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# Effects of several establishment modes of *Miscanthus × giganteus* and *Miscanthus sinensis* on yields and yield trends

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## Abstract

*Miscanthus* is a C4 perennial grass originating from East Asia, the yields of which progressively increase in the first years of growth. Several species for bioenergy have been studied since the mid-1980s in Europe, in particular (*Miscanthus × giganteus* [*M. × giganteus*]), due to its high yields. *M. × giganteus* is mainly cultivated in France and established from rhizomes. Our study aimed to assess, in field conditions, alternative establishment methods combined with an alternative species, *Miscanthus sinensis* (*M. sinensis*). We set up a multi-environment experimental network. On each trial, we tested two treatments with *M. × giganteus*, established from rhizomes (G\_r-sd) and from plantlets obtained from rhizomes (G\_p-sd), and two treatments with *M. sinensis* seedlings transplanted in single (S\_p-sd) and double density (S\_p-dd). ANOVA was performed to compare establishment and regrowth rates across treatments, as well as yields across treatments and site-years. A logistic model was used to describe yield trends and to compare the maximum yield reached and the rate of yield increase of both species. Results showed that miscanthus establishment from plantlets resulted in higher establishment (between 87% and 92%) and regrowth (between 91% and 94%) rates compared to establishment from rhizomes. Treatments with *M. × giganteus* obtained higher average yields across site-years than those with *M. sinensis*, but more variable yields across site-years. We showed a strong species effect on yields, yield components (shoot weight, shoot density and shoot number per plant) and light interception (through leaf area index). Lastly, to use *M. sinensis* established from transplanted plantlets as an alternative to *M. × giganteus*, research would be required on the breeding of *M. sinensis* sterile seeds to avoid risks of invasiveness.

## KEYWORDS

biomass, *Miscanthus*, multi-environment trial, rhizomes, transplanted plantlets, yield trend modelling

## 1 | INTRODUCTION

*Miscanthus* is a C4 perennial grass from East Asia, several species of which have been studied since the mid-1980s in Europe (Lewandowski, Scurlock, Lindvall, & Christou, 2003) and in the United States (Heaton, Dohleman, & Long, 2008). *Miscanthus* × *giganteus* (hereafter named *M.* × *giganteus*), an interspecific hybrid from *Miscanthus sinensis* (hereafter named *M. sinensis*) and *Miscanthus sacchariflorus* (hereafter named *M. sacchariflorus*), has been studied as an energy crop due to its high lignocellulose content and biomass production (Milovanovic, Drazic, Ikanovic, Jurekova, & Rajkovic, 2012; Nakajima, Yamada, Anzoua, Kokubo, & Noborio, 2018; Ussiri, Guzman, Lal, & Somireddy, 2019). Yields of *M.* × *giganteus* varied between 10 and 25 t of dry matter (DM) per ha in Europe in field experiments, with yields progressively increasing every year during the first years of growth (Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000).

*M.* × *giganteus* encounters difficulties, however, which impede its expansion in farmers' fields. First of all, the high cost of establishment (1,904–3,376 €/ha) due to rhizome costs (Xue, Kalinina, & Lewandowski, 2015) is a major constraint for farmers during the first year of production. In addition, the use of rhizomes often results in a low establishment rate because the quality of the rhizomes is not easy to check: rhizome quality depends on the rhizome harvesting period, harvesting technology, storage time between harvesting and planting, storage conditions and rhizome size (Xue et al., 2015). This low establishment rate leads to additional costs for the extra supply of rhizomes, or else to lower yields. Lastly, the genetic diversity of *M.* × *giganteus* is very limited because it is a sterile clone derived from *M. sinensis* and *M. sacchariflorus*, and only vegetative propagation is possible (Lewandowski, 1998; O'Loughlin, McDonnell, & Finnan, 2017).

*M. sinensis* has similar characteristics as *M.* × *giganteus*, including low nitrogen requirements and a high lignocellulose content. However, *M. sinensis* is an earlier species than *M.* × *giganteus* due to its lower base temperature (Zub & Brancourt-Hulmel, 2010). It is also more tolerant to water stress (Clifton-Brown & Lewandowski, 2000) and would therefore be more suited than *M.* × *giganteus* to soils with a low soil water content. Finally, *M. sinensis* is more tolerant to frost than is *M.* × *giganteus* (Clifton-Brown & Lewandowski, 2000; Farrell, Clifton-Brown, Lewandowski, & Jones, 2006). However, yields of *M. sinensis* are lower than those of *M.* × *giganteus* in temperate climate (Zub & Brancourt-Hulmel, 2010).

Several establishment methods of *M.* × *giganteus* and *M. sinensis* were studied to find alternatives to the establishment from rhizomes (Atkinson, 2009; Xue et al., 2015; Xue, Lewandowski, & Kalinina, 2017). However, the success of these methods across different types of soils has not yet been documented. Moreover, the evolution of the biomass produced from alternative establishment methods over several growth years is

not addressed in the literature (O'Loughlin et al., 2017). Only yields over the short term (first 2 years) for new establishment methods have been discussed for *M.* × *giganteus* (Boersma & Heaton, 2014a; Lewandowski, 1998).

It is therefore necessary to find alternatives to *M.* × *giganteus*, to facilitate the establishment phase and to broaden genetic diversity while maintaining satisfactory yields (Clifton-Brown et al., 2001). *M. sinensis*, despite having lower yields than *M.* × *giganteus*, could achieve more stable yields under various soil and climate conditions. It could, moreover, be interesting to explore new establishment methods to overcome the problems raised by the establishment from rhizomes. The use of transplanted plants could for instance allow for a better establishment of miscanthus and therefore less investment for re-establishment in case of non-emergence.

Our study aims to assess the effect of several modes of establishment of *M.* × *giganteus* and *M. sinensis* on yield and yield trends under field conditions and various environments in the north and centre of France.

## 2 | MATERIALS AND METHODS

### 2.1 | A multi-environment experimental network

A multi-environment experimental network was set up on seven sites in France from 2013 to 2019: six sites in the Ile de France region and one site in the Centre region.

We explored a diversity of soil types, with various textures, depths and stoniness (Table 1) to compare the yield variability of the treatments across sites. In addition to these varying soil characteristics, some trials were located in agricultural lands, that is, lands that were cultivated with annual crops before carrying out the experiment (Bioferme, La Bondue), but also in marginal lands such as Chanteloup, with a polluted soil due to the presence of Metallic Trace Elements, Polycyclic Aromatic Hydrocarbons and PolychloroBiphenyl, and Marne et Gondoire, which was located between several roads/highways and was therefore difficult to access with agricultural equipment.

Before setting up the trials, Subdray was managed as a set-aside land. La Bondue and Episy had been managed as set-aside lands since 1992. Marne et Gondoire and Evry had also been maintained as set-aside lands since 2003 and 2005 respectively. The preceding crop was winter barley (*Hordeum vulgare*) for Bioferme and Chanteloup (Table 1).

