season, nutrients stored in the rhizome and roots, which constitute the below-ground parts (BP), are
130 transferred to new buds and stems, the future above-ground parts (AP), to support their initial growth. This transfer
131 is called spring remobilisation. During the senescence, nutrients in leaves and stems are withdrawn and transferred
132 to the rhizome to be stored during winter. This transfer is called autumn remobilisation. These fluxes have been
133 quantified for *M×g* in several studies, using the apparent N fluxes method, based on the comparison of N stocks
134 in the above-ground and below-ground parts at different times during the growing season [12-14, 29-30].
135 According to the different studies, spring N remobilisation ranged from 23 to 98 kg N ha⁻¹ and autumn
136 remobilisation ranged from 45 to 134 kg N ha⁻¹ during the third growing year of *M×g*.

137 The large variability found for *M×g* spring and autumn N remobilisation can be partly explained by discrepancies
138 between the different calculation methods. Beale and Long [12] and Himken *et al.* [13] considered that N spring
139 remobilisation starts at emergence, perhaps underestimating spring remobilisation because N is probably
140 transferred to new buds in formation before emergence, during the winter. Strullu *et al.* [14] considered the
141 beginning of remobilisation to take place before emergence in February. This later proposition seems to be fairer
142 when considering the beginning of spring remobilisation. In the same way, the autumn N remobilisation can be
143 calculated either from AP or BP N stocks and using different starting dates. Beale and Long [12] used BP N stock
144 and defined the starting date of autumn remobilisation as the date when BP N stock was at its minimum, in summer,
145 while Himken *et al.* [13], Dierking *et al.* [30] and Strullu *et al.* [14] used AP N stocks and considered the beginning
146 of autumn remobilisation when AP N stock was at its maximum and starting to decrease, in autumn. As the increase
147 in BP N stock in summer can reflect soil N absorption and possible storage in the rhizome, we consider that the
148 beginning of autumn remobilisation should be taken at the beginning of N withdrawal from AP, in autumn.
149 Regardless of the compartment taken into account in the calculation (AP or BP), the end of autumn remobilisation
150 was taken in February or March of the year $n+1$ for Beale and Long [12], Himken *et al.* [13] and Strullu *et al.* [14]

151 in the case of a late harvest, while Dierking *et al.* [31] preferred to use the date of maximum BP N stock in
152 November. We consider that the most accurate suggestion is to define the end of autumn remobilisation at the
153 point when the rhizome is full, *i.e.* maximum observed BP N content. Furthermore, comparing the above methods
154 based on AP stocks or BP stocks highlighted higher N fluxes when calculated based on AP N stocks rather than
155 BP N stocks. As N in the fallen leaves was taken into account in the calculation, this suggests that a part of above-
156 ground N was “lost”, as it was not found in the rhizome and roots during winter. This point regarding total N
157 content of the whole plant was already underlined by Beale and Long [12], who calculated a loss of 111 kg N ha⁻¹,
158 *i.e.* 33% of whole plant maximum N stock, between July and February of the following year in a fertilised trial.
159 Hence, all these discrepancies between studies in N apparent flux calculation and results emphasized the
160 importance of homogenising the calculation method.

161 This study deals with N management by miscanthus, with a focus on the comparison between *Mxg* and two
162 genotypes of *Msin*. The first objective of the study was to characterise and compare N endogenous recycling, *i.e.*
163 spring and autumn remobilisation periods and fluxes between *Mxg* and *Msin* by using the most relevant apparent
164 N flux calculation methods from the literature. As *Mxg* and *Msin* present the same N requirement to produce
165 biomass [28], and considering that *Mxg* produces more biomass than *Msin*, we hypothesised that the N apparent
166 fluxes of *Mxg* were higher than those of *Msin*. On the other hand, based on the same facts, we hypothesised that
167 N recycling efficiencies, *i.e.* the apparent N fluxes relative to the maximum N quantity in AP, can be equivalent
168 between species. Our second objective was to compare N management between miscanthus species, taking into
169 account not only endogenous fluxes but also exogenous N fluxes during acquisition (N uptake and fertiliser
170 recovery) and estimated N losses during autumn and winter for these three genotypes. For the reasons cited above,
171 we expected higher exogenous fluxes for *Mxg* than *Msin*, but comparable proportions of N uptake relative to
172 biomass produced, or comparable N losses relative to total N content of the plant.

2. Material and methods

1. Experimental site and trial design

The field was located in northern France, at the French National Research Institute for Agriculture, Food and Environment in Estrées-Mons (49°87 N, 3°01 E) with a deep silt loam soil (Haplic Luvisol, IUSS Working Group WRB, 2006). During the last ten years (2007-2017), the oceanic climate has been characterized by an average temperature of 11.0°C and precipitation of 640 mm. The four growing years, 2014 to 2017, corresponded to these mean values with average temperatures of 11.9, 11.3, 11.0 and 11.4°C and average precipitation of 755, 631, 675 and 531 mm, respectively. Three contrasted genotypes were planted by hand in spring 2014 at a density of 2.08 plants m⁻²: *Miscanthus × giganteus* (*M×g*) from ADAS, Yorkshire, UK, *Miscanthus sinensis* Goliath (*Msin* Goliath) and *Miscanthus sinensis* Malepartus (*Msin* Malepartus) both from Plant Estate, Netherlands. The field was divided into four parts which corresponded to the four growing years studied, in order to sample plants each year without destroying the entire trial. Within each part, the three genotypes were planted in three blocks according to a complete block design with border plants between sampling zones to maintain equal competition throughout the growing season. This study focused on the third (2016) and fourth (2017) growing years because plants were supposed to have reached their maximum biomass production under these pedo-climatic conditions.

Crop emergence was determined when 50% of the observed plants had sprouted, with at least one bud emerged. It occurred on April 5th in 2016 (third growing year). In 2017 (fourth growing year), emergence occurred at the end of March but a frost event destroyed the young shoots. Therefore, a second emergence occurred on April 23rd 2017. The entire trial was harvested each year in the early spring. Chemical control was carried out in the first year to prevent competition with weeds. All plots were irrigated during the four years of cultivation to create non-limiting growing conditions: the annual amount of water added was 68, 250, 467 and 427 mm from 2014 to 2017. At the end of the first year, the entire trial was cut down and the above-ground parts were shred and left in the field. The trial was unfertilised during the first and second years. N fertiliser was applied at the beginning of May 2016 and 2017 at the rate of 120 kg N ha⁻¹ as a urea ammonium nitrate (UAN) solution. The soil mineral N content was measured each year in March or April over 0-150 cm. It was 85, 85, 53 and 54 kg N ha⁻¹ on average in 2014, 2015, 2016 and 2017, respectively. During each plant sampling campaign, soil N content was measured in each sampling zone within a 0-30 cm depth. The Nitrogen Nutrition Index (NNI) calculated according to the critical N dilution curve [28] showed that plants had never been N deficient.

201 To determine fertilisation efficiency, a ^{15}N -labeled UAN fertiliser uniformly labelled on urea, NH_4^+ and NO_3^- , with
202 a ^{15}N excess atom fraction of 0.125%, was applied on the experimental plots used for sampling on May 11th 2016
203 and May 3rd 2017. Plant isotopic excess was measured at each sampling date and ^{15}N natural abundance was
204 analysed in control plants planted the same year, grown near the others but without fertilisation and irrigation.

205

206 **2. Plant sampling**

207 Sixteen whole plant sampling campaigns, separated into above-ground parts (AP: stems and leaves) and below-
208 ground parts (BP: rhizome and associated roots), were carried out between February and November, in 2016 and
209 in 2017, approximately every ten days during the full vegetation period. At each sampling campaign, the number
210 of shoots of every seventh plants of the sampling line was counted and the median number of shoots per plant was
211 determined for each block and genotype. For each sampling and block, the three plants whose shoot number were
212 closest to the median value were collected in the morning, providing nine sampled plants per genotype. Two
213 additional plants per genotype and block, also with a shoot number close to the median, were sampled only for
214 their above-ground organs. Hence, nine plants were sampled for below-ground parts and 15 plants for above-
215 ground parts, for each genotype, at each sampling date. The stems and leaves (AP) of each plant were immediately
216 weighed. The rhizome and associated roots (BP) of each plant were washed with cold water, dried with paper
217 towels and weighed fresh. Then, AP and BP of the plants were pooled by block and genotype. Subsamples of AP
218 and BP of each block and genotype were dried at 65°C during 96 hours and weighed to determine above-ground
219 and below-ground biomass (*WA* and *WB*), water content and dry matter. They were used to determine N content
220 and ^{15}N isotopic excess.

