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

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Abstract
Nitrogen (N) recycling is a key mechanism to ensure the sustainability of miscanthus production with no or small fertiliser inputs, but little is known on the subject in miscanthus species other than the most cultivated Miscanthus × giganteus. This field experiment on Miscanthus × giganteus and Miscanthus sinensis quantified plant biomass and N stock dynamics during two years. Endogenous net N fluxes, calculated from the evolution of plant N content throughout time, were higher in Miscanthus × giganteus than in Miscanthus sinensis. Indeed, 79 kg N ha⁻¹ and 105 to 197 kg N ha⁻¹ were remobilised during spring and autumn, respectively, for Miscanthus × giganteus, as opposed to 13 to 25 kg N ha⁻¹ and 46 to 128 kg N ha⁻¹ for Miscanthus sinensis. However, N recycling efficiency, defined as the ratio between N remobilisation fluxes and the maximum above-ground N content, did not differ significantly between the two species. N recycling efficiency ranged from 8 to 27% for spring remobilisation and from 63 to 74% and 24 to 38% for autumn remobilisation calculated on above-ground and below-ground N, respectively. Exogenous N, the main source of N to constitute maximum plant N content for all genotypes, was provided by fertilisation (22 to 24%) and organic matter mineralisation or other sources (43 to 59%). During winter, 42 to 56% of plant N content was lost. Only a small part of these plant N losses was due to abscised leaves (6–12% of the maximum plant N content). Our results show that Miscanthus sinensis is as efficient as Miscanthus × giganteus in N recycling and N use efficiency and as performant as other perennial species.

Keywords Perennial crop · Lignocellulosic biomass crop · Nitrogen fluxes · Nitrogen economy · Nitrogen losses · Nitrogen uptake

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AP | Above-ground parts |
| BP | Below-ground parts |
| DM | Dry matter |
| M×g | Miscanthus × giganteus |
| Msin | Miscanthus sinensis |
| Msin Goliath | Miscanthus sinensis Goliath |
| Msin Malepartus | Miscanthus sinensis Malepartus |
| N | Nitrogen |
| Ndff | Plant N content derived from fertiliser (kg N ha⁻¹) |
| Nother | N uptake by the plant not derived from fertiliser (kg N ha⁻¹) |
| Nrec | Proportion of fertiliser-N recovered in the plant (%) |
| NUE | Nitrogen use efficiency (kg DM kg⁻¹ N) |
| NA | N content of the above-ground parts (kg N ha⁻¹) |
| NA1 | N content of the above-ground parts when N content in the below-ground parts is minimal (kg N ha⁻¹) |
| NA2 | Maximum N content of the above-ground parts (kg N ha⁻¹) |
| NA3 | N content of the above-ground parts when N content in the whole plant is maximal (kg N ha⁻¹) |

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NA, N content of the above-ground parts when N content in the below-ground parts is maximal (kg N ha\(^{-1}\))

NB, N content of the below-ground parts (kg N ha\(^{-1}\))

NT, N content of the whole plant (kg N ha\(^{-1}\))

WA, Biomass of above-ground parts (t DM ha\(^{-1}\))

WB, Biomass of below-ground parts (t DM ha\(^{-1}\))

WT, Biomass of the whole plant (t DM ha\(^{-1}\))

Introduction

Nowadays, environmental issues are major concerns because anthropogenic activities have accentuated global warming and the degradation of ecosystems. Extraction and use of fossil resources largely contribute to greenhouse gas emissions, and their non-renewable stocks are rapidly decreasing. In the last decades, sustainable resources have been developed [1, 2] and biomass is the most studied [3]. To be acceptable, lignocellulosic biomass crops have to produce high biomass per unit area, generate low environmental impacts and avoid competition with food crops as much as possible. Several perennial crops appear to be promising, as they combine high biomass production and low environmental impacts, with particularly low nitrogen (N) fertiliser requirements [4], which are known to be a major source of pollution in agriculture [5–7]. The perennial rhizomatous grass *Miscanthus × giganteus* (*M × g*) is a good candidate [4, 8, 9]: it reaches its maximum biomass production after three to six years depending on locations [10] and 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 important to note that winter harvest requires less exogenous N thanks to nutrient recycling by the plant [12–14] and through leaf fall in winter which constitutes organic mulching. In contrast, the autumn harvest of non-totally senesced plants in October does not allow plants to entirely recycle nutrients [14, 15] and prevents the accumulation of senescent leaves on the soil surface (mulch) which otherwise limits competition with weeds [15].

