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Malick Ouattara, Anabelle Laurent, Fabien Ferchaud, Magali Berthou, Elsa Borujerdi, Arnaud Butier, Pierre Malvoisin, Dominique Romelot, Chantal Loyce

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Evolution of soil carbon stocks under *Miscanthus* × *giganteus* and *Miscanthus sinensis* across contrasting environmental conditions

Malick S. Ouattara[1] | **Anabelle Laurent**[1] | **Fabien Ferchaud**[2] | **Magali Berthou**[3] | **Elsa Borujerdi**[4] | **Arnaud Butier**[1] | **Pierre Malvoisin**[5] | **Dominique Romelot**[6] | **Chantal Loyce**[1]

[1]Université Paris-Saclay, INRAE, AgroParisTech, UMR Agronomie, Thiverval-Grignon, France

[2]BioEcoAgro Joint Research Unit, INRAE, Université de Liège, Université de Lille, Université de Picardie Jules Verne, Barenton-Bugny, France

[3]Phytorestore, La Brosse Montceaux, France

[4]Communauté Urbaine Grand Paris Seine & Oise, Immeuble Autoneum, Aubergenville, France

[5]AELRED, Evry, France

[6]AXEREAL INNOVATIONS, Olivet, France

Correspondence
Malick S. Ouattara, Université Paris-Saclay, AgroParisTech, INRAE, Agronomie, 78850 Thiverval-Grignon, France.
Email: malick.ouattara@inrae.fr

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Abstract

Miscanthus is a C4 bioenergy perennial crop characterized by its high potential yield. Our study aimed to compare the carbon storage capacities of *Miscanthus sinensis* (*M. sinensis*) with that of *Miscanthus* × *giganteus* (*M.* × *giganteus*) in field conditions in different types of soils in France. We set up a multi-environment experimental network. On each trial, we tested two treatments: *M.* × *giganteus* established from rhizomes (Gr) and *M. sinensis* transplanted seedlings (Sp). We quantified the soil organic carbon (SOC) stock at equivalent soil mass for both genotypes in 2014 and 2019 and for two sampling depths: L1 (ca. 0–5 cm) and L1-2 (ca. 0–30 cm). We also calculated the total and annual variation of the SOC stock and investigated factors that could explain the variation and the initial state of the SOC stock. ANOVAs were performed to compare the SOC stock, as well as the SOC stock variation rates across treatments and soil layers. Results showed that the soil bulk density did not vary significantly between 2014 and 2019 for both treatments (Gr and Sp). The SOC concentration (i.e. SOC expressed in g/kg) increased significantly between 2014 and 2019 in L1, whereas no significant evolution was found in L2 (ca. 5–30 cm). The SOC stock (i.e. SOC expressed in t/ha) increased significantly in the superficial layer L1 for *M.* × *giganteus* and *M. sinensis,* by 0.48 ± 0.41 and 0.54 ± 0.25 t ha^{-1} $year^{-1}$ on average, respectively, although no significant change was detected in the layer L1-2 for both genotypes. Moreover, SOC stocks in 2019 did not differ significantly between *M.* × *giganteus* and *M. sinensis* in the soil layers L1 and L1-2. Lastly, our results showed that the initial SOC stock was significantly higher when miscanthus was grown after set-aside than after annual crops.

KEYWORDS

carbon storage, miscanthus, multi-environment trial, set-aside land, soil organic carbon

1 | INTRODUCTION

Miscanthus is a C4 bioenergy crop with a high potential yield (Lewandowski et al., 2000, 2003) and low nitrogen requirements (Cadoux et al., 2014). Environmental assessments of bioenergy crops are required to understand their assets, especially concerning greenhouse gas (GHG) emissions (Morandi et al., 2016). Carbon storage in the soil is one of the most discussed topics in these assessments. Carbon storage by *Miscanthus* × *giganteus* (hereafter named *M.* × *giganteus*) has been studied and results showed contrasted evolutions between sites. Some authors found significant positive soil organic carbon (SOC) storage (Dondini et al., 2009; Dufossé et al., 2014), while others found no significant change (e.g. Ferchaud et al., 2016; Richter et al., 2015). Poeplau and Don (2014) suggested that this variability could be partly due to methodological bias such as differences in baseline SOC stocks between miscanthus and control plots. Using ^{13}C measurements and modelling, these authors proposed an average SOC storage rate of 0.40 ± 0.20 t ha^{-1} year^{-1} for miscanthus plantations on former cropland. These studies also showed that most of the new C4 SOC (i.e. SOC originating from miscanthus) accumulated under miscanthus was found in the topsoil (0–30 cm). Furthermore, although it is known that calculating SOC stocks on an equal depth basis can lead to erroneous values, particularly when comparing situations with different tillage practices (e.g. Palm et al., 2014), only few studies used calculations at equivalent soil mass (ESM) to take into account changes in soil bulk density (Ferchaud et al., 2016; Richter et al., 2015).