221 The abscised leaves were picked up weekly from the ground each year from September to February. They were
222 dried and weighed each week and pooled at the end of the year for analysis.

223 Because of a strong wind causing *Mxg* lodging in 2017, the *Mxg* plants studied were chosen among the plants that
224 remained standing and sampling was no longer representative of the plots, as competition for light was largely
225 modified for these plants. Hence, the corresponding data will not be shown.

226

3. Plant nitrogen stocks and nitrogen derived from fertiliser

All plant samples were finely ground (< 500 µm) and their N concentration was determined using an elemental analyser (FLASH EA 1112 series, Thermo Electron, Germany). ¹⁵N abundance was measured with the ANCA-IRMS technique, using the elemental analyser linked to a mass spectrometer (DELTA V Advantage, Thermo Electron, Bremen, Germany).

The plant N stocks (N accumulated in the plant) were calculated using the following equations:

$$NA = WA \cdot [N_A] \quad (1a)$$

$$NB = WB \cdot [N_B] \quad (1b)$$

$$NT = NA + NB \quad (1c)$$

where *NA*, *NB* and *NT* are the amounts of N (kg ha⁻¹) contained in the AP, BP and in the whole plant respectively; *WA* and *WB* are the amounts of dry matter (t ha⁻¹) in the AP and BP, and [*N_A*] and [*N_B*] are their N concentrations (g N kg⁻¹ DM), respectively.

The amount of N derived from the ¹⁵N fertiliser (*Ndff*, kg N ha⁻¹) in the plant (AP + BP) was determined using the slightly modified equation by Hauck and Bremner [32]:

$$Ndf = NT \frac{p-q}{f-q} \quad (2)$$

where *p* is the excess atom fraction of the labelled plant, *q* the excess atom fraction of a control plant and *f* the excess atom fraction of the labelled fertiliser.

The fertiliser-N recovery (*N_{rec}*, %), *i.e.* the proportion of the fertiliser-N recovered in the plant, was:

$$Nrec = 100 \frac{Ndf}{F} \quad (3)$$

where *F* is the amount of fertiliser-N (here *F* = 120 kg ha⁻¹).

4. Net nitrogen fluxes

According to the literature, different methods can be used to calculate net N fluxes. We chose the most relevant methods based on our observations and assumptions and compared two methods for autumn N remobilisation.

Based on the evolution of N stocks in AP and BP, we were able to define six key dates (Figure 1): date 0

252 corresponded to the dormancy phase (February) of year n ; date 1 was the time when the N content of BP (NB) was
253 minimal; date 2 was the time when the N content of AP (NA) was maximal; date 3 was the time when the N content
254 of the whole plant (NT) was maximal; date 4 was the time when NB was maximal; date 5 corresponded to the
255 dormancy phase of year $n+1$.

256 Spring remobilisation (SR , kg N ha⁻¹) corresponded to the upward transfer of N from BP to AP to support the
257 growth of new shoots at the beginning of the growing season. It was calculated according to Strullu *et al.* [14]:

$$258 \quad SR = NB_0 - NB_1 \quad (4)$$

259 where NB_0 and NB_1 represent the N content of below-ground parts at dates 0 and 1, respectively.

260 Autumn remobilisation (kg N ha⁻¹) corresponded to the downward transfer of N from AP to BP in autumn. It can
261 be calculated using two methods, according to Dierking *et al.* [31]. The first method is based on NA variations:

$$262 \quad ARa = NA_2 - NA_4 \quad (5a)$$

263 where NA_2 and NA_4 are the N content of the above-ground parts at dates 2 and 4 respectively. The second method
264 is based on NB variations:

$$265 \quad ARb = NB_4 - NB_2 \quad (5b)$$

266 where NB_4 and NB_2 are the N content of the below-ground parts at dates 4 and 2 respectively.

267 The maximum N uptake (U , in kg N ha⁻¹) was calculated as follows:

$$268 \quad U = NT_3 - NB_0 \quad (6)$$

269 where NT_3 is the N content of the whole plant at date 3.

270 Finally, N losses of the whole plant (L , in kg N ha⁻¹) were:

$$271 \quad L = NT_3 - NT_5 \quad (7)$$

272 where NT_5 is the N content of the whole plant at date 5.

273 The key dates in Figure 1 were determined for each genotype and year according to the observed dynamics of NA ,
274 NB and NT (shown in Figure 4). Dates 2 and 3 were often found at the same time. Each N flux was calculated for
275 each block, year and genotype, and then the values of the three blocks were averaged by genotype and year to
276 determine the fluxes for each genotype and each year.

277

278 **5. Nitrogen Use Efficiencies**

279 The Nitrogen Use Efficiency (*NUE*, in kg DM kg⁻¹ N) represents the amount of biomass produced per unit of N
280 accumulated in the plant. It can be calculated by considering the whole plant but we chose to consider AP to be
281 able to compare our results with other studies. Two *NUE* calculations were made according to Ra *et al.* [54], Olson
282 *et al.* [55] or Dierking *et al.* [56] at two different dates:

$$283 \quad \text{NUE}_1 = \frac{WA_{max}}{NA_2} \quad (8a)$$

284 where WA_{max} is the maximum biomass accumulated in the above-ground parts during the cycle (kg DM ha⁻¹) and
285 NA_2 is the N content of the above-ground parts at date 2;

$$286 \quad \text{NUE}_2 = \frac{WA_5}{NA_5} \quad (8b)$$

287 where WA_5 is the biomass accumulated in the above-ground parts at date 5 (kg DM ha⁻¹) and NA_5 is the N content
288 of the above-ground parts at date 5.

289 NUE_1 represents the ability of the plant to produce above-ground biomass using N remobilized in the spring and
290 N absorbed from the soil. NUE_2 is the amount of biomass that can be harvested per unit of N exported.

291

292 **6. Statistical analysis**

293 Statistical analyses were performed using R Software version 3.5.1 (R Core Team, 2018). Linear models
294 (ANOVA) and Tukey tests were used to determine: (i) differences between genotypes for each variable during the
295 third and fourth growing years with genotype and block as fixed effects and (ii) differences between years for *Msin*
296 Goliath and *Msin* Malepartus with the year as a fixed effect. It is important to note that possible differences between
297 years could be attributed to age, climate or sampling effects that we were not able to identify. Unilateral student
298 tests were used to determine whether N fluxes were significantly different from 0.

299

3. Results

300

1. Biomass production and plant development

301 The dynamics of above-ground and whole plant biomass (*WA* and *WT*) followed the same pattern for the three
302 genotypes: it increased from emergence to reach a maximum in autumn and decreased between autumn and
303 February of the year $n+1$ (Figure 2a and c). Between February of year n and summer (June or July), below-ground
304 biomass (*WB*) decreased for *Msin* Goliath and *M×g* (Figure 2b, squares and circles) but stayed unchanged or
305 slightly increased for *Msin* Malepartus. *WB* then increased for all genotypes to reach a maximum around November
306 (later than the observed WA_{max}) (Figure 2b). *M×g* produced about twice as much *WA* than *Msin* with a maximum
307 of 43 t DM ha⁻¹ (vs 26 and 18 t DM ha⁻¹ for *Msin* Goliath and *Msin* Malepartus respectively) during the third
308 growing year (2016). *WA* decreased during winter to reach 22 t DM ha⁻¹ in February for *M×g* (13 and 10 t DM ha⁻¹
309 for *Msin* Goliath and *Msin* Malepartus, respectively). These yields were similar between years for both *Msin*
310 genotypes. The *WB* of *M×g* was also much higher than that of the other genotypes: in 2016 it reached a maximum
311 of 16 t DM ha⁻¹, compared to 7 and 5 t DM ha⁻¹ for *Msin* Goliath and *Msin* Malepartus respectively.