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

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

Concerning crop N requirements, Zapater et al. [26] showed that the critical N dilution curve, *i.e.* the minimum shoot N concentration required to maximise above-ground biomass production, was the same between *M × g* and *Msin*. However, although endogenous N recycling is globally well characterised for *M × g* [12–14], to our knowledge, no study dealing with *Msin* has been published. At the beginning of the growing season, nutrients stored in the rhizome and roots, which constitute the below-ground parts (BP), are transferred to new buds and stems, the future above-ground parts (AP), to support their initial growth. This transfer is called spring remobilisation [14]. During senescence, nutrients in leaves and stems are withdrawn and transferred to the rhizome to be stored during winter. This transfer is called...
autumn remobilisation [14]. These fluxes have been quantified for $M \times g$ in several studies, using the apparent N fluxes method, based on the comparison of N stocks in the above-ground and below-ground parts at different times during the growing season [12–14, 27, 28]. According to different studies, spring N remobilisation ranged from 23 to 98 kg N ha$^{-1}$ and autumn remobilisation ranged from 45 to 134 kg N ha$^{-1}$ during the third growing year of $M \times g$.

The large variability found in $M \times g$ spring and autumn N remobilisations in the literature can be partly explained by discrepancies between different calculation methods, based either on the above-ground or the below-ground compartments and considering different periods in plant development for the calculations [12–14, 29]. This emphasised the importance of homogenising the calculation method.

The present study deals with N economy by miscanthus, through re-using the N from vegetative parts of the plant, with a focus on the comparison between $M \times g$ and two genotypes of $Msin$. The first objective of the study was to finely characterise and compare N endogenous recycling, i.e. spring and autumn remobilisation periods and fluxes between $M \times g$ and $Msin$ through frequent samplings, to allow the precise determination of key dates and to compare and determine the most convenient recycling calculation method. As $M \times g$ and $Msin$ present the same N requirement to produce biomass [26], and considering that $M \times g$ produces more biomass than $Msin$, we hypothesised that the N apparent fluxes of $M \times g$ were higher than those of $Msin$. On the other hand, based on the same facts, we hypothesised that N recycling efficiencies, i.e. the apparent N fluxes relative to the maximum N quantity in AP, can be equivalent between species. Our second objective was to compare plant N economy between miscanthus species, taking into account not only endogenous fluxes but also exogenous N fluxes during acquisition (N uptake and fertiliser recovery) and estimated N losses during autumn and winter for these three genotypes. For the reasons cited above, we expected higher exogenous fluxes for $M \times g$ than $Msin$, but comparable proportions of N uptake relative to biomass produced, or comparable N losses relative to total N content of the plant.

**Material and Methods**

**Experimental Site and Trial Design**

The field was located in northern France, at the French National Research Institute for Agriculture, Food and Environment (INRAE) 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 of the experimental site has been characterised by an average annual temperature of 11.0 °C and annual precipitation of 640 mm. The four growing years of the study, 2014 to 2017, corresponded to these mean values with average temperatures of 11.9, 11.3, 11.0 and 11.4 °C and an annual 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 ($M \times g$) from ADAS, Yorkshire, UK, Miscanthus sinensis Goliath ($Msin$ Goliath) and Miscanthus sinensis Malepartus ($Msin$ Malepartus) both from Plant Estate, Netherlands. The choice of these two varieties of $Msin$ is based on the good ability to produce biomass for $Msin$ Goliath as reviewed in Arnoult et al. 2015 [10] and on the fact that $Msin$ Malepartus was a well-studied genotype in our team as it is a parent of two genetic mapping populations [30, 31]. The experimental 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 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 above-ground biomass of the entire field was cut, 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 a 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 [26], showed that plants had never been N deficient.

To determine fertilisation efficiency, a $^{15}$ N-labelled UAN fertiliser uniformly labelled on urea, NH$_4^+$ and NO$_3^-$, with a $^{15}$ N excess atom fraction of 0.125%, was applied on the...
experimental plots used for sampling on May 11th 2016 and May 3rd 2017. Plant isotopic excess was measured at each sampling date, and $^{15}$N natural abundance was analysed in control plants planted the same year, grown near the others but without fertilisation and irrigation.

**Plant Sampling**

Sixteen sampling campaigns were carried out between February and November, in 2016 and in 2017, approximately every ten days during the full vegetation period each year. At each sampling campaign, the number of shoots of the seven potentially harvestable plants of the sampling line was counted and the median number of shoots per plant was determined for each block and genotype. For each sampling and block, the three plants whose shoot number were closest to the median value (of the seven potentially harvestable plants) were collected in the morning, providing nine sampled plants per genotype. Plants were separated into aboveground parts (AP: stems and leaves) and below-ground parts (BP: rhizome and associated roots). The rhizome and associated roots were sampled to a maximum depth of ca. 30–40 cm, which corresponded to the maximum rhizome depth. Only the roots surrounding the rhizome were sampled. Two additional plants per genotype and block (amongst the seven’s potentially harvestable), also with a shoot number close to the median, were sampled only for their above-ground organs. Hence, nine plants were sampled for below-ground parts and 15 plants for above-ground parts, for each genotype, at each sampling date. The stems and leaves (AP) of each plant were immediately weighed. The rhizome and associated roots (BP) of each plant were washed with cold water, dried with paper towels and weighed fresh. Then, AP and BP of the plants were pooled by block and genotype. Subsamples of AP and BP of each block and genotype were dried at 65 °C during 96 h and weighed to determine above-ground and below-ground biomass (WA and WB), water content and dry matter. They were used to determine N content and $^{15}$N isotopic excess.