New miscanthus genotypes could be explored by taking into account the ability of miscanthus to store carbon in the soil (Nakajima et al., 2018). Miscanthus domestication is still in its infancy and genotypes can be found or bred to suit a wider range of ecological conditions and increase the efficiency of carbon sequestration (Clifton-Brown et al., 2007). Only the interspecific hybrid *M.* × *giganteus* is grown by farmers, which results in low genetic diversity (Zub & Brancourt-Hulmel, 2010). The focus of research on this single variety has led to a lack of interest in other varieties belonging to the same species, such as *Miscanthus sinensis* (hereafter named *M. sinensis*; Clifton-Brown et al., 2001).

Compared to *M.* × *giganteus*, the carbon storage capacity of *M. sinensis* genotype is seldom studied. Miscanthus could store carbon in the soil through: (a) aboveground carbon inputs (i.e. leaves that fall during winter and losses during the harvest; Chimento et al., 2016); (b) the turnover of the belowground biomass (rhizomes and roots; Gregory et al., 2018); and (c) its perennial nature that makes tillage operations almost non-existent, potentially resulting in slower soil carbon mineralization through physical protection (Chimento et al., 2016). However, *M. sinensis* has a lower aboveground biomass production compared to *M.* × *giganteus* (Clifton-Brown & Lewandowski, 2002), which could result in lower aboveground carbon inputs. Thus, a lower SOC storage capacity could be expected under *M. sinensis* than under *M.* × *giganteus*. However, while *M.* × *giganteus* achieved high yields on soils with a high soil water content (Milovanovic et al., 2012), *M. sinensis* is more tolerant to water stress (Clifton-Brown & Lewandowski, 2000). *Miscanthus sinensis* could therefore be better suited to soils with a low water availability, which could result in higher carbon storage on these soils through a higher production. Lastly, changes in SOC stocks due to bioenergy crops depend not only on the crop type and management practices, but also on the land use history (Ferchaud et al., 2016).

Hence, there is a need to assess several genotypes—particularly on carbon storage capacity—that are potential candidates as bioenergy crops in various pedo-climatic areas (Clifton-Brown et al., 2001; Lewandowski et al., 2000) and in soils in which different crops were cultivated before miscanthus (such as set-aside or annual crops). *Miscanthus sinensis* could be an interesting genotype for bioenergy plant breeding because it is more ubiquitous (Clifton-Brown & Lewandowski, 2000; Lewandowski et al., 2003). Therefore, our study aimed to compare, in field conditions, the SOC storage capacity at ESM of one genotype of *M. sinensis* with that of one genotype of *M.* × *giganteus* over different types of soils in France, and with different previous crops.

2 | MATERIALS AND METHODS

2.1 | Site characteristics

A multi-environment experimental network was set up on six sites in France from 2013 to 2019: five sites in the Ile-de-France region and one site in the Centre region (Figure 1).

This network was characterized by a diversity of soil types, with various textures, depths, stoniness and chemical properties (Table 1). It was located in a temperate climatic zone, with mean annual temperatures ranging from 10.5 to 13.4°C between 2013 and 2018 and across sites. Annual rainfall ranged from 262 to 567 mm between 2013 and 2018 and across sites. Soils were close to neutral or slightly basic (with pH varying from 7.8 to 8.3) with $CaCO_3$ contents lower than 50 g/kg. In addition to these varying soil characteristics, trials were either located in agricultural lands, *that is*, lands that were cultivated with annual crops before carrying out the experiment or in marginal lands, *that is*, lands that were previously maintained as set-aside lands, mainly because they were located between several roads/highways and therefore difficult to get to with agricultural equipment.