312 *M×g* produced only one group of shoots (cohort) at emergence, reaching a maximum of 37 shoots per plant in June
313 (Figure 3, circles), which then decreased to 26 shoots per plant in August due to shoot regression. In contrast to
314 *M×g*, *Msin* Goliath and *Msin* Malepartus (Figure 3, squares and triangles) emitted an initial cohort at emergence,
315 then a second from late summer onwards which reached a high number of shoots, with a maximum of 55 shoots
316 per plant for *Msin* Malepartus and 77 for *Msin* Goliath in October in 2016. These additional cohorts were also
317 observed during the fourth growing year (2017) for *Msin* genotypes. However, while there were two periods of
318 shoot emissions in 2016, shoot emission appeared to be continuous from emergence to September or October 2017,
319 with higher maximum shoot numbers than in 2016 (69 and 107 shoots per plant for *Msin* Malepartus and *Msin*
320 Goliath respectively). *Msin* Goliath emitted more shoots than *Msin* Malepartus and seemed to emit new shoots
321 later than *Msin* Malepartus, particularly during the fourth year (2017). The highest number of shoots emitted by
322 *Msin* Goliath also corresponded to a higher *WA* than *Msin* Malepartus.

323

2. Seasonal variation of above-ground and below-ground nitrogen contents

All the studied genotypes presented similar N content dynamics during the two years of the experiment (Figure 4). N content in above-ground parts (*NA*) increased from emergence to reach a maximum level in late summer or in autumn, then decreased until February (Figure 4a). The maximum level was determined in September in 2016 and late August in 2017 (Table 1). N content in below-ground parts (*NB*) decreased from the beginning of the growing season to summer and then increased to reach a maximum level in late autumn (Figure 4b). During winter *NB* presented contrasted evolutions depending on the year: in 2016, *NB* appeared to decrease during winter whereas it remained stable in 2017. *NB* was minimal in June or July (Table 1). Whole plant N content (*NT*) evolution in time (Figure 4c) showed the same dynamics as *NA* during the vegetative season: an increase from emergence to autumn followed by a decrease.

M×g NA, *NB* and *NT* in 2016 (Figure 4a, b and c, circles) were globally higher than for both *Msin*. The maximum *NA* was 273 kg N ha⁻¹ for *M×g* versus 158 to 213 kg N ha⁻¹ for *Msin* Malepartus and Goliath, respectively. *NB* was twice as high for *M×g* as for *Msin* during the whole 2016 year. It reached a maximum of 176 kg N ha⁻¹ versus 80 kg N ha⁻¹ for *Msin*. Minimum *NB* levels were observed during the summer (date 1), and were 47 and 25 kg N ha⁻¹ for *M×g* and *Msin* respectively in 2016. *Msin NB* were higher in 2017 than in 2016 and similar between the two genotypes. This higher N quantity in 2017 corresponded to a higher biomass and suggests that *Msin* growth had not yet reached the plateau of biomass production in 2016. Interestingly, at the end of the growing season and in the February of the year *n+1* (dates 4 and 5) *NA* presented similar differences between genotypes for both years: more N remained in *Msin* Goliath's AP (47 and 63 kg N ha⁻¹ in 2016 and 2017) than in *Msin* Malepartus (about 30 kg N ha⁻¹ for both years).

3. Nitrogen fluxes and plant nitrogen functioning

Endogenous nitrogen fluxes

During both years, there was no significant difference between genotypes concerning N spring remobilisation (*SR*) fluxes (Eq. 4) and efficiencies (Table 2), probably due to the rather large variability in *WB* measurements. However, N spring remobilisation in 2016 was greater for *M×g* than *Msin*, in terms of quantity (79, 25 and 13 kg N ha⁻¹ for *M×g*, *Msin* Goliath and *Msin* Malepartus respectively) and efficiency (27%, 12% and 8% of the *NA*₂ for

352 the three species, respectively). This indicated that 33 to 59% of the N stock present in below-ground parts at the
353 end of winter (NB_0) was remobilised during spring according to the genotype. Spring remobilisation fluxes
354 appeared to be higher for *Msin* Goliath than for *Msin* Malepartus in both years, although statistically not
355 significantly different.

356 The autumn remobilisation fluxes calculated based on NA (AR_a , Table 2) did not differ significantly between
357 genotypes either, although they seemed to be higher in $M \times g$ (197 kg N ha⁻¹ versus 128 and 117 kg N ha⁻¹ for *Msin*
358 Goliath and *Msin* Malepartus in 2016). During the autumn 2016, NA decreased by 63 to 74% compared to the
359 maximum stock (NA_2), with no significant differences between genotypes. In 2017, even if the autumn
360 remobilisation flux was not significantly higher for *Msin* Goliath than for *Msin* Malepartus, the efficiency was
361 significantly lower ($p < 0.10$) for *Msin* Goliath (57%) than for *Msin* Malepartus (75%).

362 Autumn remobilisation calculated based on NB (AR_b , Table 2) was significantly higher ($p < 0.05$) for $M \times g$ (105
363 kg N ha⁻¹) than for *Msin* Goliath and Malepartus (46 and 48 kg N ha⁻¹ respectively). A significant difference ($p <$
364 0.10) was also found in terms of efficiency (*i.e.* the proportion of NA_2) which was higher for $M \times g$ (38%) than for
365 *Msin* Goliath (24%). For *Msin*, autumn remobilisation calculated with this method appeared to be lower in 2017
366 than in 2016. The difference between years was significant for *Msin* Goliath ($p < 0.05$). In 2017, *Msin* Goliath
367 stored significantly less N into BP (21 kg N ha⁻¹ *i.e.* 10% of NA_2) than *Msin* Malepartus (37 kg N ha⁻¹ *i.e.* 17% of
368 NA_2).

369 Autumn remobilisation calculated based on NB was systematically lower than autumn remobilisation calculated
370 based on NA . In 2016, the increase in NB corresponded only to 40-56% of the decrease in NA during the same
371 period, with no significant difference between genotypes. It was even lower for both *Msin* in 2017 (18% and 23%
372 for *Msin* Goliath and Malepartus respectively).

373

374 ***Exogenous nitrogen fluxes and whole plant nitrogen balance***

375 Nitrogen uptake (U) was calculated using NT and NB as indicated in Eq. 6. In 2016, it reached 243 kg N ha⁻¹ for
376 $M \times g$, 183 kg N ha⁻¹ for *Msin* Goliath and 159 kg N ha⁻¹ for *Msin* Malepartus (Table 3). Even if there was no
377 significant difference between the two years, U seemed to be higher for both *Msin* in 2017 than in 2016, with 213
378 and 240 kg N ha⁻¹ for *Msin* Goliath and *Msin* Malepartus respectively.

379 In 2016, the N derived from fertiliser (N_{dff}) was significantly higher ($p < 0.10$) for $M \times g$ (87 kg N ha⁻¹) than for
380 both $Msin$ (57 and 43 kg N ha⁻¹ for $Msin$ Goliath and $Msin$ Malepartus, respectively) which suggested a better
381 utilisation of the applied fertiliser for $M \times g$ (Table 3). Indeed, N recovery (N_{rec}) reached 72% for $M \times g$ versus only
382 36% for $Msin$ Malepartus and 48% for $Msin$ Goliath. There was no difference in N_{dff} and N_{rec} between the two
383 $Msin$ genotypes in 2017 or in 2016.

384 The maximum N stock in the whole plant (NT_3) originates from endogenous N which corresponds to the initial N
385 stock in BP in February (N_{B_0}) and has been partly remobilised during spring, and from exogenous N uptake (U)
386 which either comes from N fertiliser (N_{dff}) or from other sources (N_{other}) (Figure 5). For all genotypes and both
387 growing years, endogenous N (N_{B_0} remobilised and not remobilised) constituted the smallest proportion of NT_3
388 (19-34%). It was however higher for $M \times g$ than for the two other genotypes in 2016 (34 versus 19-22%). The
389 exogenous N uptake represented 66 to 81% of NT_3 . N_{dff} in proportion to NT_3 was quite similar between genotypes
390 and years (16-24%). Finally, 43 to 65% of NT_3 came from the soil (other source than the rhizome or fertiliser, *i.e.*
391 initial soil mineral N, soil organic N mineralised, etc.) (Figure 5). This proportion was significantly higher for
392 $Msin$ Malepartus than $M \times g$ in 2016 (59 vs. 43%).