Nylon nets were placed on the soil surface of the 16th sampling line of each block and genotype before leaf abscission in order to collect abscised leaves during the senescence. The net covered an area of five plants corresponding to 2.4 m$^2$ (400 cm long × 60 cm large) and a height of 40 cm with a mesh size of 1 cm × 1 cm. The abscised leaves were picked up weekly from the ground each year from September to February. They were dried and weighed each week and pooled at the end of the year for analysis.

Because of a strong wind on 6th of June 2017 (just before the fourth sampling) causing $M \times g$ lodging, the $M \times g$ plants studied thereafter were chosen among the plants that remained standing and sampling was no longer representative of the plots, as competition for light was largely modified for these plants. Hence, the corresponding data will not be shown.

**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). $^{15}$N 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:

\[
NA = WA \times [N_A] \\
NB = WB \times [N_B] \\
NT = NA + NB
\]

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 $^{15}$N fertiliser ($Ndff$, kg N ha$^{-1}$) in the plant (AP + BP) was determined using the slightly modified equation by Hauck and Bremner [32]:

\[
Ndff = NT \frac{P - q}{f - q}
\]

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:

\[
N_{rec} = 100 \frac{Ndff}{F}
\]

where $F$ is the amount of fertiliser-N (here $F = 120$ kg ha$^{-1}$).

**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 (Fig. 1): date 0 corresponded to the dormancy phase (February) of year $n$; date 1 was the...
time when the N content of BP (NB) was minimal; date 2 was the time when the N content of AP (NA) was maximal; date 3 was the time when the N content of the whole plant (NT) was maximal; date 4 was the time when NB was maximal; date 5 corresponded to the dormancy phase of year n + 1.

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

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

where $NB_0$ and $NB_1$ represent the N content of below-ground parts at dates 0 and 1, respectively. Autumn remobilisation (kg N ha$^{-1}$) corresponded to the downward transfer of N from AP to BP in autumn. It can be calculated using two methods, according to Dierking et al. [29]. The first method is based on NA variations:

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

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

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

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

The maximum N uptake ($U$, in kg N ha$^{-1}$) was calculated as follows:

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

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

Finally, N losses of the whole plant ($L$, in kg N ha$^{-1}$) were calculated as:

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

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

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

Nitrogen Use Efficiencies

The Nitrogen Use Efficiency ($NUE$, in kg DM kg$^{-1}$ N) represents the amount of biomass produced per unit of N accumulated in the plant. It can be calculated by considering the whole plant, but we chose to consider AP to be able to compare our results with other studies. Two $NUE$ calculations were made according to Ra et al. [33], Olson et al. [34] or Dierking et al. [35] at two different dates:

$$NUE_1 = \frac{WA_{max}}{NA_2}$$  \( (8a) \)

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

$$NUE_2 = \frac{WA_5}{NA_5}$$  \( (8b) \)

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 of the above-ground parts at date 5.

$NUE_1$ represents the ability of the plant to produce above-ground biomass using N remobilised in the spring and N
absorbed from the soil. NUE$_2$ is the amount of biomass that can be harvested per unit of N exported.

**Statistical Analysis**

Statistical analyses were performed using R Software version 3.5.1 (R Core Team, 2018). Linear fixed effects models were fitted for each calculated variable described in Tables 2, 3 and 4 using the lm function. Then, the functions Anova (car package) and TukeyHSD were used to determine: (i) differences between genotypes, during the third (dataset considering only 2016) and fourth (dataset considering only 2017) growing years with genotype and block as fixed effects and (ii) differences between years for Msin Goliath and Msin Malepartus with the year as a fixed effect. It is important to note that possible differences between years could be attributed to age, climate or sampling effects that we were not able to identify. Unilateral student tests were used to determine whether N fluxes were significantly different from zero.