Before setting up the trials, the Subdray site was managed as set-aside land. The Episy site had been left as set-aside land since 1992. The Marne et Gondoire and Evry sites had

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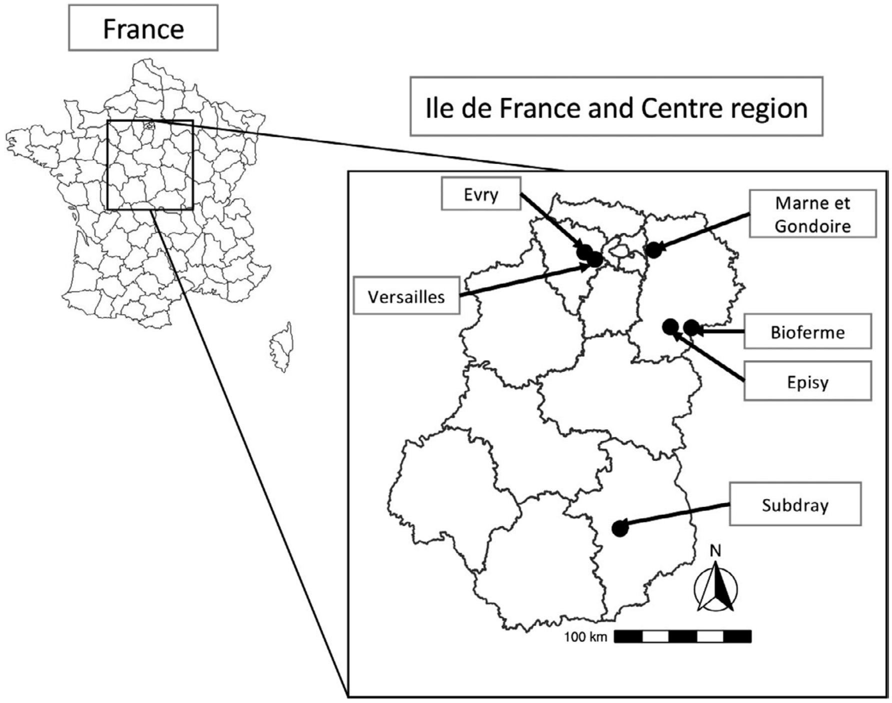
FIGURE 1 Localization of the sites



also been maintained as set-aside lands since 2003 and 2005 respectively. Set-aside management consisted in biomass shredding without exportation. The preceding crop was winter barley (*Hordeum vulgare*) at the Bioferme site and corn (*Zea mays* L.) at the Versailles site (Table 1).

2.2 | Treatments

Each trial of the multi-environment network was composed of two strips, with an area of 141 m^2 (27.2 m × 5.2 m) for each strip. Two treatments corresponding to the two strips were established in the trial network: (a) *M.* × *giganteus* (clone, origin ADAS) established from rhizomes (Gr); and (b) *M. sinensis* K1399 (population variety, origin University of Wageningen) established from seedlings transplanted from seeds (Sp).

2.3 | Crop management strategies

The establishment of miscanthus was carried out manually in March 2013 for all sites. After clearing by using herbicides (for set-aside land) or by ploughing (for Marne et Gondoire, Versailles, Bioferme and Episy sites), soil preparation at all sites consisted of shallow tillage 1 or 2 days before establishment with a tine. During the first year of growth, all treatments were protected against weeds. Weed management was chemical and/or manual depending on the extent of the weed growth observed in the trial. Given the low N requirements of miscanthus, no nitrogen fertilizer was applied throughout the experiment. However, 30 and 300 kg/ha of P_2O_5 and K_2O, respectively, were applied after harvest time in the third year of growth, except for the Bioferme and Episy sites, which were fertilized in the fourth growth year. Miscanthus was not harvested the first year; the aboveground biomass was chopped and left on the soil surface. The following years, miscanthus was harvested in late winter (i.e. in February or March) either using a manual cutting tool (brushcutter; Subdray, Marne et Gondoire and Evry sites) or with a silage harvester (Bioferme, Episy and Versailles sites).

2.4 | Measurements

2.4.1 | Soil bulk density

Soil bulk density was measured on each plot (strip) of the trial network during the first growth year (from February to March 2014) and 5 years later (from February to March 2019). We used one of two methods, depending on the soil layer. For the layer 0–5 cm, bulk density was measured using steel cylinders (length: 5 cm; diameter: 5 cm) inserted vertically into the soil. For the layer 5–30 cm, bulk density was measured using soil cores of 5 cm in diameter. The soil cores were inserted into the ground with a hydraulic gauge and a plastic tube to preserve the initial soil structure. We then measured the soil cores with a measuring tape to keep only the 5–30 cm layer. Six samples were taken in each plot, from the 0–5 and 5–30 cm layers, *that is*, 12 samples per site and sampling date. Samples were dried in an oven at 105°C for 96 hr. The resulting dry samples were weighed and we