393 The N balance calculated for the whole plant (NT , Figure 4c) indicated that significant plant N losses occurred in
394 autumn and winter (Table 3). They represented large amounts: from 103 ±51 kg N ha⁻¹ in $Msin$ Malepartus 2016
395 to 207±106 kg N ha⁻¹ in $M \times g$ in 2016. Through statistical analyses, we verified that these losses were significantly
396 different from 0. It is interesting to note that, when expressed as a proportion of the maximum NT (NT_3), these
397 losses were rather similar between genotypes and years (42 to 56%). These calculated N losses did not take into
398 account the N measured in abscised leaved during autumn and winter. The cumulative biomass of abscised leaves
399 varied from 3 to 4 t DM ha⁻¹ between genotypes and years, with a significant difference between $Msin$ Malepartus
400 and $M \times g$ in 2016 (4 and 3 t DM ha⁻¹ respectively). The corresponding N quantities ranged from 19 to 27 kg N ha⁻¹
401 (Table 3) depending on genotypes and years, which represented 6 to 12% of NT_3 .

402

403 ***Nitrogen Use Efficiencies***

404 The maximum above-ground biomass found in in $M \times g$ (43 t DM ha⁻¹) was significantly higher than $Msin$ Goliath
405 (26 t DM ha⁻¹) which was itself significantly higher than $Msin$ Malepartus (18 t DM ha⁻¹) (Table 4). The above-
406 ground biomass decreased during winter and was almost halved for the three genotypes. NUE_i did not differ
407 significantly between genotypes in 2016 and 2017. In 2016, it ranged between 138 and 161 kg DM kg⁻¹ N. In 2017,

408 NUE_1 was similar to its 2016 values for *Msin* Goliath (121 kg DM kg⁻¹ N) but was significantly lower ($p < 0.05$)
409 than in 2016 for *Msin* Malepartus (97 kg DM kg⁻¹ N). The N use efficiency NUE_2 was significantly higher than
410 NUE_1 . This is due to the decrease in NA in autumn. It was higher in 2016 for $M \times g$ (653 kg DM kg⁻¹ N) than *Msin*
411 (287-338 kg DM kg⁻¹ N) and similar between years for *Msin*.

4. Discussion

Studying the N functioning (*i.e.* endogenous and exogenous N fluxes and N use linked with biomass production) of *Msin* is important in order to appreciate the fact that this species can contribute to the expansion of the varietal offer of miscanthus to produce biomass with low environmental impacts. With this fine temporal study of *M×g* and *Msin* N stocks and biomass dynamics over two years, we estimated N recycling fluxes and showed similar recycling efficiencies between these two species. This point is the first to be discussed below. We also highlighted the importance of N uptake in constituting the whole plant N stock, and consequent N losses during winter which have to be further investigated. These points were discussed in the second section below. Finally, *NUE* values calculated for these two species allowed us to compare their performance with other annual and perennial plant species in the third section of the discussion.

4.1. Nitrogen recycling efficiency is rather similar in *Miscanthus × giganteus* and *Miscanthus sinensis*

Our study consisted in a detailed temporal analysis of the evolution of biomass and N content in above-ground and below-ground parts of three contrasted miscanthus genotypes. Sixteen plant sampling campaigns were carried out each year during two years, in contrast to four to eight sampling campaigns per year in other studies [12-14, 28, 30, 31, 36]. The short time steps between the sampling campaigns allowed us to identify precisely the periods of spring and autumn N remobilisations. We found that the duration of remobilisation phases was similar between genotypes. The only difference was relative to the end of spring remobilisation which occurred about three weeks earlier for *Msin* Goliath. Assuming that autumn remobilisation starts when *NA* is at its maximum level, we found that *M×g* remobilisation began in the first part of September, whereas Strullu *et al.* [14] and Dierking *et al.* [31] observed it slightly earlier in August. But they only carried out three sampling campaigns between July and October or November respectively, whereas we carried out 11 between the beginning of July and the end of November, each year.

The N remobilisation flux found in *M×g* during spring 2016 (79 kg N ha⁻¹) was slightly lower than that obtained (98 kg N ha⁻¹) by Strullu *et al.* [14] with similar crop management. Similarly, N efficiency (relative to *NA*₂) was smaller (27% vs 44%). The N remobilisation flux found in *M×g* during autumn 2016 using *NA* (197 kg N ha⁻¹) was comparable to Strullu *et al.* [14], but three to four times greater than Dierking *et al.* [31] who observed a

440 decline in NA of 46-67 kg N ha⁻¹ depending on the fertilisation rate. The flux calculated using the evolution of NB
441 was also higher in our study (105 kg N ha⁻¹) compared to that obtained by Dierking *et al.* [31] who observed an
442 increase in NB of 40-60 kg N ha⁻¹, but their work concerned two year-old plants that produced 15-19 t DM ha⁻¹,
443 *i.e.* half as much as in our experiment, which suggests they may not have reached their maximum biomass
444 production.

445 No study has been reported in the literature concerning N fluxes in *Msin*. In our study, N fluxes were smaller in
446 *Msin* than in *Mxg*, due to lower biomass and N stocks in *Msin*. However, N remobilisation efficiency, relative to
447 the maximum above-ground N content (NA_2), did not differ significantly between *Mxg* and *Msin* both in spring
448 and autumn. Autumn N remobilisation efficiency appeared to be greater for *Msin* Malepartus than *Msin* Goliath:
449 a greater part of the N contained in the AP was transferred into the BP in *Msin* Malepartus compared to *Msin*
450 Goliath in autumn 2017 ($p < 0.10$). This difference can be explained by the presence of stay-green shoots of the
451 last cohort during winter observed in greater number for *Msin* Goliath than for *Msin* Malepartus. These green
452 shoots could explain the higher NA in winter for *Msin* Goliath in 2017 and lower autumn N remobilisation.
453 Moreover, these stay-green shoots exhibit re-growth at the time of the emergence of new buds, and may provide
454 photo-assimilates at an early stage. The emission of the shoots of the different cohorts is known to be dependent
455 on climatic conditions and particularly temperature [26]. Indeed, spring was warmer in 2017 than in 2016 (the
456 mean temperature over May-June was 17.0°C in 2017 and 15.4°C in 2016) but late summer was cooler (the mean
457 temperature in August-September was 16.5 and 18.5°C respectively). This suggests that variations in autumn N
458 remobilisation for genotypes that presented cohort phenomena such as *Msin* could depend on climate.

459 In summary, *Mxg* and *Msin* appeared to have relatively similar periods of remobilisation and similar N recycling
460 efficiency. The remobilisation fluxes (SR and AR , in kg N ha⁻¹) were higher for *Mxg* because of its higher biomass
461 and N content. Since our trial was fertilised during two years out of four, our results may not apply to unfertilised
462 marginal lands and poor soils in which further studies have to be conducted.