### 2.2 | Treatments: Miscanthus species combined with establishment mode

Each trial of the multi-environment network was composed of four strips, with an area of 141.4 m<sup>2</sup> (27.2 m × 5.2 m) for

**TABLE 1** Main characteristics of the site-years

Sites	Lat. and Long.	Soil texture (0–30 cm)	Rooting depth (cm)	PS (%)	Preceding crop	Year	Rainfall <sup>a</sup> (mm)	DD (°C)
La Bondue	48°19'20"N, 3°2'13"E	Sandy clayey loam	78.5	48	Set-aside	2013	459	1,848
						2014	485	1,985
						2015	359	1,981
						2016	514	1,957
						2017	556	2,077
						2018	354	2,318
Episy	48°21'37"N, 2°49'36"E	Clayey sand	75	19	Set-aside	2013	459	1,848
						2014	485	1,985
						2015	359	1,981
						2016	514	1,957
						2017	556	2,077
						2018	354	2,318
Evry	48°51'7"N, 1°59'6"E	Clayey sandy loam	81.5	37	Set-aside	2013	420	1,926
						2014	500	2,053
						2015	334	2,041
						2016	464	1,985
						2017	408	2,161
						2018	262	2,383
Subdray	47°1'50"N, 2°19'52"E	Loamy sandy clay	48	18	Set-aside	2013	521	2,135
						2014	540	2,269
						2015	415	2,272
						2016	471	2,063
						2017	483	2,331
						2018	358	2,608
Marne et Gondoire	48°51'58"N, 2°39'45"E	Clayey sandy loam	77.5	15	Set-aside	2013	439	2,018
						2014	516	2,147
						2015	370	2,136
						2016	492	2,121
						2017	459	2,293
						2018	494	2,478
Chanteloup	48°57'46"N, 2°2'11"E	Sand	102	15	Winter barley	2013	360	1,964
						2014	372	2,081
						2015	327	2,041
						2016	393	2,027
						2017	358	2,186
						2018	361	2,314
Bioferme	48°21'17"N, 3°1'57"E	Clayey sandy loam	142	0	Winter barley	2013	459	1,848
						2014	485	1,985
						2015	359	1,981
						2016	514	1,957
						2017	556	2,077
						2018	354	2,318

Abbreviations: DD, Degree-days (base temperature: 7°C); PS, Proportion of stones in volume over the rooting depth.

<sup>a</sup>Between March 1 and October 15.

each strip. Four treatments were tested (one per strip): two treatments with *M. × giganteus* established from rhizomes (G\_r-sd) or from plantlets (G\_p-sd) and two treatments with *M. sinensis* K1399 (population variety, i.e. composed of diverse genotypes, whose seeds were provided by Wageningen University & Research) from plantlets in single (S\_p-sd) and double (S\_p-dd) density, i.e. 3.33 plants per m<sup>2</sup> instead of 1.56 plants per m<sup>2</sup>. Seedlings were raised in greenhouse before transplanting. G\_r-sd was the control treatment: it was used as a basis for comparison with the three other treatments. S\_p-sd and S\_p-dd were *M. sinensis* treatments with transplanting plants from seedlings that were sown in early February 2013. S\_p-dd allowed us to evaluate the effect of doubling the density on *M. sinensis* yields and yield trends. Lastly, G\_p-sd was *M. × giganteus* established from plantlets grown from rhizome tips. This treatment allowed us to evaluate the feasibility of this method to establish *M. × giganteus*.

Three sub-plots were positioned on each strip after the establishment phase. For single density (G\_r-sd, S\_p-sd, G\_p-sd), five rows and five plants per row were selected for a sub-plot area of 16 m<sup>2</sup> (distance between plants and between rows: 0.8 m). For the double density (S\_p-dd), the sub-plot area was 15 m<sup>2</sup> with five rows and 10 plants per row (distance between plants: 0.5 m; distance between rows: 0.6 m).

### 2.3 | Crop management over the whole trial

The establishment of miscanthus was carried out manually in March 2013 for all sites. During the first year of growth, all treatments were protected against weeds. Weed management was chemical and/or manual, depending on the amount of weeds observed in the trial. Given the low N requirements of miscanthus, no nitrogen fertilizer was applied throughout the experiment. 30 and 300 kg/ha of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively, were applied after harvest time in the third year of growth, except for the Bioferme, Episy and La Bondue sites, which were fertilized in the fourth growth year.

### 2.4 | Measurements

The aerial biomass of the whole trial was shredded the first year (end of winter 2014) and left on the soil as a mulch. To assess this initial aerial biomass (AB<sub>1</sub>) without modifying the effect of the mulch inside the sub-plots, 10 plants inside each strip (treatment) but outside the sub-plots were selected, cut at 15 cm from the ground and weighed (SW<sub>s</sub>). We counted the shoot number (SN<sub>s</sub>) for these 10 plants. A 2–3 kg sample was put in the oven at 80°C for 48 hr to determine the DM. The number of plants on each sub-plot was counted (SN<sub>p</sub>) and the yields measured in kg/m<sup>2</sup>. The sub-plot area (A<sub>p</sub>) was 15 or 16 m<sup>2</sup>. We multiplied by 10 the formula described

below to express the aerial biomass in t/ha. The aerial biomass produced during the first year was obtained as follows:

$$AB_1 = \frac{SW_s}{SN_s} \times \frac{SN_p}{A_p} \times \frac{DM}{100} \times 10,$$

where AB<sub>1</sub> is the aerial biomass the first year of production (t/ha of DM), SN<sub>s</sub> is the shoot number per m<sup>2</sup> of the sample, SW<sub>s</sub> is the shoot weight of the sample (kg), SN<sub>p</sub> is the shoot number of the sub-plot (kg), A<sub>p</sub> is the area of the sub-plot (m<sup>2</sup>) and DM is the dry matter (%).

The establishment rate was measured in July 2013 (i.e. the first growth year) by determining the average ratio (over the three sub-plots of a given treatment) between the number of plants that have emerged or survived and the number of transplanted plants (for S\_p-sd, S\_p-dd and G\_p-sd) or planted rhizomes (G\_r-sd). For each sub-plot, 25 plants at single density and 50 plants for double density were considered for this measurement.

The regrowth rate was measured at the beginning of the second growing year at leaf development stage 1–12 according to the BBCH scale (Tejera & Heaton, 2017) to assess the quality of the regrowth of miscanthus. This corresponds for each treatment to the average ratio between the number of plants at leaf development stage in the second year and the number of plants at leaf development stage in the first growth year (over three sub-plots of a given treatment).

The LAI was assessed each growth year (i.e. from 2013 to 2018) between mid-July and mid-August. We collected all the green leaves of 10 shoots from each plot. The length and width of each leaf were measured and the leaf area index was calculated as follows:

$$LAI = \frac{k \times L \times W \times SN}{10},$$

where LAI is the leaf area index, *L* is the length (m), *W* is the width (m), SN is the shoot number per m<sup>2</sup>, *k* is the correction coefficient for plant leaves varying across species. *k* amounts to 0.68 (Clifton-Brown, Neilson, Lewandowski, & Jones, 2000) and 0.61 (Laurent, pers. comm.), respectively, for *M. × giganteus* and *M. sinensis*.

Every year, from the second year of growth (i.e. 2015) until 2019, yield and yield components (shoot weight, shoot number per plant, shoot height, plant density) were estimated from the three sub-plots of each treatment before the harvest of the entire trial (i.e. in February or March). To assess yields, on each sub-plot we cut all shoots higher than 1 m (15 cm from the ground). The fresh matter of the biomass (FM) was calculated in the field using a scale. A sample of 1.5–2 kg of fresh matter (FW) was taken. This sample was immediately weighed in the laboratory to avoid water loss. The sample was then put in the oven for 48 hr at 80°C and weighed to get the dry weight of the sample. Sub-plot yields were in kg/m<sup>2</sup>, and



the plot area ( $A$ ) was 15 or 16 m<sup>2</sup>. We multiplied the formula described below by 10 to express yields in t/ha. The following equation was used to calculate the yield in t of DM per ha:

$$Y = \frac{FM \times (DW/FW) \times 10}{A},$$

where  $Y$  is the yield (t/ha of DM), FM is the fresh matter of the sub-plot (kg), FW is the fresh weight of the sample (kg), DW is the dry weight of the sample (kg) and  $A$  is the sub-plot area (m<sup>2</sup>).