TABLE 1 Main characteristics of the sites and soil properties

Sites	Lat. and Long.	Soil texture (0–30 cm)	Preceding crop	Rooting depth (cm)	PB (%)	C (%)	Si (%)	S (%)	pH	$CaCO_3$ (g/kg)	Year	Rainfall[a] (mm)	*T* (°C)
Versailles	48°48′19.9″N, 2°5′09.2″E	Clayey sandy loam	Annual crop	161	0	19	61	20	8.0	<1	2013	602	15.04
											2014	612	16.75
											2015	666	16.76
											2016	580	12.54
											2017	633	12.13
											2018	684	11.93
Episy	48°21′37″N, 2°49′36″E	Clayey sand	Set-aside	75	0	15	16	69	8.4	36	2013	459	10.52
											2014	485	12.02
											2015	359	11.84
											2016	514	11.18
											2017	556	11.17
											2018	354	12.32
Evry	48°51′7″N, 1°59′6″E	Clayey sandy loam	Set-aside	81	0	27	29	44	7.8	<1	2013	420	10.91
											2014	500	12.28
											2015	334	12.00
											2016	464	11.30
											2017	408	10.66
											2018	262	12.53
Subdray	47°1′50″N, 2°19′52″E	Loamy sandy clay	Set-aside	48	0	35	41	24	8.3	48	2013	521	11.68
											2014	540	13.11
											2015	415	11.78
											2016	471	11.71
											2017	483	12.41
											2018	358	13.44
Marne et Gondoire	48°51′58″N, 2°39′45″E	Clayey sandy loam	Set-aside	77	0	28	55	17	8.3	13	2013	439	11.57
											2014	516	12.77
											2015	370	12.54
											2016	492	11.98
											2017	459	12.50
											2018	494	13.07

(Continues)

TABLE 1 (Continued)

Sites	Lat. and Long.	Soil texture (0–30 cm)	Preceding crop	Rooting depth (cm)	PB (%)	C (%)	Si (%)	S (%)	pH	$CaCO_3$ (g/kg)	Year	Rainfall[a] (mm)	*T* (°C)
Bioferme	48°21′17″N, 3°1′57″E	Clayey sandy loam	Annual crop	142	0	27	56	17	8.1	5	2013	459	10.52
											2014	485	12.02
											2015	359	11.84
											2016	514	11.18
											2017	556	11.17
											2018	354	12.32

Abbreviations: C, clay content; PB, proportion of pebbles in volume over the layer 0–30 cm; S, sand content; Si, silt content; *T*, temperature.
[a]Between March 1 and October 15.

subtracted the mass of the cylinder to get the dry soil mass of the sample (WS). Knowing the volume of the sample (VS), bulk density was obtained as follows:
If proportion of pebbles = 0

$$\mathrm{BD} = \frac{\mathrm{WS}}{\mathrm{VS}}. \quad (1)$$

If proportion of pebbles ≠ 0

$$\mathrm{BD} = \frac{\mathrm{WS}}{\mathrm{VS}} \times \left(1 - \frac{P_{\mathrm{m}}}{100}\right), \quad (2)$$

where BD is the bulk density (g/cm^3), WS is the sample dry weight (g), VS is the sample volume (cm^3) and P_m is the mass content of pebbles (%).

2.4.2 | SOC concentration and stock

Soil samples were taken for measuring SOC concentration in 2014 and 2019, at the same time as the bulk density measurements. Soil cores 5 cm in diameter were extracted and divided into two layers (0–5 and 5–30 cm). Three samples were collected following the diagonal of each plot and mixed to obtain a composite sample for each layer. We thereby obtained four composite soil samples per site-year (one composite sample for 0–5 cm, one for 5–30 cm, for each treatment). Plant residues and roots were then manually removed from the samples. Soil samples were dried in an oven at 40°C for 96 hr. Samples were then sieved (2 mm) and a sub-sample was finely ground. The total carbon concentration was analysed using the dry combustion method (NF ISO 10694). The $CaCO_3$ concentration was also measured and, in case of the presence of $CaCO_3$, was used to calculate the SOC concentration.

Soil organic carbon stocks were calculated at ESM (Ferchaud et al., 2016) for each layer and for the years 2014 and 2019.

The soil mass was calculated following the equation:

$$M = 10 \times \mathrm{BD} \times L, \quad (3)$$

where *M* is the mass of dry soil (t/ha), BD is the bulk density (g/cm^3) and *L* is the layer thickness (mm).

The cumulative SOC stock (SOC_m) was obtained according to the following equation:

$$\mathrm{SOC_m} = 0.001 \sum_{n=1}^{2} M(n) \times \mathrm{OC}(n), \quad (4)$$

where *n* is the soil layer (0–5 or 5–30 cm), SOC_m is the soil organic carbon stock (t/ha), *M* is the mass of dry soil (t/ha) and OC is the soil organic carbon concentration (g/kg).