463

464 **4.2. Components of nitrogen uptake and fertiliser nitrogen recovery**

465 The N content of the whole plant (NT) varied widely throughout the year. Part of this variation was due to the
466 significant N uptake (U) which occurred for the three miscanthus genotypes particularly during the first part of the
467 growing season, from late May to early September. N uptake, estimated with Eq. 6, ranged from 159 to 243 kg N
468 ha⁻¹ (Table 3, Figures 4c and 5). This exogenous N flux represented 66% to 81% of the maximum whole plant N

469 stock (NT_3). It came partly from N fertiliser which corresponded to 43-87 kg N ha⁻¹ and mainly from other sources:
470 initial soil mineral N stock, irrigation water, atmospheric deposition and soil organic matter mineralisation. The
471 initial soil mineral N (measured in early spring) was rather stable between years (*ca.* 50 kg N ha⁻¹ in 0-150 cm and
472 30 kg N ha⁻¹ in 0-30 cm) and reached a minimum value of *ca.* 10 kg N ha⁻¹ in 0-30 cm (it was not measured below
473 a 30 cm depth during the growing season), so it may have contributed at least 20 kg N ha⁻¹ to the N uptake.
474 According to the mean nitrate content measured in the irrigation water, N input through irrigation represented *ca.*
475 30 kg N ha⁻¹. Atmospheric deposition represented about 9 kg N ha⁻¹ during the year [37]. If we take into account
476 these contributions (N from fertilizer, initial soil mineral N, irrigation and atmospheric deposition), there remains
477 57 to 132 kg N ha⁻¹, which would have come from soil organic N mineralisation. This range of organic N
478 mineralisation is in agreement with Mary *et al.* [38] who estimated that annual N mineralisation was around 140
479 kg N ha⁻¹ in the same soil type (but not in the same year and under bare soil). Another source of N for the plant
480 might be N-fixing bacteria. The presence of N-fixing bacteria in the miscanthus rhizosphere has been demonstrated
481 [39] but their importance for plant N-nutrition is not well known. Using a ¹⁵N experiment on first-year *M×g* plants,
482 Keymer and Kent [39] estimated that 16% of the new plant N was derived by N fixation during the growing season.
483 The fertiliser-N recovery in the whole plant in 2016 varied between 36% in *Msin* Malepartus, 48% in *Msin* Goliath
484 and 72% in *M×g*. The recovery in *Msin* was also low in 2017 (45% and 40% respectively). The lower recoveries
485 in *Msin* can be explained by a smaller N demand since *Msin* has a smaller growth than *M×g*, and/or a delay in the
486 developmental stages between genotypes, which means the date of fertiliser application for *Msin* Malepartus may
487 not have been optimal (too early), which contributed to fertiliser-N losses.

488

489 **4.3. Nitrogen balance demonstrates substantial nitrogen losses in all** 490 **species**

491 A very substantial decrease in the whole plant N content (NT) was observed between September and February for
492 all genotypes: it reached 103 ±51 to 207 ±106 kg N ha⁻¹, which corresponds to 42 to 56% of the maximum N
493 accumulated in the whole plant at the end of summer (NT_3). Indeed, the strong decrease in N accumulated in the
494 AP during autumn and winter was much greater than the increase in N stored in the BP during the same period.
495 This explains why the autumn N remobilisation calculated based on NB was much lower than the N remobilisation
496 calculated based on NA . Biomass losses also occurred during the same period (30-52% of the maximum biomass
497 of the whole plant). N losses have already been observed in miscanthus in the literature but not really investigated.

498 Calculations using data of Beale and Long [12] show that 33% of the whole plant N was lost between July and
499 February in three year-old plants of *Miscanthus × giganteus*. Himken *et al.* [13] observed a decrease of 165 to 203
500 kg N ha⁻¹ between September and February, which corresponds to 40 to 47% of NT₃ in unfertilized and fertilized
501 plants respectively. The N lost by abscised leaves, which is not included in N calculated losses, represented only
502 19 to 27 kg N ha⁻¹ in our experiment and 31 kg N ha⁻¹ in Strullu *et al.* [14], and cannot be responsible for the
503 unrecovered N. If biomass losses can be easily explained by root turnover and plant respiration, N losses
504 necessarily corresponded to N fluxes towards the soil or the atmosphere. Four main hypotheses could explain these
505 fluxes: H1) N storage in deep roots; H2) N rhizodeposition (release of organic and inorganic N from living plant
506 roots) and root turnover (due to root mortality); H3) NH₃ volatilisation into the atmosphere; H4) N₂O emissions
507 into the atmosphere.

508 H1) Roots of *M×g* and *Msin* were found down to a depth of 2.5-3.0 m (data not shown) whereas only rhizomes
509 and roots were sampled in the 30-40 first cm. However, the amounts of N contained in these roots seem to be
510 rather small. Neukirchen *et al.* [40] showed that N concentration and root dry mass decreased rapidly with depth.
511 Ferchaud *et al.* [41] found that the N content in roots of five year-old *M×g* was 56 kg N ha⁻¹ in the 0-20 cm layer
512 and only 10 kg N ha⁻¹ in the 20-60 cm layer. N storage in deeper roots was probably very low.

513 H2) The N rhizodeposition hypothesis has already been put forward by Heaton *et al.* [42] and supported by
514 Hromádka *et al.* [43] who demonstrated that autumn root exudates of *M×g* are composed of protein and are used
515 to feed the bacterial community. Rhizodeposition occurs in many plant species [44], and the amounts of N
516 rhizodeposited could be important, as in ryegrass with 94 kg N ha⁻¹ calculated over two years of cultivation [45].
517 Moreover, part of the root N also returns to the soil through root turnover. Neukirchen *et al.* [40] measured an
518 increase in the total root biomass between May and November of 3.3 t ha⁻¹ and a decrease of 2.4 t ha⁻¹ between
519 November and March. *Miscanthus* produces new roots every year [46], but the proportion of root biomass that
520 dies every year is unknown.

521 H3) The emission of N gases by plants has been put forward by many authors who observed N losses in spring
522 barley, wheat or maize. For example, N losses varying between 45 to 81 kg N ha⁻¹ were reported for maize during
523 grain filling [49]. The authors suggested that losses are linked to ammonia volatilization from the aerial parts of
524 plants [47-49]. Schjørring and Mattsson [50] concluded a two-year survey by stating that N volatilisation from
525 plants “will represent in many areas a significant input of ammonia to the atmosphere and that NH₃ losses may
526 become large enough to significantly affect crop N budgets”.

527 H4) Emissions of nitrous oxide (N₂O) directly by plants have been reported in some studies made under controlled
528 conditions. An emission rate of 0.17 and 0.11 ng N₂O g⁻¹ fresh weight week⁻¹ was measured in maize and wild
529 barley [51] and a flux of 2.8 mg N₂O-N m⁻² day⁻¹ was found in rye-grass [52]. After N fertilisation in soil, potted
530 beech (*Fagus sylvatica*) emitted between 0.4 and 2.0 µg N m⁻² leaf area h⁻¹ [53]. Chang *et al.* [54] demonstrated
531 that potted canola can emit N₂O from its aerial parts when soil is water saturated. Lenhart *et al.* [55] observed in
532 laboratory conditions that a few weeks old *Msin* produced 3 to 30 times more N₂O than other species such as maize
533 or tobacco. Even if the growing conditions are far from that of the field, further investigations have to be carried
534 out to verify this in field conditions.

535 Our results demonstrate that N uptake is the major contributor to the N accumulated in plants at the end of summer
536 and that N losses into the soil or the atmosphere can be substantial in autumn and winter. Further investigations
537 are required to elucidate the origin of such N losses. From a management point of view, the significance of these
538 losses will depend on the main processes involved. If gaseous N losses are dominant, they will have to be reduced
539 to a minimum to ensure sustainable biomass production. If losses are mainly explained by rhizodeposition and
540 root turnover, then N remains in the soil system, contributes to the build-up of soil organic matter and can be
541 available for the crop in the following years.

542

543 **4.4. *Miscanthus × giganteus* and *Miscanthus sinensis* gave as good** 544 **performances as other perennial species**

545 The low contribution of spring N remobilisation to the N accumulated in AP may lead us to believe that *M×g* and
546 *Msin* were not as efficient in terms of N recycling as expected. However, the spring remobilisation efficiency of
547 the three studied miscanthus genotypes was comparable to other herbaceous species such as big bluestem
548 (*Andropogon gerardii*) in which 46-58% of the rhizome N content was remobilised during spring [56], compared
549 to 20-59% of the *M×g* and *Msin* in our experiment. It was slightly lower than *Festuca rubra* and *Agrostis capillaris*
550 in which 34% and 45% of NA came from BP, respectively [57], compared to 8-27% in our experiment. Concerning
551 autumn remobilisation, efficiency for our *M×g*, *Msin* Goliath and *Msin* Malepartus (63-75%) was similar or even
552 higher than for big bluestem (58%) [56] or switchgrass (3-61%) [58-59]).