Shoot number counting consisted in enumerating the shoots over 1 m high on each sub-plot. We then counted the number of plants on each sub-plot to determine the crop density (number of plants per m<sup>2</sup>). Shoot height was measured using a tape measure. Lastly, shoot weight (kg) was assessed by dividing yield (expressed in kg/m<sup>2</sup>) by the shoot number per m<sup>2</sup>.

## 2.5 | Statistical analysis

The analysis consisted of two steps: (a) we analysed the effect of treatments on establishment rate, regrowth rate, yield and yield components across site-years; and (b) we modelled the temporal evolution of yields to compare yield trends between treatments. Data analysis was implemented using R software 3.5.2 (R Core Team, 2018).

### 2.5.1 | Analysing the effects of treatments on establishment rate, regrowth rate, yield and yield components

Multi-factor ANOVAs were performed to compare the effect of the treatments on establishment rate, regrowth rate and yield.

$$E_{ij} = \mu_E + \alpha_T T_i + \alpha_S S_j + e_{E,ij},$$

where  $E_{ij}$  is the establishment rate (%) in 2013 or the regrowth rate (%) in 2014 for the  $i$ th treatment of the  $j$ th site,  $T_i$  is the  $i$ th treatment,  $S_j$  is the  $j$ th site,  $\mu_E$  is the average establishment rate,  $e_{E,ij}$  is the residual associated with  $E_{ij}$  and  $\alpha_T$  and  $\alpha_S$  are the parameters associated with each factor.  $E_{ij}$  is assumed to follow independent Gaussian distribution with mean zero and constant variance.  $e_{E,ij} \sim N(0, \sigma_E^2)$ .

$$Y_{ij} = \mu_y + \alpha_T T_i + \alpha_{SA} SA_j + e_{y,ij},$$

where  $Y_{ij}$  is the yield or cumulative yield (t/ha of DM) for the  $i$ th treatment of the  $j$ th site-year,  $T_i$  is the  $i$ th treatment,  $SA_j$  is the  $j$ th site-year,  $\mu_y$  is the average yield,  $e_{y,ij}$  is the residual associated with  $Y_{ij}$  are the parameters associated with each factor.  $Y_{ij}$  is assumed to follow independent Gaussian distribution with mean zero and constant variance.  $e_{y,ij} \sim N(0, \sigma_y^2)$ .

The significance of the difference between treatments was evaluated using a Tukey test with a 0.05 confidence level. The average comparisons between treatments were performed with the multcomp package of R (release 1.4-10).

We performed single linear regressions between: (a) yield and shoot number; and (b) yield and shoot weight, to compare yield build-up between treatments and identify which yield component explains better yield variability.

These regressions were performed according to the following formula:

$$Y_i = \mu + \alpha X_i + e_i,$$

where  $Y_i$  is the explained variable (yield, LAI) for the  $i$ th site,  $X_i$  is the explanatory variable (shoot number, shoot weight, LAI, shoot height) for the  $i$ th site,  $\mu$  is the global average of the explained variable and  $\alpha$  is the parameter associated with the explanatory variable.  $Y_i$  is assumed to follow independent Gaussian distribution with mean and constant variance.  $e_i \sim N(0, \sigma^2)$ .

Clifton-Brown et al. (2000) assumed the yield to be a logistic function of the LAI. We used the SSlogis function of stats package of R (released 3.5.2) to parameterize this relationship. Logistic growth function  $g$  describing miscanthus yield trends over LAI.  $g$  is defined as follows:

$$g(LAI_i, P_{1,i}, P_{2,i}, P_{3,i}) = \frac{P_{1,i}}{1 + \exp((P_{2,i} - LAI)/P_{3,i})},$$

where  $LAI_i$  is the leaf area index for the  $i$ th site.  $P_{1,i}$ ,  $P_{2,i}$  and  $P_{3,i}$  are function parameters for the  $i$ th site. As we will not use the parameters of the function  $g$ , we will not detail them.

### 2.6 | Using a statistical model to compare yield trends across treatments

We used a logistic growth function  $f$  (Laurent, Pelzer, Loyce, & Makowski, 2015; Miguez, Villamil, Long, & Bollero, 2008) to describe miscanthus yield trends over time ( $T$ ).

Based on the data design (repeated yield measurements over time for each site), the model was defined as a mixed effects model, that is, with one (or more) fixed and random parameters. In this model, three random parameters have been defined for the logistic growth:

$$f(T_{ij}, \boldsymbol{\varphi}_i) = \frac{y_{\max,i}}{1 + \exp((\boldsymbol{\varphi}_{1,i} - T)/\boldsymbol{\varphi}_{2,i})}, \quad (1)$$

where  $T_{ij}$  is the  $j$ th year of growth for the  $i$ th site.  $\boldsymbol{\varphi}_i$  is the function parameter vector ( $y_{\max,i}$ ,  $\boldsymbol{\varphi}_{1,i}$ ,  $\boldsymbol{\varphi}_{2,i}$ ) for the  $i$ th site. The first parameter ( $y_{\max,i}$ ) is the deviation asymptote from the curve towards the plateau, that is, the maximum yield for a mature

miscanthus crop. The second parameter ( $\varphi_{1,i}$ ) represents the inflection point, that is, the year in when the crop has reached half of its maximum yield. The third parameter ( $\varphi_{2,i}$ ) gives an indication of the spread of the function, but it can also be interpreted as the time elapsed between reaching the second and the third quarter of the maximum yield.

Equation (2) describes the distribution of yields within a given group (i.e. within a site-year). In this formulation, the sites were nested within the years to calculate the average values of the associated parameters.  $Y_{ij}$  is the  $j$ th yield data collected in the  $i$ th site-year.  $Y_{ij}$  is linked to the time of year since establishment  $T_{ij}$  as follows:

$$Y_{ij} = f(T_{ij}, \varphi_i) + e_{ij}, \quad e \sim N(0, \sigma^2), \quad (2)$$

where  $Y_{ij}$  is the yield of the miscanthus (t/ha of DM),  $f$  is the function linking  $Y_{ij}$  to  $T_{ij}$  and to a set of specific parameters  $\varphi_i$  and  $e_{ij}$  is the residual normally distributed term. All residuals are assumed to be independent, their distribution following a variance  $\sigma^2$ .

Equation (3) describes  $\varphi_k$  (i.e.  $y_{\max}$ ,  $\varphi_1$  and  $\varphi_2$  parameters) by distinguishing fixed and random effects:

$$\varphi_k = \beta_k + b_k, \quad b \sim N(0, \psi). \quad (3)$$

The fixed effect  $\beta_k$  represents the average values of the parameter  $\varphi_k$  on all sites and  $b_k$  represents the local  $\varphi_k$  estimated deviation. It was assumed that the random effect  $b$  of the different parameters in  $\varphi_i$  was related by a multivariate normal distribution of estimated variance–covariance matrix  $\psi$ .