**Results**

**Biomass Production and Plant Development**

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

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

**Seasonal Variation in Above-ground and Below-ground Nitrogen Contents**

The three studied genotypes presented similar N content dynamics during the two years of the experiment (Fig. 4). N content in above-ground parts (NA) increased from emergence to reach a maximum level in late summer or in autumn and then decreased until February (Fig. 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 (Fig. 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 with time (Fig. 4c) showed the same dynamics as NA during the vegetative season: an increase from shoot emergence to autumn followed by a decrease. M×g NA, NB and NT in 2016 (Fig. 4a, b and c, circles) were globally higher than for both Msin. 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 NB 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 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).
Nitrogen Fluxes and Plant Nitrogen Functioning

Endogenous Nitrogen Fluxes

During both years, there were statistically significant differences 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 $M\times g$ than $M_{sin}$, in terms of quantity (79, 25 and 13 kg N ha$^{-1}$ for $M\times g$, $M_{sin}$ Goliath and $M_{sin}$ Malepartus, respectively) and efficiency (27%, 12% and 8% of $NA_2$ for the three species, respectively). This indicated that 33 to 59% of the N stock present in below-ground parts at the end of winter ($NB_0$) was remobilised during spring according to the genotype. Spring remobilisation fluxes appeared to be
higher for \textit{Msin} Goliath than for \textit{Msin} Malepartus in both years, although statistically not significantly different.

The autumn remobilisation fluxes calculated on the basis of \textit{NA} (\textit{AR}_g, Table 2) did not differ significantly between genotypes either, although they seemed to be higher in \textit{M} \times \textit{g} (197 kg N ha\(^{-1}\) \textit{versus} 128 and 117 kg N ha\(^{-1}\) for \textit{Msin} Goliath and \textit{Msin} Malepartus in 2016). During the autumn 2016, \textit{NA} decreased by 63 to 74% relatively to the maximum stock (\textit{NA}_2), with no significant differences between genotypes. In 2017, even if the autumn remobilisation flux was not significantly higher for \textit{Msin} Goliath than for \textit{Msin} Malepartus, the efficiency was significantly lower (\textit{p}<0.10) for \textit{Msin} Goliath (57%) than for \textit{Msin} Malepartus (75%).

Autumn remobilisation calculated on the basis of \textit{NB} (\textit{AR}_p, Table 2) was significantly higher (\textit{p}<0.05) for \textit{M} \times \textit{g} (105 kg N ha\(^{-1}\)) than for \textit{Msin} Goliath and Malepartus (46 and 48 kg N ha\(^{-1}\)), respectively. A significant difference (\textit{p}<0.10) was also found in terms of efficiency \textit{(i.e. the proportion of \textit{NA}_2)} which was higher for \textit{M} \times \textit{g} (38%) than for \textit{Msin} Goliath (24%). For \textit{Msin}, autumn remobilisation calculated with this method appeared to be lower in 2017 than in 2016. The difference between years was significant for \textit{Msin} Goliath (\textit{p}<0.05). In 2017, \textit{Msin} Goliath stored significantly less N into BP (21 kg N ha\(^{-1}\) \textit{i.e.} 10% of \textit{NA}_2) than \textit{Msin} Malepartus (37 kg N ha\(^{-1}\) \textit{i.e.} 17% of \textit{NA}_2).

Autumn remobilisation calculated on the basis of \textit{NB} was systematically lower than autumn remobilisation calculated on the basis of \textit{NA}. In 2016, the increase in \textit{NB} corresponded only to 40–56% of the decrease in \textit{NA} during the same period, with no significant difference between genotypes. It was even lower for both \textit{Msin} in 2017 (18% and 23% for \textit{Msin} Goliath and Malepartus, respectively).

**Exogenous Nitrogen Fluxes and Whole Plant Nitrogen Balance**

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

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

The maximum \textit{N} stock in the whole plant (\textit{NT}_3) originates from endogenous \textit{N}, which corresponds to the initial \textit{N} stock in BP in February (\textit{NB}_0) and has been partly remobilised during spring, and from exogenous \textit{N} uptake (\textit{U}) which either comes from \textit{N} fertiliser (\textit{Ndff}) or from other sources (\textit{N}_{\text{other}}) (Fig. 5). For all genotypes and both growing years, endogenous \textit{N} constituted the smallest proportion of \textit{NT}_3 (19–34%). It was, however, higher for \textit{M} \times \textit{g} than for the two other genotypes in 2016 (34 versus 19–22%). Exogenous \textit{N} uptake represented 66 to 81% of \textit{NT}_3. \textit{Ndff} in proportion to \textit{NT}_3 was quite similar between genotypes and years (16–24%). Finally, 43 to 65% of \textit{NT}_3 came from \textit{N} sources others than rhizome or fertiliser, \textit{i.e.} initial soil mineral N, soil organic N mineralised, etc.) (Fig. 5). This proportion was significantly higher for \textit{Msin} Malepartus than \textit{M} \times \textit{g} in 2016 (59 vs. 43%).