553 Another criterion to characterize the performance of N use by plants is the Nitrogen Use Efficiency (NUE). In our
554 experiment, NUE was calculated as the amount of above-ground biomass produced per unit of above-ground N

555 content. It did not differ significantly between $M \times g$ and $Msin$ at WA_{max} but was higher for $M \times g$ than $Msin$ in
556 February because of a more important decrease in NA for $M \times g$. Dierking *et al.* [35] calculated the NUE of four
557 clones of $M \times g$, as the ratio between WA and NA in January. Their values were much smaller than ours, with 126
558 to 297 kg DM kg⁻¹ N on average between unfertilized and fertilized treatments, in contrast to 653 kg DM kg⁻¹ N
559 in February for our $M \times g$ (NUE_2). This difference can be explained by yields half as high as ours because of younger
560 plants (one and two years old). Ra *et al.* [33] and Olson *et al.* [34] calculated NUE in different plants dedicated to
561 biomass production as the ratio between WA and NA in autumn when WA was at its maximum. According to this
562 method, our three genotypes presented NUE from 97 to 161 kg DM kg⁻¹ N (NUE_1), comparable to Johnsongrass
563 (*Sorghum halepense*), napiergrass (*Cenchrus purpureus*), sugarcane and sorghum (70 to 125 kg DM kg⁻¹ N) and
564 better than *Erianthus*, switchgrass and maize (76 to 97 kg DM kg⁻¹ N) [33]. Olson *et al.* [34] worked with a hybrid
565 of sorghum that produced 50 t DM ha⁻¹ and thus presented a higher NUE of 370 kg DM kg⁻¹ N. We conclude that
566 $M \times g$ and $Msin$ had similar N recycling efficiencies and NUE to other perennial and annual species dedicated to
567 biomass production.

568 **5. Conclusion**

569 This study provides the first experimental comparison of N pools and N fluxes (endogenous and exogenous) in
570 *Miscanthus × giganteus* and *Miscanthus sinensis*. According to our first hypothesis, *M×g* has higher net N fluxes
571 than *Msin* due to higher biomass. As a result, our second hypothesis was also verified: because of the same N
572 requirement to produce a unit of biomass between species, the latter presented similar N recycling efficiencies. As
573 it appeared to be as efficient in terms of N recycling as *Miscanthus × giganteus*, *Miscanthus sinensis* can become
574 an alternative to *M×g* for producing lignocellulosic biomass. Even if miscanthus is at least as efficient as other
575 perennial crops concerning N recycling, it is able to sustain high N uptake which is the main source of the whole
576 plant N stock at the end of summer. Consequent plant N losses were also concurrently measured at the end of the
577 growing season. Although they have already been observed in miscanthus and other plant species, the processes
578 involved have to be investigated, particularly the possible transfer on N from plant to soil through rhizodeposition
579 and root turnover. To complete the understanding of N recycling mechanism in miscanthus, particularly
580 *Miscanthus sinensis*, the link with development stages and growing season length associated with climate response
581 has to be studied to determine mechanism triggers. Finally, to avoid competition with land-use for food production,
582 miscanthus should be grown as much as possible on marginal lands. Further studies on N functioning on poor or
583 degraded soils have to be carried out to help choose the most appropriate genotypes that combine decent yields
584 and low environmental impacts.

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Figures and Tables Captions

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Fig1. Seasonal evolution of the nitrogen stocks in the above-ground (NA) and below-ground parts (NB) during the key periods for N recycling over the course of one year in Miscanthus × giganteus (based on Strullu (2011) and Dierking et al. (2017))

Fig2. Seasonal dynamics of above-ground (a), below-ground (b) and total (c) biomass in Miscanthus × giganteus (circles), Miscanthus sinensis Goliath (squares) and Malepartus (triangles) during the third (2016) and fourth (2017) growing years (mean ± standard error)

Fig3. Seasonal dynamics of shoot numbers in Miscanthus × giganteus (circles), Miscanthus sinensis Goliath (squares) and Malepartus (triangles) during the third (2016) and fourth (2017) growing years (mean ± standard error)

Fig4. Seasonal dynamics of above-ground (a), below-ground (b) and whole plant (c) nitrogen content in Miscanthus × giganteus (circles), Miscanthus sinensis Goliath (squares) and Malepartus (triangles) during the third (2016) and fourth (2017) growing years (mean ± standard error)

Fig5. Nitrogen sources at date 3 (around September) in Miscanthus × giganteus, Miscanthus sinensis Goliath and Malepartus: mean values calculated over two successive years.

Table 1. Observed dates 1 (Below-ground N stock is minimum) and 2 (Above-ground N stock is maximum) for Miscanthus × giganteus, Miscanthus sinensis Goliath and Malepartus during the third (2016) and fourth (2017) growing years. See Figure 1 for abbreviations.

787 **Table 2. Endogenous N fluxes in *Miscanthus × giganteus*, *Miscanthus sinensis* Goliath and *Malepartus*:**
788 **mean values calculated over two successive years. See Figure 1 for abbreviations. Standard errors are**
789 **indicated in parentheses. Letters indicate the results of Tukey tests applied to the comparison between genotypes**
790 **for a given year (A,B $p < 0.05$, a,b $p < 0.10$). Asterisks indicate the significant difference in N fluxes from 0 (***
791 **$p < 0.10$).**

792

793 **Table 3. Exogenous N fluxes and N balance in *Miscanthus × giganteus*, *Miscanthus sinensis* Goliath and**
794 ***Malepartus*: mean values calculated over two successive years. Standard errors are indicated in parentheses.**
795 **Letters indicate the results of Tukey tests applied to the comparison between genotypes for a given year (A,B**
796 **$p < 0.05$, a,b $p < 0.10$). Asterisks indicate the significant differences in N fluxes from 0 (* $p < 0.10$).**

797

798 **Table 4. Nitrogen Use Efficiencies (NUE) by *Miscanthus × giganteus*, *Miscanthus sinensis* Goliath and**
799 ***Malepartus*: mean values calculated over two successive years. Standard errors are indicated in parentheses.**
800 **Letters indicate the results of Tukey tests applied to the comparison between genotypes for a given year (A,B**
801 **$p < 0.05$, a,b,c $p < 0.10$).**