This model was previously adapted to *M. × giganteus* by Miguez et al. (2008) and Laurent et al. (2015). We validated its use for *M. × giganteus* and its extension to *M. sinensis* by verifying that residuals did not have a particular structure and that their distribution was normal (Shapiro–Wilk test). We

also compared the prediction error of the model by using the root mean square error (RMSE) obtained for the two species.

Finally, we performed an ANOVA on the estimated model parameters ( $y_{\max}$ ,  $\varphi_1$ ,  $\varphi_2$ ) to assess the differences in the multi-year trends across treatments.

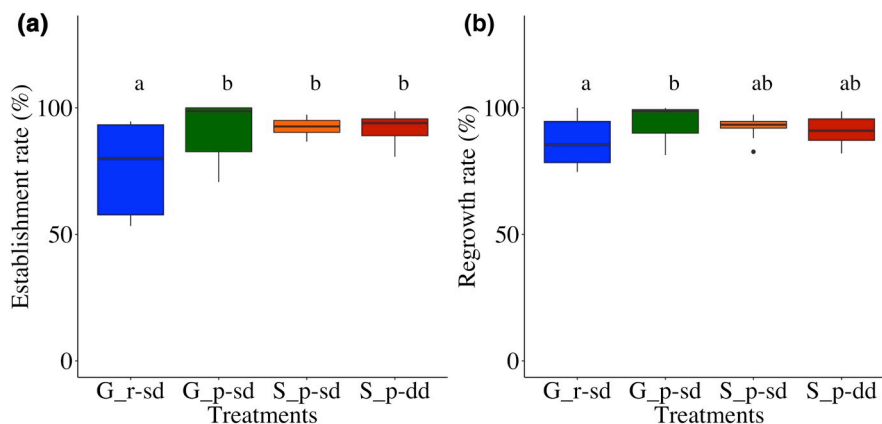
## 3 | RESULTS

### 3.1 | Establishment and regrowth rates

Establishment rates of all sites were higher than 75%. However, the establishment rate of *M. × giganteus* from rhizomes (77%) was significantly lower ( $p = .008$ ) than the establishment rate of *M. × giganteus* from transplanted plants (87%; Figure 1a). The establishment rates of both *M. sinensis* treatments (S\_p-sd and S\_p-dd) were not significantly different and were similar to the establishment rate of *M. × giganteus* from transplanted plants (Figure 1a).

One year after the crop establishment, the regrowth rate of *M. × giganteus* established from rhizomes (86%) was significantly lower ( $p = .001$ ) compared to the regrowth rate of *M. × giganteus* established from plants (94%; Figure 1b). Regrowth rates of both *M. sinensis* treatments (92% and 91%, respectively, for single and double density) were not significantly different.

Lastly, establishment and regrowth rates of *M. × giganteus* from rhizomes were more variable across sites than the treatments established from transplanted plants. The coefficients of variation of G\_r-sd were 23% and 11%, respectively, for establishment and regrowth rates. These coefficients of variation were higher than those of G\_p-sd (14% and 8% for establishment and regrowth rates, respectively) and much higher than those of S\_p-sd (4% and 5% for establishment and regrowth rates, respectively) and S\_p-dd (7% and 6% for establishment and regrowth rates, respectively).



**FIGURE 1** Establishment rate in spring 2013 (a) and regrowth rate in spring 2014 (b) for each treatment across eight sites. Boxplots with the same letter are not significantly different at the 5% threshold. G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density

### 3.2 | Effects of treatments, sites and years on yields

Yields obtained for each treatment varied across sites and years (Figure 2). Note that the ‘yield’ in 2014 corresponds to the quantity of shredded biomass for the first growth year. Yield trends of G<sub>p</sub>-sd treatment in Chanteloup site are missing because the establishment of this treatment did not succeed (Figure 2b).

Years, sites and treatments had a significant effect on yields when taken alone and considering their interaction in pairs (Table 2). Yields were indeed affected by treatment × sites ( $p = 3.92 \times 10^{-15}$ ), treatment × year ( $p = 1.39 \times 10^{-7}$ ) and year × sites ( $< 2.2 \times 10^{-16}$ ; Table 2). We did not do a third level of interaction (treatment × sites × year) because we did not have enough degrees of freedom to do so.

G<sub>r</sub>-sd obtained both the highest yield (20.5 t/ha of DM) and the lowest yield (2 t/ha of DM) of the trial network. G<sub>p</sub>-sd yields ranged from 2.1 to 19.5 t/ha of DM across site-years. S<sub>p</sub>-sd and S<sub>p</sub>-dd obtained yields ranging from 1.8 to 11 t/ha of DM and from 1.4 to 11.9 t/ha of DM, respectively, across site-years (Figure 2).

Over the site-years, G<sub>r</sub>-sd and G<sub>p</sub>-sd (both in *M. × giganteus*) achieved similar yields (Figure 3a). We conclude that *M. × giganteus* can be established using

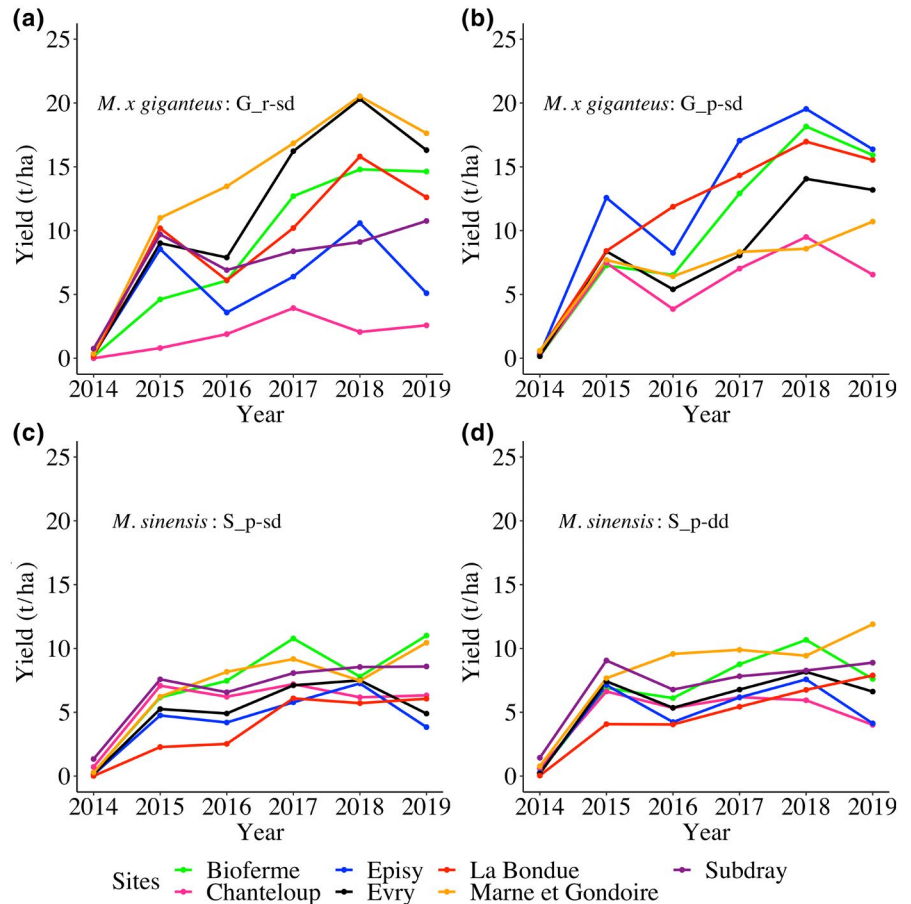
transplanted plants from rhizomes without impacting yields compared to conventional establishment (from rhizomes).