The \textit{N} balance calculated for the whole plant indicated that significant \textit{N} losses occurred in autumn and winter (Table 3). These losses represented large amounts: from 103 ± 51 kg N ha\(^{-1}\) in \textit{Msin} Malepartus 2016 to 207 ± 106 kg N ha\(^{-1}\) in \textit{M} \times \textit{g} in 2016 and were significantly different from 0. When expressed as a proportion of the maximum \textit{N} stock (\textit{NT}_3), \textit{N} losses were high and rather similar between genotypes and years (42 to 56%). These calculations did not take into account abscised fallen leaves before the last sampling date in February (Date 5). Cumulated fallen leaves represented each year 3 to 4 t DM ha\(^{-1}\), with a
significant difference between \textit{M}sin Malepartus and \textit{M} \times \textit{g} in 2016 (4 and 3 \text{ t DM ha}^{-1}, respectively). The corresponding N quantities ranged from 19 to 27 \text{ kg N ha}^{-1} (Table 3) depending on genotypes and years, which represented 6 to 12\% of \textit{NT}_3. Thus, fallen leaves were responsible for only a small fraction of the plant N losses (9–21\%).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig4.png}
\caption{Seasonal dynamics of above-ground (a), below-ground (b) and whole plant (c) nitrogen content in \textit{Miscanthus} \times \textit{giganteus} (GIG, green symbols), \textit{Miscanthus} \textit{sinensis} Goliath (GOL, blue) and Malepartus (MAL, orange) during the third (2016) and fourth (2017) growing years (mean \pm standard error).
}
\end{figure}

\section*{Nitrogen Use Efficiencies}

The maximum above-ground biomass was significantly higher in \textit{M} \times \textit{g} (43 \text{ t DM ha}^{-1}) than in \textit{M}sin Goliath (26 \text{ t DM ha}^{-1}), itself significantly higher than \textit{M}sin Malepartus (18 \text{ t DM ha}^{-1}) (Table 4). The above-ground biomass
decreased during winter and was almost halved for the three genotypes. \( NUE_1 \) did not differ significantly between genotypes in 2016 and 2017. In 2016, it ranged between 138 and 161 kg DM kg\(^{-1}\) N. In 2017, \( NUE_1 \) was similar to its 2016 values for \( Msin \) Goliath (121 kg DM kg\(^{-1}\) N) but was significantly lower (\( p < 0.05 \)) than in 2016 for \( Msin \) Malepartus (97 kg DM kg\(^{-1}\) N). The N use efficiency \( NUE_2 \) was significantly higher than \( NUE_1 \). This is due to the decrease in \( NA \) in autumn. \( NUE_2 \) was higher in 2016 for \( M \times g \) (653 kg DM kg\(^{-1}\) N) than \( Msin \) (287–338 kg DM kg\(^{-1}\) N) and similar between years for \( Msin \).

**Discussion**

Studying plant N economy (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 detailed temporal study of \( M \times g \) 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, we compared N economy (N remobilisation efficiency and N used to produce biomass) of these two species with other annual and perennial plant species in the third section of the discussion.

**Nitrogen Recycling Efficiency is Rather Similar in Miscanthus \( \times \) Giganteus and Miscanthus Sinensis despite Larger N Net Fluxes**

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 with four to eight sampling campaigns per year in other studies [12–14, 26, 28, 29, 36]. These fine temporal N dynamics in above and below-ground parts allow to refine periods to take into account in N remobilisation calculation, on physiological basis. When looking at the literature, these methods vary from one study to another, both for the compartments (AP or BP) used in the calculations and for the periods taken into account for N remobilisation. 

### Table 3

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

| Genotype | Crop age | Plant uptake (U) and fertiliser recovery | Plant losses (L) | N in abscised leaves, kg N ha⁻¹ |
|----------|----------|-----------------------------------------|-----------------|---------------------------------|
| M × g    | 3        | 243 (55) A a * 87 (22) A a 72% (18%) A a | 207 (106) A a * 53% (14%) A a | 19 (4) A a * |
| M sin Goliath | 3   | 183 (24) A a * 57 (12) AB b 48% (10%) AB b | 129 (33) A a * 56% (13%) AB b | 27 (4) A a * |
| M sin Malepartus | 3  | 159 (45) A a * 43 (12) B b 36% (10%) B b | 103 (51) A a * 50% (15%) B b | 22 (5) A a * |
| M sin Goliath | 4   | 213 (26) A a * 54 (10) A a 45% (8%) A a | 123 (61) A a * 42% (19%) A a | 26 (4) A a * |
| M sin Malepartus | 4  | 240 (40) A a * 49 (11) A a 40% (9%) A a | 151 (52) A a * 50% (11%) A a | 21 (2) A a * |