Figures

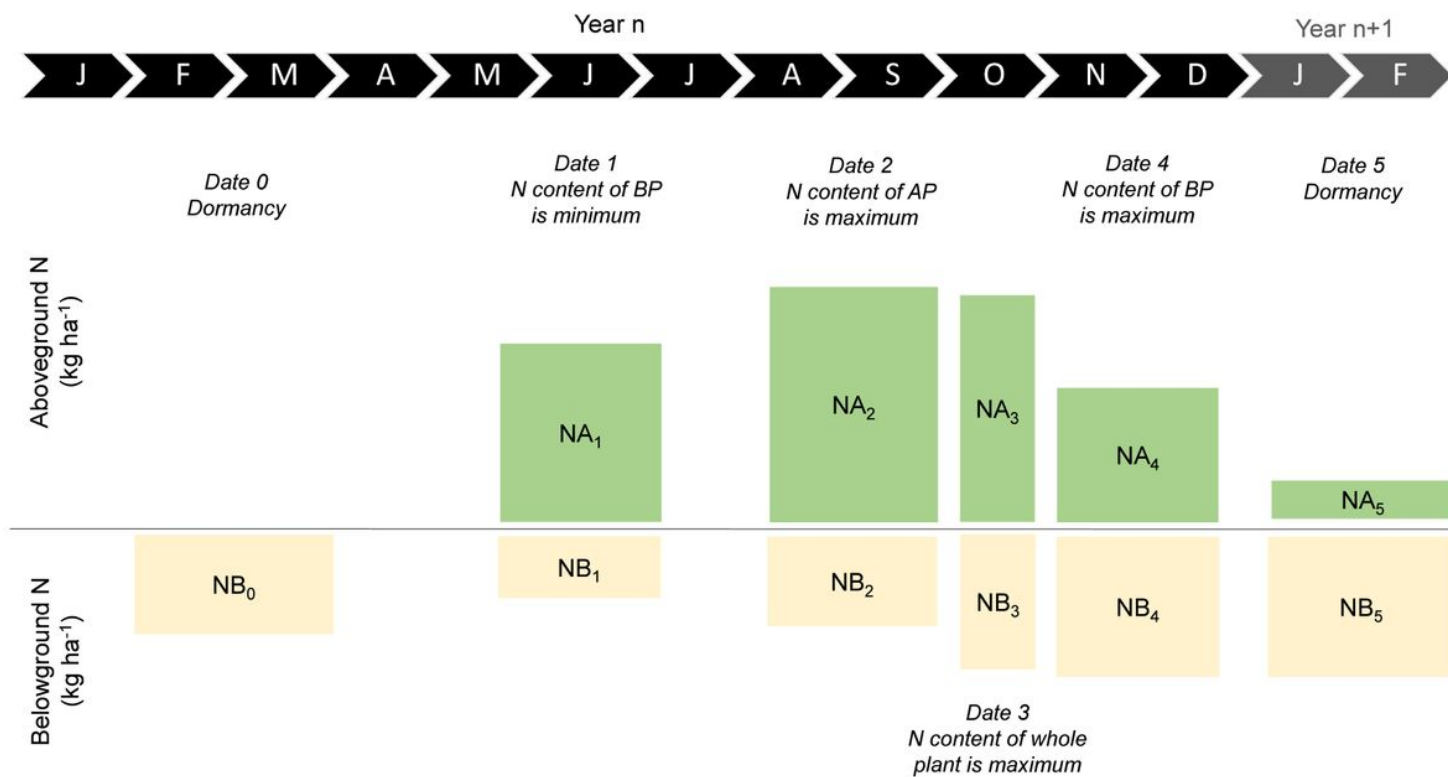


Figure 1

Seasonal evolution of the nitrogen stocks in the above-ground (NA) and below-ground parts (NB) during the key periods for N recycling over the course of one year in *Miscanthus x giganteus* (based on Strullu (2011) and Dierking et al. (2017))

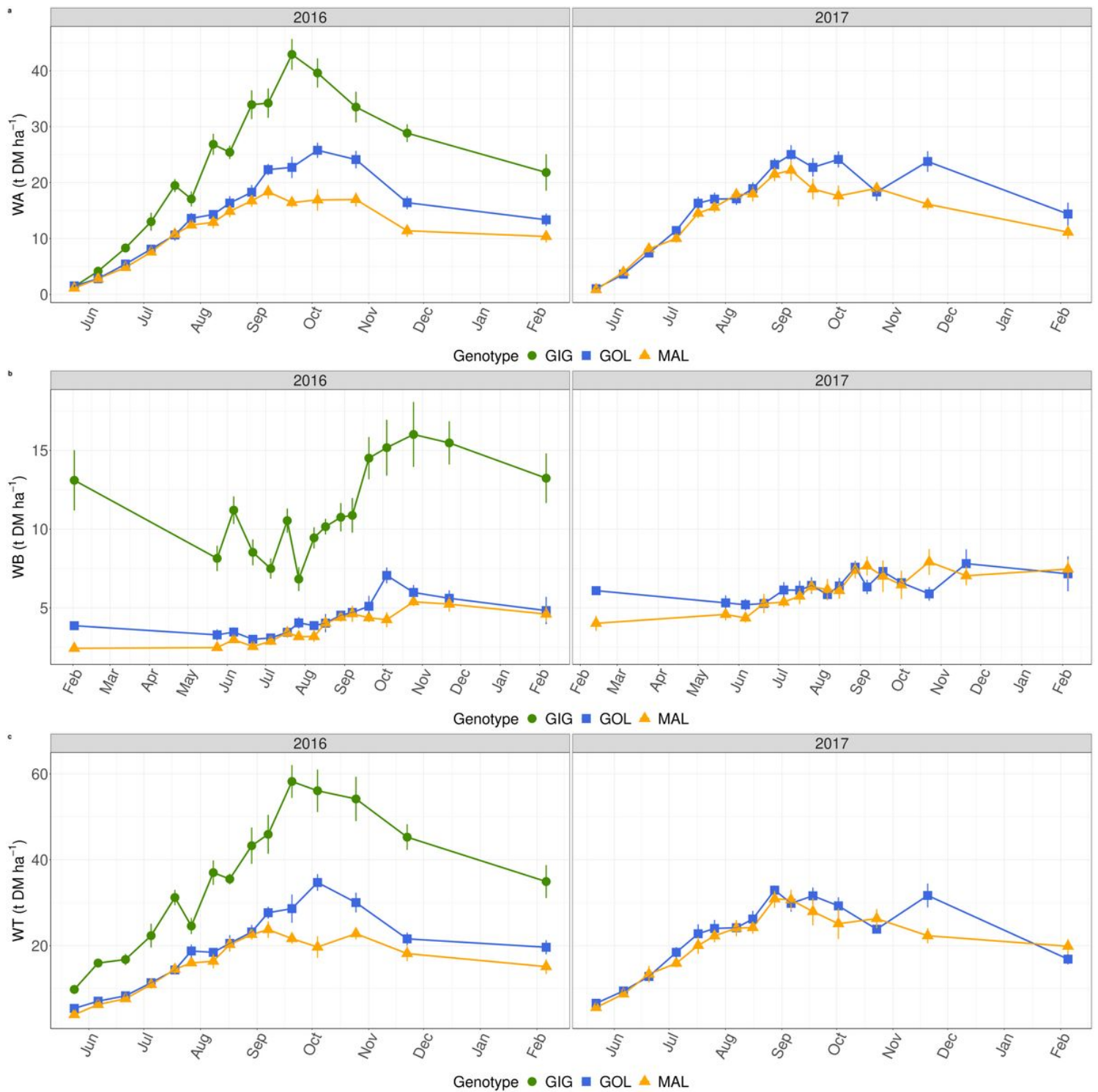


Figure 2

Seasonal dynamics of above-ground (a), below-ground (b) and total (c) biomass in *Miscanthus × giganteus* (circles), *Miscanthus sinensis* Goliath (squares) and *Malepartus* (triangles) during the third (2016) and fourth (2017) growing years (mean ± standard error)

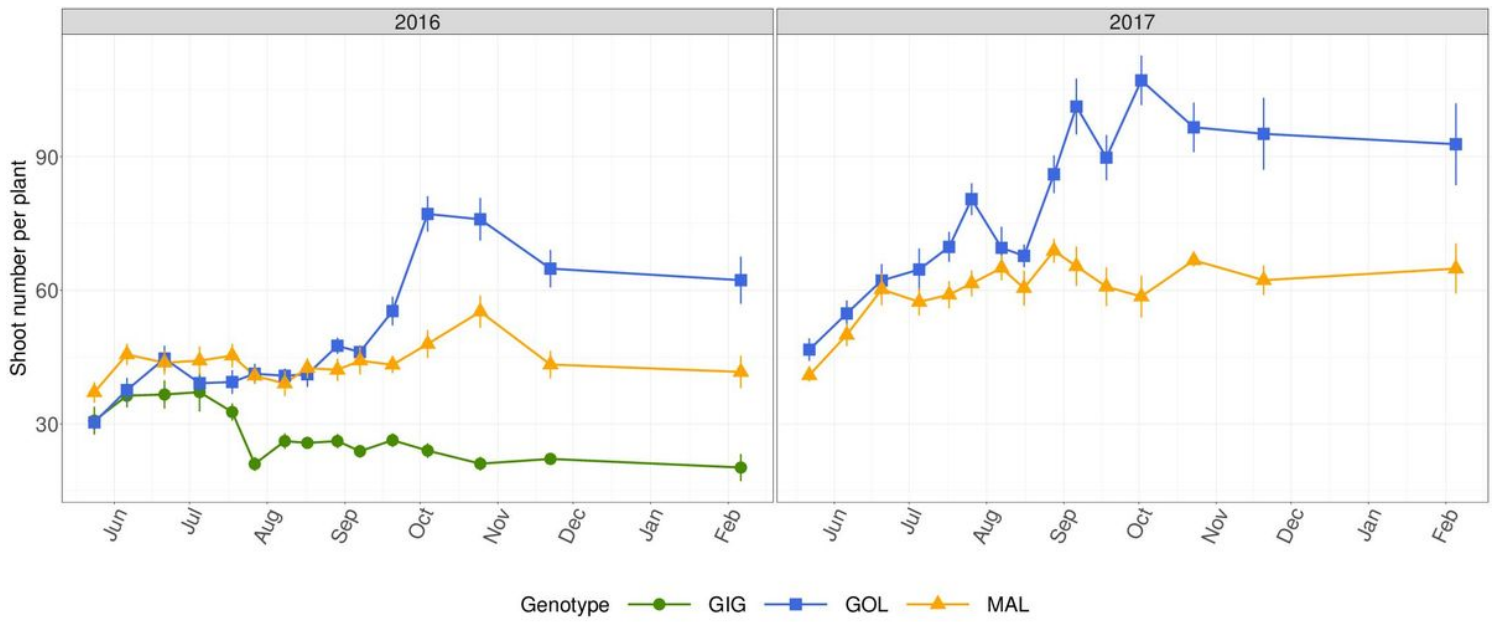


Figure 3

Seasonal dynamics of shoot numbers in *Miscanthus × giganteus* (circles), *Miscanthus sinensis* Goliath (squares) and *Malepartus* (triangles) during the third (2016) and fourth (2017) growing years (mean ± standard error)