There were, moreover, no significant differences in yields between S<sub>p</sub>-sd and S<sub>p</sub>-dd treatments (Figure 3). As a result, transplanting *M. sinensis* plants at double density (S<sub>p</sub>-dd) did not produce higher yields than transplanting it at single density (S<sub>p</sub>-sd).

Average yields of treatments with *M. × giganteus* (G<sub>r</sub>-sd and G<sub>p</sub>-sd) were significantly higher than those with *M. sinensis* (S<sub>p</sub>-sd and S<sub>p</sub>-dd): they amounted to 8.1 and 8.6 t/ha of DM for G<sub>r</sub>-sd and G<sub>p</sub>-sd, respectively, compared to 5.6 and 5.8 t/ha of DM for S<sub>p</sub>-sd and S<sub>p</sub>-dd, respectively

**TABLE 2** ANOVA results concerning the effects of treatments, sites and years on yields

Factors	Yield (t/ha of DM) <i>p</i> value
Treatment	.002
Site	.003
Year	$< 2.2 \times 10^{-16}$
Treatment × Site	$3.92 \times 10^{-15}$
Treatment × Year	$1.39 \times 10^{-7}$
Site × Year	$< 2.2 \times 10^{-16}$



**FIGURE 2** Yield trends for G<sub>r</sub>-sd (a), G<sub>p</sub>-sd (b), S<sub>p</sub>-sd (c) and S<sub>p</sub>-dd (d) from 2014 to 2019 for the different sites of the trial network. G<sub>r</sub>-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G<sub>p</sub>-sd: *M. × giganteus* established from plantlets. S<sub>p</sub>-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S<sub>p</sub>-dd: *M. sinensis* K1399 established from plantlets at double density



(Figure 3a). Lastly, yield variability was high for the G\_r-sd (coefficient of variation = 73%) and G\_p-sd (coefficient of variation = 64%) compared to S\_p-sd (coefficient of variation = 55%) and S\_p-dd (coefficient of variation = 54%). Both coefficients of variation of *M. sinensis* treatments showed that yields were more stable than *M. × giganteus* treatments.

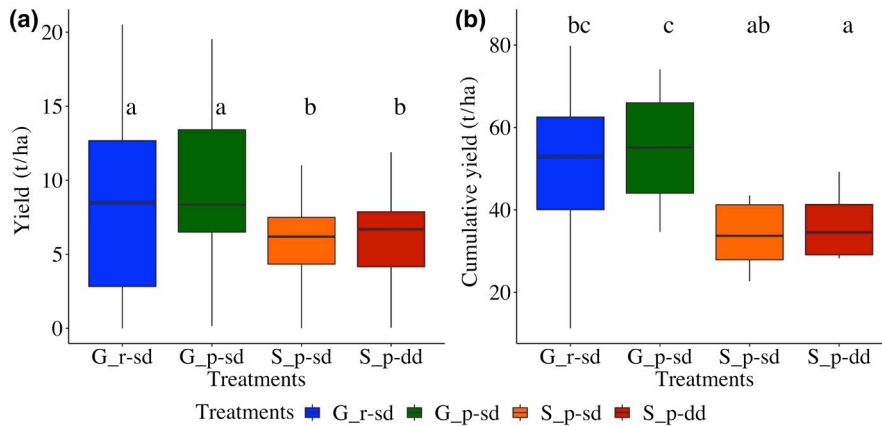
Cumulative yields of G\_r-sd (49.9 t/ha) and G\_p-sd (54.8 t/ha) treatments were not significantly different (Figure 3b). There were also no significant differences between the cumulative yield of both treatments of *M. sinensis* (S\_p-sd = 34 t/ha and S\_p-dd = 36.1 t/ha). The cumulative yield of G\_p-sd was significantly higher than the cumulative yields of both treatments of *M. sinensis* (S\_p-sd and S\_p-dd), whereas the cumulative yield of G\_r-sd was significantly higher than the cumulative yield of S\_p-sd.

### 3.3 | Treatments effect on yield components

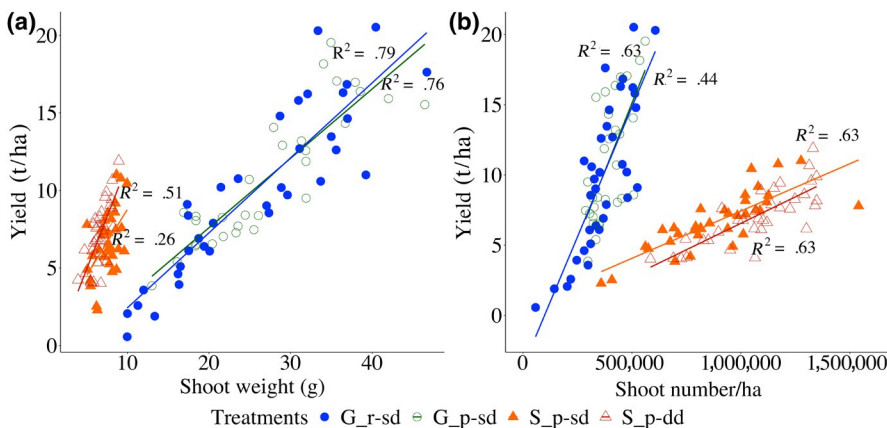
We observed a strong species effect on the relationships between yield and shoot weight (Figure 4a), between yield

and shoot number per ha (Figure 4b), between shoot number per ha and plant density (Figure 5a), between yield and LAI (Figure 6a) and between LAI and shoot height (Figure 6b). *M. × giganteus* G\_r-sd and G\_p-sd modalities did indeed have similar yield build-up, differing from *M. sinensis* S\_p-sd and S\_p-dd modalities, which also had similar yield build-up.

Yields of *M. × giganteus* were more correlated to shoot weight ( $R^2 = .79$  and  $.76$ , respectively, for G\_r-sd and G\_p-sd) than to shoot number ( $R^2 = .63$  and  $.44$ , respectively, for G\_r-sd and G\_p-sd). Yields of *M. sinensis* were more correlated to shoot number per ha ( $R^2 = .63$  and  $.63$ , respectively, for S\_p-sd and S\_p-dd) than to shoot weight ( $R^2 = .26$  and  $.51$ , respectively, for S\_p-sd and S\_p-dd). The slopes of the linear regressions described in Figure 4a (0.50 for G\_r-sd and 0.43 for G\_p-sd) showed that yields of *M. × giganteus* increased more slowly with shoot weight than yields of *M. sinensis* (0.86 for S\_p-sd and 1.31 for S\_p-dd). The slopes of the linear relationship between yield and the shoot number showed that yields of *M. × giganteus* increased more rapidly with shoot weight ( $4.04 \times 10^{-5}$  and  $3.88 \times 10^{-5}$  for G\_r-sd and G\_p-sd) than those of *M. sinensis* ( $6.62 \times 10^{-6}$  and  $7.32 \times 10^{-6}$  for S\_p-sd and S\_p-dd; Figure 4b).