### Table 4

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

| Genotype | Crop age | WA₂max, t DM ha⁻¹ | WA₂, t DM ha⁻¹ | NUE₁, kg DM kg⁻¹ N (Eq. 8a) | NUE₂, kg DM kg⁻¹ N (Eq. 8b) |
|----------|----------|-------------------|----------------|-------------------------------|-------------------------------|
| M × g    | 3        | 43 (5) A a | 22 (6) A a | 161 (32) A a | 653 (99) A a |
| M sin Goliath | 3   | 26 (3) B b | 13 (3) AB ab | 149 (19) A a | 287 (70) B b |
| M sin Malepartus | 3  | 18 (3) B c | 10 (2) B b | 138 (10) A a | 338 (25) B b |
| M sin Goliath | 4   | 25 (3) A a | 14 (7) A a | 121 (17) A a | 219 (69) A a |
| M sin Malepartus | 4  | 22 (3) A a | 11 (3) A a | 97 (3) A a | 359 (45) A a |
account: while Beale and Long [12] and Himken et al. [13] considered that N spring remobilisation starts at emergence, perhaps underestimating spring remobilisation because N is probably transferred to new buds in formation before emergence, Strullu et al. [14] considered the beginning of remobilisation to take place before emergence in February. In this study, we followed this later proposition that seems to be physiologically fairer when considering the beginning of spring remobilisation. Regarding autumn remobilisation, we assumed that it starts when NA is at its maximum level (beginning of N withdrawal) as proposed by Himken et al. [13], Dierking et al. [29] and Strullu et al. [14], and not when BP N stock was at its minimum as proposed by Beale and Long 1997 [12], as the increase in BP N stock in summer can reflect soil N absorption and possible storage in the rhizome. In the same way, the end of autumn remobilisation was taken following Dierking et al. [29] proposition which is the date of maximum BP N stocks (full rhizome) as we consider this a more accurate suggestion than the use of the date of February or March of n + 1 as proposed by Beale and Long [12], Himken et al. [13] and Strullu et al. [14].

Based on the above described methodology, 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. Furthermore, the short time steps between successive samplings allowed us to identify precisely the periods of spring and autumn N remobilisations: hence, we found that autumn remobilisation takes place for M × g in the first part of September, whereas Strullu et al. [14] and Dierking et al. [29] observed it slightly earlier in August, probably because 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 NA₂) 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. [29] who observed a decline in NA of 46–67 kg N ha⁻¹ depending on the fertilisation rate. The flux calculated using the evolution of NB was also higher in our study (105 kg N ha⁻¹) compared to that obtained by Dierking et al. [29] who observed an 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, i.e. half as much as in our experiment, which suggests they may not have reached their maximum biomass production.

No study has been reported in the literature concerning N fluxes in Msin. In our study, N fluxes were smaller in Msin than in M × g, due to lower biomass and N stocks in Msin. However, N remobilisation efficiency, relative to the maximum above-ground N content (NA₂), did not differ significantly between M × g and Msin both in spring and autumn. Autumn N remobilisation efficiency appeared to be greater for Msin Malepartus than Msin Goliath: a greater part of the N contained in the AP was transferred into the BP in Msin Malepartus compared to Msin Goliath in autumn 2017 (p < 0.10). This difference can be explained by the presence of stay-green shoots of the last cohort during winter observed in greater number for Msin Goliath than for Msin Malepartus. These green shoots could explain the higher NA in winter for Msin Goliath in 2017 and lower autumn N remobilisation. Moreover, these stay-green shoots exhibit re-growth at the time of the emergence of new buds and may provide photo-assimilates at an early stage. Emission of different shoot cohorts throughout the growing season in Msin has already been reported [37, 38] and is known to be dependent on climatic conditions, and particularly temperature [37]. Indeed, spring was warmer in 2017 than in 2016 (the mean temperature over May–June was 17.0 °C in 2017 and 15.4 °C in 2016), but late summer was cooler (the mean temperature in August–September was 16.5 and 18.5 °C, respectively). This suggests that variations in autumn N remobilisation for genotypes that presented cohort phenomena such as Msin could depend on climate.

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

Components of Nitrogen Uptake and Fertiliser Nitrogen Recovery

The N content of the whole plant (NT) varied widely throughout the year. Part of this variation was due to the significant N uptake (U) which occurred for the three Miscanthus genotypes particularly during the first part of the growing season, from late May to early September. N uptake, estimated with Eq. 6, ranged from 159 to 243 kg N ha⁻¹ (Table 3, Figs. 4c and 5). This exogenous N flux represented 66% to 81% of the maximum whole plant N stock (NT). It came partly from N fertiliser which corresponded to 43–87 kg N ha⁻¹ and mainly from other sources: initial soil mineral N stock, irrigation water, atmospheric deposition and soil organic matter mineralisation. The initial soil mineral N (measured in early spring) was rather stable between years (ca. 50 kg N ha⁻¹ in 0–150 cm and 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 a 30 cm depth during the growing season), so it may have contributed at least 20 kg N ha⁻¹ to the N uptake. According to the mean nitrate
content measured in the irrigation water, N input through irrigation represented ca. 30 kg N ha$^{-1}$. Atmospheric deposition represented about 9 kg N ha$^{-1}$ during the year [39]. If we take into account these contributions (N from fertiliser, initial soil mineral N, irrigation and atmospheric deposition), there remains 57 to 132 kg N ha$^{-1}$, which would have come from soil organic N mineralisation. This range of organic N mineralisation is in agreement with Mary et al. [40] who estimated that annual N mineralisation was around 140 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 might be N-fixing bacteria. The presence of N-fixing bacteria in the miscanthus rhizosphere has been demonstrated [39], but their importance for plant N-nutrition is not well known. Using a $^{15}$N experiment on first-year M × g plants, Keymer and Kent [41] estimated that 16% of the new plant N was derived by N fixation during the growing season.