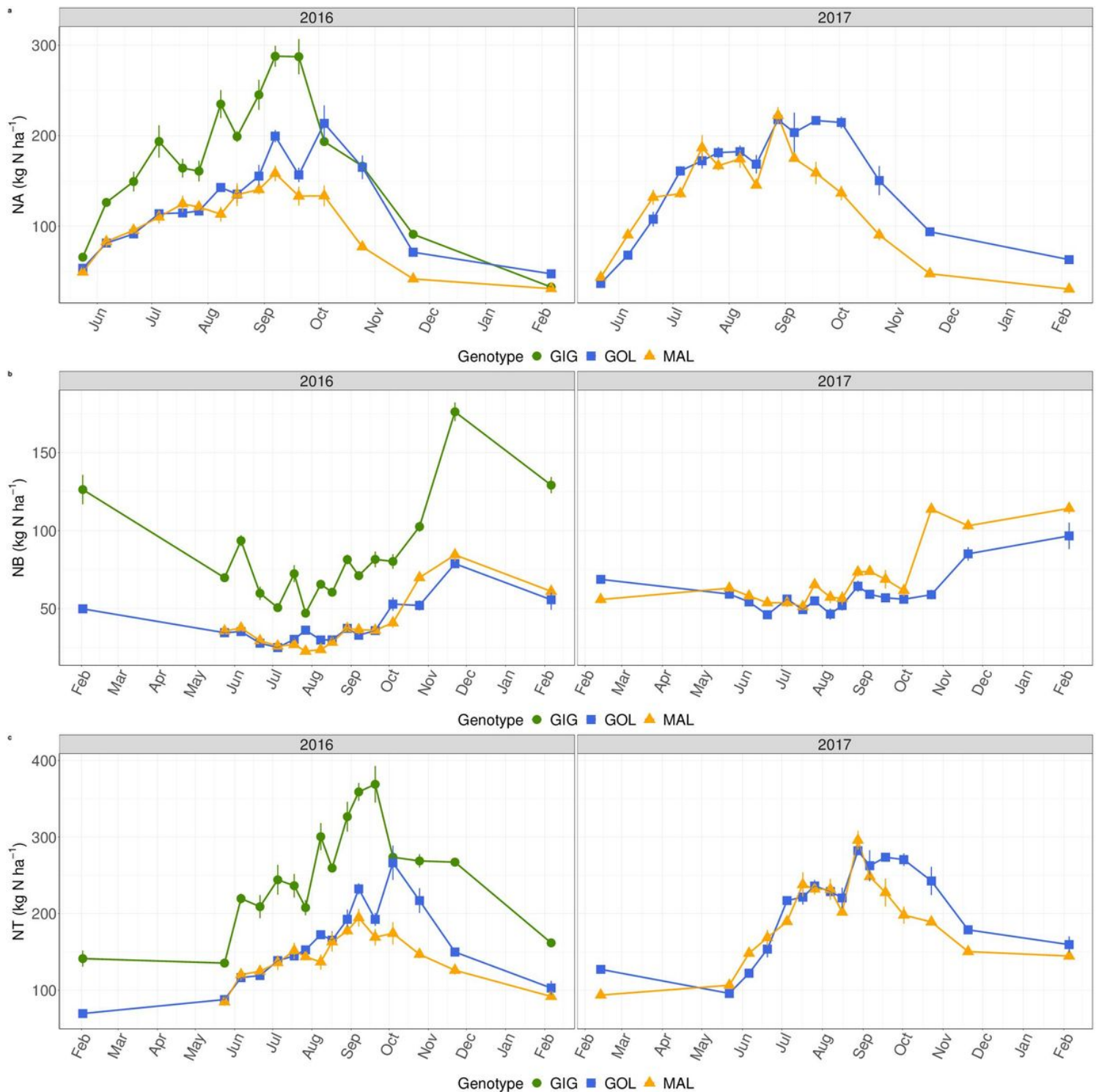


Figure 4

Seasonal dynamics of above-ground (a), below-ground (b) and whole plant (c) nitrogen content in *Miscanthus × giganteus* (circles), *Miscanthus sinensis* Goliath (squares) and Malepartus (triangles) during the third (2016) and fourth (2017) growing years (mean \pm standard error)

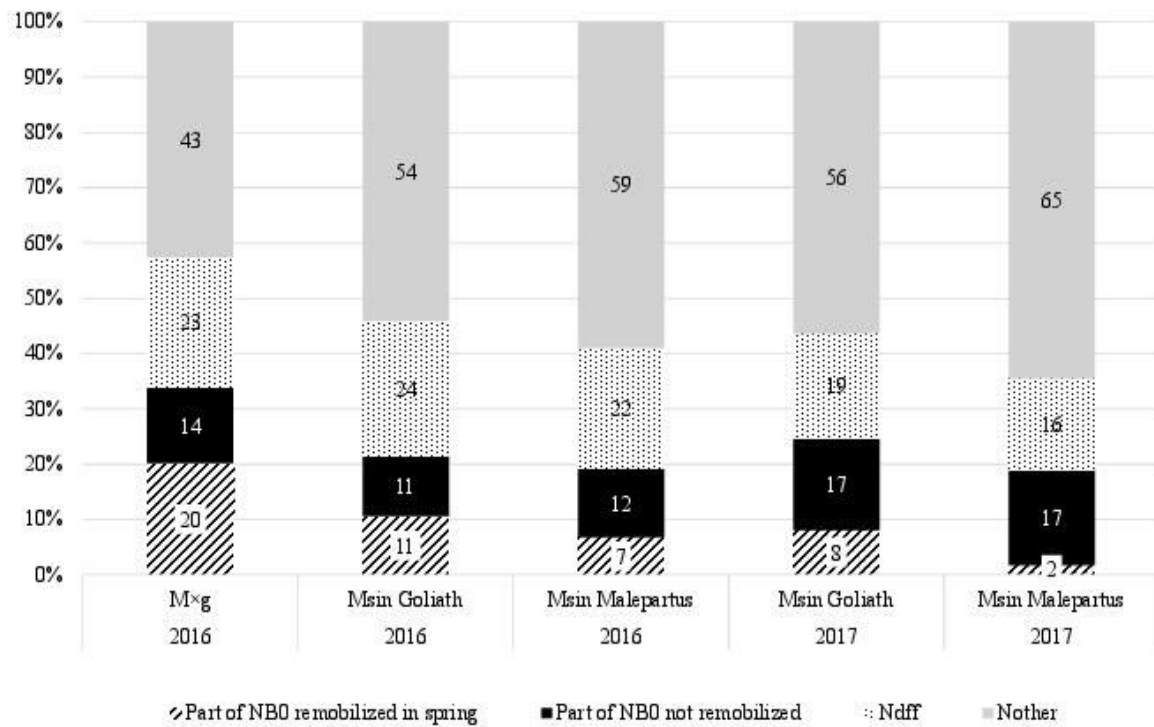


Figure 5

Nitrogen sources at date 3 (around September) in *Miscanthus × giganteus*, *Miscanthus sinensis* Goliath and Malepartus: mean values calculated over two successive years.