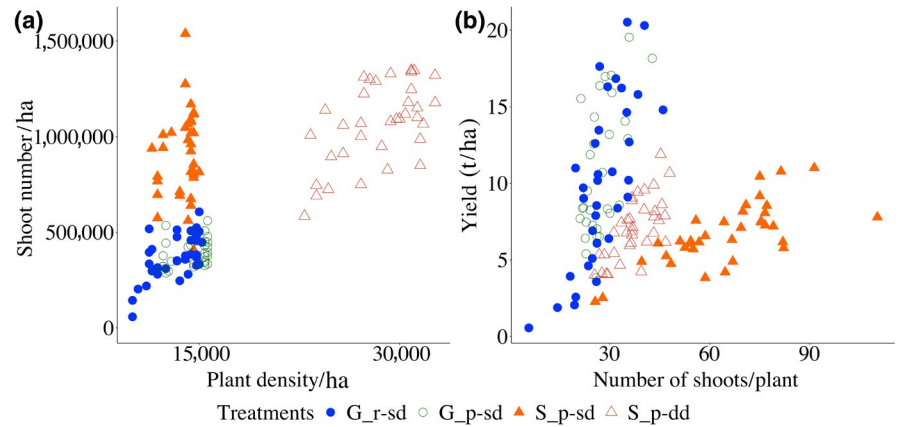


**FIGURE 3** Effects of treatments on miscanthus average yields (a) and cumulative yields (b) from seven sites across 5 years (2015–2019). Boxplots with the same letter are not significantly different at the 5% threshold. G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density

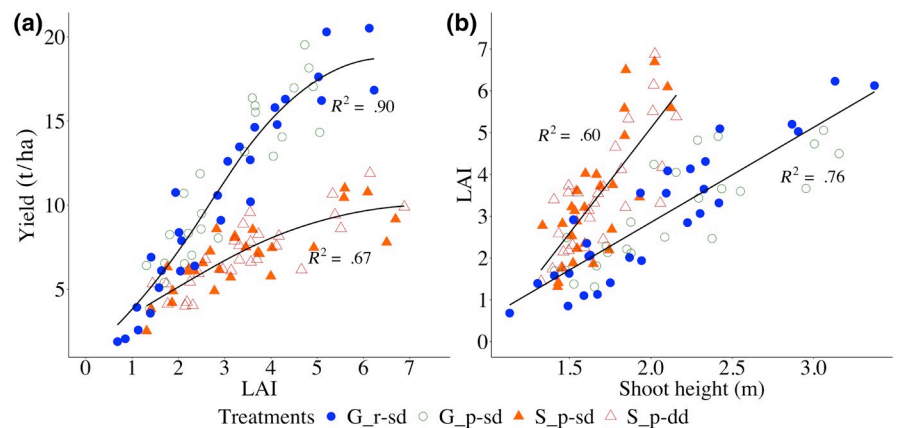


**FIGURE 4** Relationship between yield and shoot weight (a), and between yield and number of shoots per ha (b). G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density

**FIGURE 5** Relationship between shoot number per ha and plant density (a) and between yield and number of shoots per plant (b). G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density



**FIGURE 6** Relationship between LAI and yield (a) and between LAI and shoot height (b). G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density



Plant densities ranged from 10,000 to 15,625 plants/ha for the treatments in single density, that is, G\_r-sd (from 10,000 to 15,208 plants/ha), G\_p-sd (from 11,458 to 15,625 plants/ha) and S\_p-sd (from 11,458 to 15,000 plants/ha). Plant densities of S\_p-dd ranged from 22,888 to 32,666 plants/ha (Figure 5a). At the same plant density, *M. sinensis* (in S\_p-sd) produced more shoots per ha than *M. × giganteus* (in G\_r-sd and G\_p-sd; Figure 5a). Lastly, despite an increased plant density, the number of shoots per ha of *M. sinensis* at double density (S\_p-dd) was similar as *M. sinensis* at single density (S\_p-sd; Figure 5a).

The number of shoots per plant (between 5 and 46 shoots per plant) of *M. × giganteus* was lower compared to that of *M. sinensis* (between 25 and 110 shoots per plant; Figure 5b). G\_r-sd and G\_p-sd treatments obtained similar numbers of shoots per plant. *M. sinensis* at single density produced more shoots per plant than *M. sinensis* at double density (Figure 5b).

*M. × giganteus* observed yields were better correlated with simulated yields following a logistic function of the LAI ( $R^2 = .90$ ) than were *M. sinensis* observed yields ( $R^2 = .67$ ; Figure 6a). At similar LAI values, *M. × giganteus* (G\_r-sd and G\_p-sd) obtained higher biomass than did *M. sinensis* (S\_p-dd and S\_p-sd; Figure 6). The highest yields were obtained for LAI values greater than 5 for both species.

**TABLE 3** Assessment of the model capacity to predict yield for each treatment

Treatments	RMSE (t/ha of DM)	RRMSE (%)
G_r-sd ( <i>Miscanthus × giganteus</i> established from rhizomes)	2.3	39
G_p-sd ( <i>M. × giganteus</i> from plantlets)	2.3	41
S_p-sd ( <i>Miscanthus sinensis</i> established from plantlets at single density)	1.1	37
S_p-dd ( <i>M. sinensis</i> established from plantlets at double density)	1.2	38

Abbreviations: RMSE, root mean square error; RRMSE, ratio root mean square error.

Shoot heights of *M. × giganteus* were more positively correlated to LAI ( $R^2 = .76$ ) than those of *M. sinensis* ( $R^2 = .60$ ). The slope of the regression line of *M. × giganteus* (2.28) showed in Figure 5b was lower than that of *M. sinensis* (5.04). The highest LAIs were obtained for the largest shoot heights. This relationship could be explained by leaf production, which increased as the plant grew (Figure 6b).