From the calculation of SOC_m, which is a classical stock calculation, we calculated the SOC stock at ESM. The average soil mass calculated in 2014 at each site was considered as the reference mass (M_{ref}) for the calculation of the SOC stock at ESM (Table 2). We considered 2014 as the reference period because it was the initial state of our experimental ground, with homogeneity of all the soil at the same site.

The cumulative SOC stock at ESM (SOC) was calculated as follows:

If $M \geq M_{ref}$

$$\mathrm{SOC}(n) = \mathrm{SOC_m}(n) - 0.001\left(M(n) - M_{ref}\right) \times \mathrm{OC}(n). \quad (5)$$

If $M < M_{ref}$

$$\mathrm{SOC}(n) = \mathrm{SOC_m}(n) + 0.001\left(M_{ref} - M(n)\right) \times \mathrm{OC}(n+1), \quad (6)$$

where SOC is the cumulative soil organic carbon stock at ESM (t/ha), M_{ref} is the cumulative reference soil mass (t/ha), M is the cumulative mass of dry soil (t/ha) and OC is the soil organic carbon concentration (g/kg).

The SOC concentration on an ESM basis was then obtained by dividing the SOC stock at ESM by the reference soil mass of the layer.

In the following, SOC concentrations and SOC stocks are systematically presented on an ESM basis. Soil layers are referred to as L1 and L2 (corresponding to roughly 0–5 and 5–30 cm) and the whole sampling soil layer is called L1-2 (corresponding to roughly 0–30 cm). The equivalent soil depths for each situation, that is, the soil depths corresponding to the reference soil mass according to the bulk density measurements, are presented in Table 2.

The calculation of the change in SOC stocks between 2014 and 2019 was done separately for the L1 (top layer) and L1-2 layers (both L1 and L2 layers) for each treatment. The SOC stock variation between 2014 and 2019, and the annual SOC storage rate was obtained as follows:

$$\Delta\mathrm{SOC} = \mathrm{SOC}_{2019} - \mathrm{SOC}_{2014}, \quad (7)$$

$$\Delta\mathrm{SOC}_{annual} = \frac{\Delta\mathrm{SOC}}{2019 - 2014}, \quad (8)$$

TABLE 2 Reference soil mass (M_{ref}) used for calculations at ESM for each treatment and site and corresponding equivalent soil depth in 2014 and 2019

Layer	Treatment	Site	Reference soil mass (t/ha)	Equivalent soil depth in 2014 (cm)	Equivalent soil depth in 2019 (cm)
L1	Gr	Bioferme	722	4.8	4.7
		Episy	786	5.0	4.7
		Evry	724	5.1	4.8
		Marne et Gondoire	685	4.7	4.5
		Subdray	789	4.9	5.6
		Versailles	743	4.9	4.7
	Sp	Bioferme	722	5.1	4.7
		Episy	786	4.9	5.0
		Evry	724	4.8	4.8
		Marne et Gondoire	685	5.2	5.1
		Subdray	789	5.1	5.9
		Versailles	743	5.0	4.7
L1-2	Gr	Bioferme	4,356	29.6	29.8
		Episy	4,607	29.9	31.4
		Evry	4,653	30.9	30.1
		Marne et Gondoire	4,308	29.2	28.3
		Subdray	4,705	29.6	31.9
		Versailles	4,185	30.1	28.3
	Sp	Bioferme	4,356	30.3	30.8
		Episy	4,607	30.0	30.0
		Evry	4,653	29.0	31.1
		Marne et Gondoire	4,308	30.8	28.8
		Subdray	4,705	30.3	33.8
		Versailles	4,185	29.8	28.1

where ΔSOC is the SOC stock variation (t/ha), ΔSOC_{annual} is the annual SOC storage rate (t ha^{-1} $year^{-1}$) and SOC_{year} is the SOC stock for the considered year (t/ha).

2.4.3 | Soil physical proprieties and chemical analysis

Soil samples were collected during the first growth year of miscanthus (from February to March 2014) to characterize the soils and to assess their chemical fertility. A composite soil sample was made in each site by mixing 10 soil cores collected on a diagonal across the entire trial. These cores were collected with a motorized auger over the 0–30 cm layer. The samples were dried in an oven at 40°C for 96 hr. The following analyses were carried out: soil texture, pH, P_2O_5, exchangeable K, $CaCO_3$, CaO and MgO.