The fertiliser-N recovery in the whole plant in 2016 varied between 36% in Msin Malepartus, 48% in Msin Goliath and 72% in M × g. The recovery in Msin was also low in 2017 (45% and 40%, respectively). The lower recoveries 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 developmental stages between genotypes, which means the date of fertiliser application for Msin Malepartus may not have been optimal (too early), which contributed to fertiliser-N losses.

**Nitrogen Balance Demonstrates Substantial Nitrogen Losses in the Two Species**

A very substantial decrease in the whole plant N content ($NT_j$) was observed between September and February for all genotypes: it reached 103 ± 51 to 207 ± 106 kg N ha$^{-1}$, which corresponds to 42 to 56% of the maximum N accumulated in the whole plant at the end of summer ($NT_j$). Indeed, the strong decrease in N accumulated in the AP during autumn and winter was much greater than the increase in N stored in the BP during the same period. Note that the decrease in biomass and N quantities observed at belowground scale between November 2016 and February 2017 (Figs. 2 and 4) was not statistically different. This explains why the autumn N remobilisation calculated based on NB was much lower than the N remobilisation calculated based on NA. Biomass losses also occurred during the same period (30–52% of the maximum biomass of the whole plant). N losses have already been observed in miscanthus in the literature but not really investigated. Calculations using data of Beale and Long [12] show that 33% of the whole plant N was lost between July and February in three-year-old plants of Miscanthus × giganteus. Himken et al. [13] observed a decrease of 165 to 203 kg N ha$^{-1}$ between September and February, which corresponds to 40 to 47% of $NT_j$ in unfertilised and fertilised plants, respectively. The N lost by abscised leaves represented 19 to 27 kg N ha$^{-1}$ in our experiment (and 31 kg N ha$^{-1}$ in Struluv et al. [14]) and therefore only a small fraction of the calculated plant N losses. Even taking into account these abscised leaves, the amounts of unrecovered N were still large (81 to 188 kg N ha$^{-1}$).

If biomass losses can be easily explained by root turnover and plant respiration, N losses necessarily corresponded to N fluxes towards the soil or the atmosphere. Four main hypotheses could explain these fluxes: H1) N storage in deep roots; H2) N rhizodeposition (release of organic and inorganic N from living plant roots) and root turnover (due to root mortality); H3) NH$_3$ volatilisation into the atmosphere; H4) N$_2$O emissions into the atmosphere.

Regarding 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 and roots were sampled in the 30–40 first cm in the present study. However, the amounts of N contained in these roots seem to be rather small. Neukirchen et al. [42] showed that N concentration and root dry mass decreased sharply with depth. Ferchaud et al. [43] found that root N content of five-year-old M × g was 56 kg N ha$^{-1}$ in the 0–20 cm layer and only 10 kg N ha$^{-1}$ in the 20–60 cm layer. N storage in deeper roots was probably very low.

The N rhizodeposition hypothesis (H2) has already been put forward by Heaton et al. [44] and supported by Hromádko et al. [45] who demonstrated that autumn root exudates of M × g are composed of protein and are used to feed the bacterial community. Rhizodeposition occurs in many plant species [46], and the amounts of N rhizodeposited could be important, as in ryegrass with 94 kg N ha$^{-1}$ calculated over two years of cultivation [47]. Moreover, part of the root N also returns to the soil through root turnover. Neukirchen et al. [42] measured an 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 November and March. Miscanthus produces new roots every year [48], but the proportion of root biomass that dies every year is unknown.

Emission of N gases by plants (H3) has been put forward by many authors who observed N losses in spring barley, wheat or maize. For example, N losses varying between 45 and 81 kg N ha$^{-1}$ were reported for maize during grain filling [49]. The authors suggested that losses are linked to ammonia volatilisation from the aerial parts of plants [49–51]. Schjørring and Mattsson [52] concluded a two-year survey by stating that N volatilisation from plants “will represent in many areas a significant input of ammonia to the atmosphere and that NH$_3$ losses may become large enough to significantly affect crop N budgets”.