**TABLE 4** Comparison of model parameters as a function of treatments

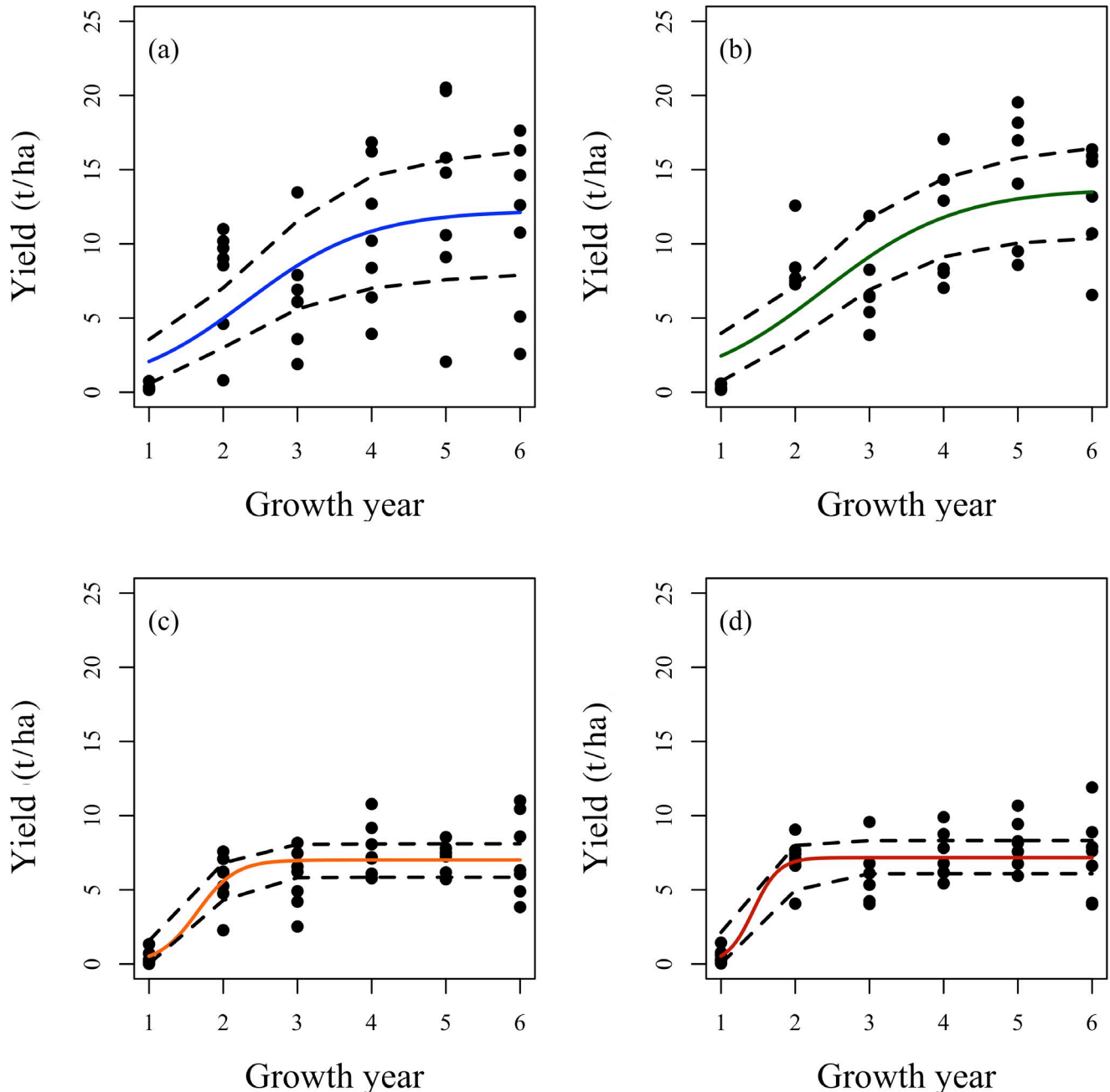
Treatments	$y_{\max}$	$\varphi_1$	$\varphi_2$
G_r-sd	12.2 a	2.3 a	0.8 a
G_p-sd	13.7 a	2.4 b	0.9 b
S_p-sd	7 b	1.6 c	0.3 c
S_p-dd	7.2 b	1.4 d	0.2 d

Note: Values followed by the same letter on the same column are not significantly different at the 5% threshold.

### 3.4 | Modelling the multi-year trend of miscanthus yields

#### 3.4.1 | Assessment of the model

The model used to describe the multi-year trend was evaluated for all treatments (Table 3). The residuals of the model had no particular structure, and followed a normal distribution (Shapiro test > 0.05). RMSE value was 2.4 and 2.3 t/ha of DM for *M. × giganteus* (respectively, for G\_r\_sd



**FIGURE 7** Miscanthus yield trends for G\_r-sd (a), G\_p-sd (b), S\_p-sd (c) and S\_p-dd (d) across growth years. G\_r-sd: *Miscanthus × giganteus* (*M. × giganteus*) established from rhizomes. G\_p-sd: *M. × giganteus* established from plantlets. S\_p-sd: *Miscanthus sinensis* K1399 established from plantlets at single density. S\_p-dd: *M. sinensis* K1399 established from plantlets at double density

and G<sub>p</sub>-sd). *M. sinensis* had an RMSE of 1.1 and 1.2 t/ha of DM, respectively, for S<sub>p</sub>-sd and S<sub>p</sub>-dd. The prediction error ratio (RRMSE between 37% and 41%) is close for both species, suggesting that the model is just as efficient for *M. sinensis* yields as it is for *M. × giganteus* yields, or even better.

### 3.4.2 | Multi-year yield trends

Neither of the two parameters ( $y_{\max}$ ; representing the maximum yields achieved) were significantly different (a) between *M. × giganteus* treatments or (b) between both treatments of *M. sinensis*. Additionally,  $y_{\max}$  values of G<sub>r</sub>-sd (12.2) and G<sub>p</sub>-sd (13.7) were significantly higher than those of S<sub>p</sub>-sd (7) and S<sub>p</sub>-dd (7.2; Table 4).

Parameters  $\varphi_1$  and  $\varphi_2$ , indicating the crop biomass evolution rate, were significantly different across treatments. *M. × giganteus*—G<sub>r</sub>-sd and G<sub>p</sub>-sd took longer than *M. sinensis* (S<sub>p</sub>-sd and S<sub>p</sub>-dd) to reach its maximum yield (Table 4).

*M. × giganteus* yields were beginning to reach the plateau in the sixth growth year. *M. sinensis* achieved a plateau between the second and third year of growing and then remained stable.

*M. sinensis* established from plantlets at double density tended to reach its maximum yield slightly faster than *M. sinensis* established from plantlets at single density (Figure 7). However, the maximum yield was not significantly different for both treatments.

## 4 | DISCUSSION

### 4.1 | Benefits of establishing miscanthus from plantlets

*M. × giganteus* established from rhizomes resulted in higher losses (23%) compared to the treatments established from plantlets (13%). Boersma and Heaton (2014a) found a similar result (equal to 23%) for establishment from rhizomes. To overcome such losses, Christian, Yates, and Riche (2009) recommend using 14% more rhizomes. Losses of plants during the establishment from rhizomes have been explained by intrinsic properties of the rhizomes, lack of active buds, small rhizome size, loss of germination capacity due to bacterial or fungal attacks during storage (Covarelli, Beccari, & Tosi, 2012; Xue et al., 2015) and soil and climate conditions (Boersma & Heaton, 2014b). Establishment from plantlets overcomes these difficulties. Transplanted plantlets also eliminate the need for a re-establishment worksite and make it possible to avoid a significant delay in the stage between the plants resulting from the establishment and the re-establishment.

The regrowth rate of miscanthus established from transplanted plantlets is also higher than establishment from rhizomes. The order of magnitude of regrowth rate of miscanthus established from rhizomes observed in our study (88%) is consistent with the one estimated by Pyter, Dohleman, and Voigt (2010), who found a regrowth rate of 88%. In contrast, Boersma and Heaton (2014a) found a regrowth rate higher than those we encountered (98.8%). This discrepancy with our results could be explained by the management method chosen by these authors, who replaced the dead plants before the winter so as to have 100% survival at the end of the first year. Plant losses can be due to drought or frost. The main source of plant losses during regrowth is due to winter frost. Devastating winter losses have been widely discussed in the literature (Clifton-Brown, 1997; Clifton-Brown, Breuer, & Jones, 2007; Kucharik, VanLoocke, Lenters, & Motew, 2013; Purdy et al., 2013). Losses related to the establishment from rhizomes could lead to gross margin reductions of up to 50% over the whole miscanthus production period (Zimmermann, Styles, Hastings, Dauber, & Jones, 2014). The better establishment from transplanted plants could reduce these economic losses.

### 4.2 | A strong genotype effect on yield build-up

*M. × giganteus* obtained higher average yields than *M. sinensis*. Our results are in line with those of Clifton-Brown et al. (2001) and Lewandowski et al. (2003) obtained in European conditions. In Asian conditions, *M. sinensis* obtained higher yields than *M. × giganteus*: Nakajima et al. (2018) and Yu, Ding, Huai, and Zhao (2013) observed higher average yield for *M. sinensis* (~30 t/ha of DM) than for *M. × giganteus* (~20 t/ha of DM). Asian conditions are characterized by much higher annual rainfall (exceeding 1,000 mm) than in Northern Europe (where average annual rainfall amounted to 600 mm), which could explain the opposite yield differences between *M. sinensis* and *M. × giganteus*. Higher yields of *M. × giganteus* could also be explained by its lateness, as late genotypes have been shown to achieve higher yields (Gauder, Graeff-Hönniger, Lewandowski, & Claupein, 2012; Jensen et al., 2013; Lewandowski et al., 2000).