2.4.4 | Yield

Yields were estimated every year at harvest from three subplots of each treatment. To assess yields, all shoot higher than 1 m have been cut (15 cm from the ground). Using a scale, the fresh matter of the biomass (FM) was weighed in the field. A sub-sample of 1.5–2 kg of fresh matter (FW) was taken and then put in the oven for 48 hr at 80°C and weighed to get its dry weight (DW). The following equation was used to calculate the yield in t of DM per ha:

$$Y = \frac{\mathrm{FM} \times \frac{\mathrm{DW}}{\mathrm{FW}} \times 10}{A},$$

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 sub-sample (kg), DW is the dry weight of the sub-sample (kg) and A is the subplot area (m^2).

More details on yield measurements are given in Ouattara et al. (2020).

2.5 | Statistical analysis

Data analyses were performed using the software R version 4.0.2 (R Core Team, 2020).

2.5.1 | Analysing the effects of treatments on bulk density and SOC concentration

ANOVA was performed according to the following equation to study the effect of treatments, and years on bulk density:

$$\mathrm{BD}_{ijk} = \mu_{\mathrm{BD}} + \alpha_{\mathrm{T}}\mathrm{T}_i + \alpha_{\mathrm{S}}\mathrm{S}_j + \alpha_k \mathrm{Y}_k + e_{\mathrm{BD},ijk}, \tag{9}$$

$$e_{\mathrm{BD},ijk} \sim N(0, \sigma^2_{\mathrm{BD}}),$$

where BD_{ijk} is the bulk density (g/cm^3) for the ith treatment of the jth site of kth year, T_i is the ith treatment, S_j is the jth site, Y_k is the kth year, μ_{BD} is the average BD, $e_{BD,ijk}$ is the residual associated with BD_{ijk}. $\alpha_{T,}$ α_S and α_Y are the parameters associated with each factor. Site (S) is considered as a random effect. BD_{ijk} is assumed to follow independent Gaussian distribution with mean zero and constant variance.

The effect of the treatments and years on SOC concentration was analysed with another multi-factor ANOVA as follows:

$$\mathrm{OC}_{ijk} = \mu_{\mathrm{OC}} + \alpha_{\mathrm{T}}\mathrm{T}_i + \alpha_{\mathrm{S}}\mathrm{S}_j + \alpha_k \mathrm{Y}_k + e_{\mathrm{OC},ijk}, \tag{10}$$

$$e_{OC,ijk} \sim N(0, \sigma^2_{\mathrm{OC}}),$$

where OC_{ijk} is the soil organic carbon concentration (g/cm^3) for the ith treatment of the jth site of kth year, T_i is the ith treatment, S_j is the jth site, Y_k is the kth year, μ_{oc} is the average OC, $e_{OC,ijk}$ is the residual associated with OC_{ijk}. $\alpha_{T,}$ α_S and α_Y are the parameters associated with each factor. Site (S) is considered as a random effect. OC_{ijk} is assumed to follow independent Gaussian distribution with mean zero and constant variance.

2.5.2 | Analysing the effects of treatments on SOC stock and SOC stock annual variation rate

ANOVAs were performed to study the effect of the treatments and years on SOC stock and SOC stock annual variation rate:

$$\mathrm{SOC}_{ijk} = \mu_{\mathrm{SOC}} + \alpha_{\mathrm{T}}\mathrm{T}_i + \alpha_{\mathrm{S}}\mathrm{S}_j + \alpha_k \mathrm{Y}_k + e_{\mathrm{SOC},ijk}, \tag{11}$$

$$e_{SOC,ijk} \sim N(0, \sigma^2_{\mathrm{SOC}}),$$

where SOC_{ijk} is the SOC stock (t/ha) for the ith treatment of the jth site of kth year, T_i is the ith treatment, S_j is the jth site, Y_k is the kth year, μ_{SOC} is the average SOC, $e_{SOC,ijk}$ is the residual associated with SOC_{ijk}, and α_T, α_S and α_Y are the parameters associated with each factor. Site (S) is considered as a random effect. SOC_{ijk} is assumed to follow independent Gaussian distribution with mean zero and constant variance.

$$\Delta\mathrm{SOCannual}_{ij} = \mu_{\Delta\mathrm{soc}} + \alpha_{\mathrm{T}}\mathrm{T}_i + \alpha_{\mathrm{S}}\mathrm{S}_j + e_{\Delta\mathrm{SOCannual},ij}, \tag{12}$$

$$e_{\Delta\mathrm{SOCannual},ij} \sim N(0, \sigma_{\Delta\mathrm{SOCannual}}),$$

where $\Delta SOCannual_{ij}$ is the annual SOC stock variation rate (t ha^{-1} $year^{-1}$) for the ith treatment of the jth site, T_i is the ith treatment, S_j is the jth site, $\mu_{\Delta SOC}$ is the average SOC, $e_{\Delta SOC,ij}$ is the residual associated with $\Delta SOCannual_{ij}$, and α_T and α_S

are the parameters associated with each factor. Site (S) is considered as a random effect. $\Delta SOCannual_{ij}$ is assumed to follow independent Gaussian distribution with mean zero and constant variance.