Emissions of nitrous oxide (N$_2$O) directly by plants (H4) have been reported in some studies under controlled conditions. An emission rate of 0.17 and 0.11 ng N$_2$O g$^{-1}$ fresh
weight week\(^{-1}\) was measured in maize and wild barley [53], and a flux of 2.8 mg N\(_2\)O-N m\(^{-2}\) day\(^{-1}\) was found in rye-grass [54]. After N fertilisation in soil, potted beech (\textit{Fagus sylvatica}) emitted between 0.4 and 2.0 µg N m\(^{-2}\) leaf area h\(^{-1}\) [55]. Chang et al. [56] demonstrated that potted canola can emit N\(_2\)O from its aerial parts when soil is water saturated. Lenhart et al. [57] observed in laboratory conditions that a few weeks old \textit{Msin} produced 3 to 30 times more N\(_2\)O than other species such as maize or tobacco. Even if the growing conditions are far from that of the field, further investigations have to be carried out to verify this in field conditions.

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

\textbf{Miscanthus × Giganteus and Miscanthus Sinensis Presented as Good Nitrogen Economy as Other Perennial Species}

The low contribution of spring N remobilisation to the N accumulated in AP may lead us to believe that \(M \times g\) and \(Msin\) were not as efficient in terms of N recycling as expected. However, the spring remobilisation efficiency of the three studied miscanthus genotypes was comparable to other herbaceous species such as big bluestem (\textit{Andropogon gerardii}) in which 46–58% of the rhizome N content was remobilised during spring [58], compared to 20–59% of the \(M \times g\) and \(Msin\) in our experiment. It was slightly lower than \textit{Festuca rubra} and \textit{Agrostis capillaris} in which 34% and 45% of NA came from BP, respectively [59], compared to 8–27% in our experiment. Concerning autumn remobilisation, efficiency for our \(M \times g\), \textit{Msin} Goliath and \textit{Msin} Malepartus (63–75%) was similar or even higher than for big bluestem (58%) [58] or switchgrass (3–61%) [60, 61]).

Another criterion to characterise the economy of N by plants is the Nitrogen Use Efficiency (NUE). In our experiment, NUE was calculated as the amount of above-ground biomass produced per unit of above-ground N content. It did not differ significantly between \(M \times g\) and \textit{Msin} at \(WA_{\text{max}}\) but was higher for \(M \times g\) than \textit{Msin} in February because of a more important decrease in NA for \(M \times g\). Dierking et al. [35] calculated the NUE of four clones of \(M \times g\), as the ratio between WA and NA in January. Their values were much smaller than ours, with 126 to 297 kg DM kg\(^{-1}\) N on average between unfertilised and fertilised treatments, in contrast with 653 kg DM kg\(^{-1}\) N in February for our \(M \times g\) (NUE\(_2\)). This difference can be explained by yields half as high as ours because of younger plants (one and two years old). Ra et al. [33] and Olson et al. [34] calculated NUE in different plants dedicated to biomass production as the ratio between WA and NA in autumn when WA was at its maximum. According to this method, our three genotypes presented NUE from 97 to 161 kg DM kg\(^{-1}\) N (NUE\(_1\), comparable to johnsongrass (\textit{Sorghum halepense}), napiergrass (\textit{Cenchrus purpureus}), sugarcane and sorghum (70 to 125 kg DM kg\(^{-1}\) N) and better than \textit{Erianthus}, switchgrass and maize (76 to 97 kg DM kg\(^{-1}\) N) [33]. Olson et al. [34] worked with a hybrid 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 \(M \times g\) and \textit{Msin} had similar N recycling efficiencies and NUE to other perennial and annual species dedicated to biomass production.

\textbf{Conclusion}

This study provides the first experimental comparison of N pools and N fluxes (endogenous and exogenous) between \textit{Miscanthus sinensis} and \textit{Miscanthus × giganteus}. According to our first hypothesis, \(M \times g\) has higher net N fluxes than \textit{Msin} due to higher biomass. As a result, our second hypothesis was also verified: because of the same N requirement to produce a unit of biomass between species, the latter presented similar N recycling efficiencies. As it appeared to be as efficient in terms of N recycling as \textit{Miscanthus × giganteus}, \textit{Miscanthus sinensis} could become an alternative to \(M \times g\) for producing lignocellulosic biomass provided that these results are confirmed under situations of lower soil resources. Even if miscanthus is at least as efficient as other perennial crops concerning N recycling, it is able to sustain high N uptake which is the main source of the whole plant N stock at the end of summer. Consequent plant N losses were also concurrently measured at the end of the growing season. Although losses have already been observed in miscanthus and other plant species, the processes involved have to be investigated, particularly the possible transfer of N from plant to soil through rhizodeposition and root turnover. To complete the understanding of N recycling mechanism in miscanthus, particularly \textit{Miscanthus sinensis}, the link with development stages and growing season length associated with climate response has to be studied to determine triggers of N remobilisation. Finally, to avoid competition with land-use for food production, miscanthus should be grown as much as possible on marginal lands. Further studies on N functioning on poor or degraded soils have to be carried out to help choose the most appropriate genotypes that combine decent yields and low environmental impacts.
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Availability of Data and Material The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

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

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