Yield differences between the treatments were linked to a strong genotype effect, which in particular determined yield potential. *M. sinensis* showed variability between plants within the same strip. In particular, shoot diameter, shoot height and shoot number per plant differed across plants within a given treatment-site-year. In contrast, *M. × giganteus* showed an architectural uniformity of plants within a plot. Plant height, leaf and shoot proportion are key factors explaining the variability in biomass production



between species (Zub & Brancourt-Hulmel, 2010). Lim et al. (2014) found similar results from the analysis of 66 miscanthus genotypes: they showed that plant height and shoot weight were positively and significantly correlated with yield.

Our results showed that yields of *M. × giganteus* were linked to shoot weight while those of *M. sinensis* were essentially determined by the number of shoots per ha. A canopy architecture specific to each species is therefore established, determined by plant height (3.5 m for *M. × giganteus* compared to 2.5 m for *M. sinensis*) and by the number of shoots per plant, which was higher for *M. sinensis* (between 25 and 110 shoots per plant) than for *M. × giganteus* (between 5 and 46 shoots per plant). Similar results were found by Zapater et al. (2017) who found a shoot production of *M. sinensis* twice as high as that of *M. × giganteus*, with a higher canopy height of *M. × giganteus* compared to *M. sinensis*. Gauder et al. (2012) found a lower tillering of *M. × giganteus* (38–43 shoots per plant) than that of *M. sinensis* (116–150 shoots per plant). They also found that plant height was the most appropriate indicator to explain yield differences across *M. × giganteus* and *M. sinensis*. However, it cannot be considered as the only indicator because *M. sacchariflorus* was about 30 cm higher than *M. × giganteus* genotypes but had lower yields.

The LAI values that we observed reinforce this species effect. At similar LAI value, *M. × giganteus* produced higher yields than did *M. sinensis*. Our results showed that maximum yields were reached when LAI is higher than 5 for both species, and with a more efficient use of the solar radiation intercepted by *M. × giganteus* compared to *M. sinensis*. Beyond that value, we observed a yield plateau. Our results are consistent with those of Defra (2007) that showed that the LAI value for which miscanthus yields are maximum varied between 5 and 6. Our results are also consistent with those of Davey et al. (2017) who found maximum LAI values of 6 for *M. × giganteus* and 4.3 for *M. sinensis* with more efficient use of the incident Photosynthetic Active Radiation (PAR) of *M. × giganteus* compared to *M. sinensis*. Davey et al. (2017) noted that from an LAI of 3.5, *M. × giganteus* could intercept 90% of the incident PAR. The yield plateau we then described for higher LAI values could be due to self-shading of the leaves which limits photosynthesis and no longer allows an increase in yields (Davey et al., 2017; Defra, 2002).

### 4.3 | Yield variability across site-years

Beyond the effect of treatments, we observed a variability related to sites and years, with a higher effect of years than sites. Our results also showed a greater inter-annual and inter-site variability of *M. × giganteus* than *M. sinensis*. High

average yields of *M. × giganteus* were associated with high yield variability across sites, as shown by Lesur et al. (2013) and Miguez et al. (2008). Similar results to those of our study were obtained by Laurent et al. (2015) and Clifton-Brown et al. (2007), which showed that yields of *M. × giganteus* varied across sites and years. Kalinina et al. (2017) also found interannual and inter-site variability for several *M. sinensis* genotypes.

The inter-site variability of yields could be related to the edaphic variability of our trial network, characterized by contrasting soil types that can generate a range of crop sensitivity to stress, particularly water stress. As the annual yield variability could be linked to crop age and to climatic variability, it is not straightforward to explain them. Further studies on our trial network could be carried out to identify the main factors responsible for the variability in miscanthus yields across site-years, in order to draw conclusions for a better management of miscanthus and an anticipation of its location.

### 4.4 | Which perspectives for the management of miscanthus?

Describing yield trend curves could be useful for the management of *M. × giganteus* and *M. sinensis* established from plantlets.

Despite the losses at establishment and regrowth periods, *M. × giganteus* established from plantlets showed a yield increase rate close to that of *M. × giganteus* established from rhizomes. According to the model, the maximum yield of *M. × giganteus* is reached after 6 years of cultivation or more. Our results are consistent with those of Lesur et al. (2013) and Christian, Riche, and Yates (2008) who found that the maximum yields of *M. × giganteus* were reached after 8 years of growth. This proximity of yields trends could be explained by a similarity of the physiology of the two establishment modes of *M. × giganteus*. After the year of establishment, both treatments restarted in the same period each spring and the growth and development process were identical.

*M. sinensis* established in single and double density reached similar maximum yields. These results are consistent with those of Lesur et al. (2013) and Miguez et al. (2008) who found no effect of plant density on maximum yields for *M. × giganteus*. Atkinson (2009) obtained results which differed from ours: they showed that increasing the establishing density from 10,000 to 40,000 plants/ha increased yields. This difference with our results could be explained by the larger difference between the two densities than the one we tested, and by the use of a plant density (10,000 plants/ha) that is below the recommended plant density (around 15,000 plants/ha). The slightly faster yield increase of double density in the first years of growth for *M. sinensis* established from



plantlets could be linked to the higher density of plants. The crop therefore covered the area faster and received more light, which enabled more biomass production per area unit. However, after the first few years of growth, there would be greater competition for light, but crops would also be at the maximum of their LAI, and therefore at the maximum of their production (Bullard, Heath, & Nixon, 1995; Lewandowski et al., 2000).

The successful results of establishment from transplanted plants obtained for both species open the way to additional possibilities for miscanthus establishment.

Establishing miscanthus from transplanted plants could be an interesting approach, as it would reduce losses during the establishment phase. However, the resulting profitability of this crop management route needs to be assessed, by taking into account establishment costs and resulting yields. Xue et al. (2015) estimated the cost of establishment from rhizomes at ~1,900–3,400 €/ha and the cost of establishment from plantlets at ~4,200–4,400 €/ha. Establishment from rhizomes is therefore less expensive. However, the multiplication power of rhizomes ( $\times 10$ ) is low compared to the multiplication power of transplanted plants ( $\times 30$ ), which could create a potential gap between supply and demand for establishment from rhizomes in case of large-scale plantation (Xue et al., 2015). The high cost of establishment from plantlets is related to the cost of labour and energy for pre-growing. According to Xue et al. (2015), by improving the multiplication rate of establishment from transplanted plants, the cost of establishment could be reduced to ~2,600 €/ha which would be lower than or equivalent to the cost of establishment from rhizomes. Establishment from seedlings or direct seeding are the least expensive establishment modes (Hastings, 2017), but so far *M. sinensis* seeds are not sterile. Research is required on the breeding of *M. sinensis* sterile seeds to avoid risks of invasiveness (Clifton-Brown et al., 2017). Lastly, a study could be carried out to assess the technical feasibility of establishing miscanthus from transplanted plants in farmers' fields, by mechanizing this establishment mode.

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