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

2.5.3 | Explaining SOC stock variation and initial SOC stock using candidate variables

We used linear regressions to determine the relationship between yield (Y) and clay and silt content (CS) on one hand, and SOC stock variation on the other hand. These linear regressions were performed according to the following formula:

$$\Delta SOC_i = \mu + \alpha X_i + e_i, \quad (13)$$

$$e_i \sim N(0, \sigma^2),$$

where ΔSOC_i is SOC stock variation for the ith site, X_i is the explanatory variable (Y for the L1 and L1-2 layers, CS for L2 layer and initial SOC for L1-2 layer) for the ith site, μ is the global average of SOC stock variation and α is the parameter associated with the explanatory variable. ΔSOC_i is assumed to follow an independent Gaussian distribution with mean and constant variance.

We also evaluated the effect of preceding crop (P) and CS on the initial SOC stock. The following multiple regression model was used:

$$SOCin_i = \mu_Z + \alpha_P P_i + \alpha_{CS} CS_i + e_{SOCin,i}, \quad (14)$$

$$e_{SOCin,i} \sim N(0, \sigma^2_{SOCin}),$$

where $SOCin_i$ is the initial SOC stock for the ith site, P_i and CS_i are the explanatory variables for the ith site, μ_Z is the global average of initial SOC stock. α_P and α_{CS} are the parameters associated with the explanatory variable. $SOCin_i$ stock is assumed to follow independent Gaussian distribution with mean and constant variance.

3 | RESULTS

3.1 | Soil bulk density across site-years

Soil bulk density did not vary significantly between 2014 and 2019 and between treatments for all soil layers considered (Figure 2). Bulk densities ranged from 1.30 to 1.61 g/cm^3 in 2014 and from 1.32 to 1.65 g/cm^3 in 2019 for the 0–5 cm layer. Average bulk densities were 1.48 and 1.49 g/cm^3 in 2014 and 2019 respectively (Figure 2a). In the 5–30 cm layer, bulk densities ranged from 1.37 to 1.62 g/cm^3 with an average of 1.49 g/cm^3 in 2014 and from 1.39 to 1.55 g/cm^3 with an average of 1.48 g/cm^3 in 2019 (Figure 2b). Average bulk densities over the 0–30 cm layer were 1.49 and 1.48 g/cm^3 in 2014 and 2019 respectively (Figure 2c).

3.2 | Effect of treatments on the evolution of SOC concentration

In the layer L1, the SOC concentration significantly increased ($p < .001$) between the year 2014 (Gr = 16.1 g/kg and Sp = 15.8 g/kg) and the year 2019 (Gr = 19.9 g/kg and Sp = 19.8 g/kg; Figure 3a). There was no significant difference between treatments for a given year. The SOC

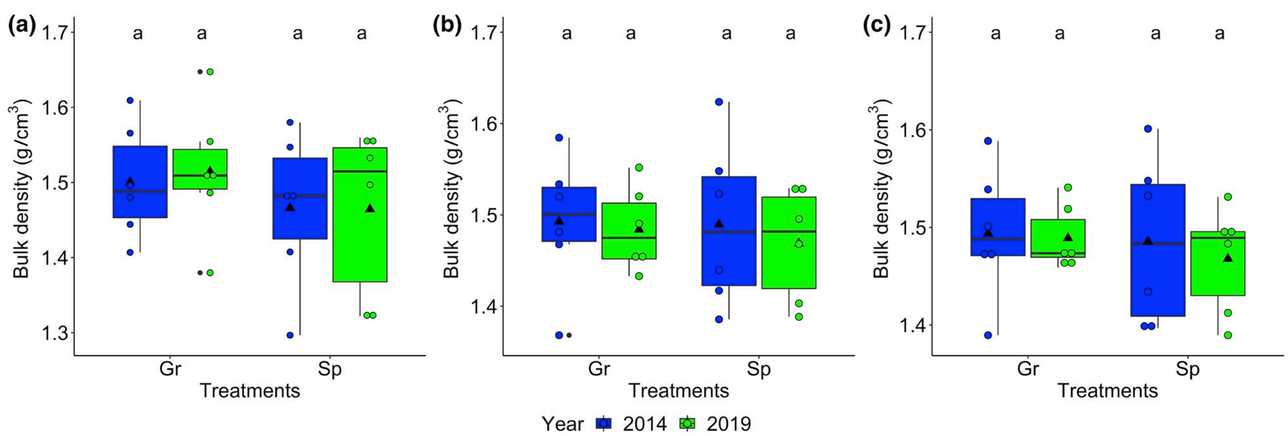


FIGURE 2 Bulk density for each treatment in the 0–5 cm (a), 5–30 cm (b) and 0–30 cm (c) soil layers. Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds. The triangle represents the average value across all sites, the bold black horizontal lines the median value, and the fine black vertical lines the 25th and 75th percentiles of bulk density. Blue dots and green dots are the individual measurement values. Boxplots with the same letter are not significantly different at the 5% threshold

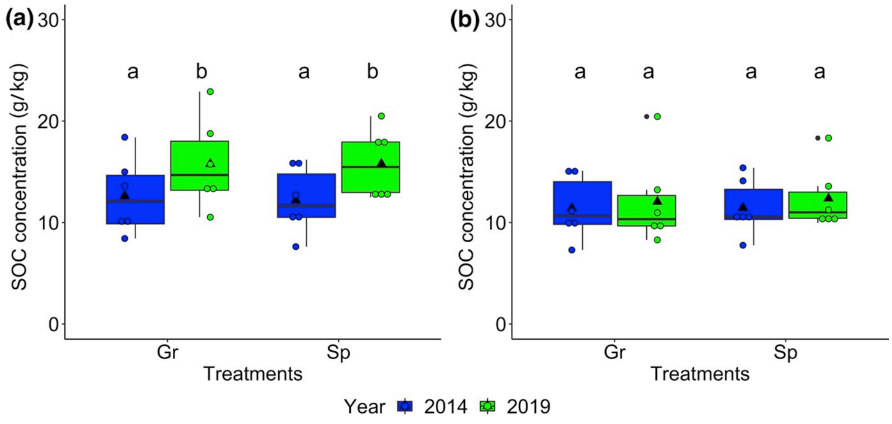


FIGURE 3 Soil organic carbon concentration for each treatment and year in the layers L1 (a) and L2 (b). Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds. The triangle represents the average value, the bold black horizontal lines the median value, and the fine black vertical lines the 25th and 75th percentiles of C concentration. Blue dots and green dots are the individual measurement values. Boxplots with the same letter are not significantly different at the 5% threshold

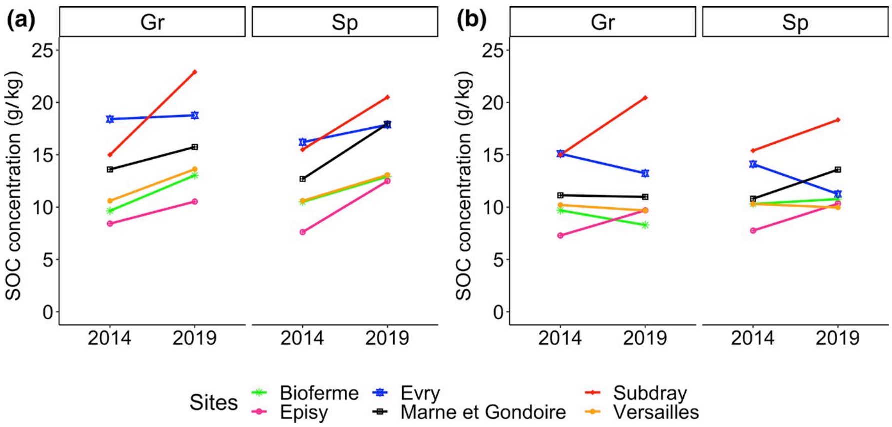


FIGURE 4 Soil organic carbon concentration trends between 2014 and 2019 for each site and each treatment in layers L1 (a) and L2 (b). Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds

concentration ranged from 7.62 to 18.4 g/kg in 2014 and from 10.53 to 22.9 g/kg in 2019 (Figure 3a).

In the layer L2, there was no significant difference ($p = .68$) between 2014 and 2019, nor across treatments (Figure 3b).

In the layer L1, we found that SOC concentration increased at all sites (Figure 4a) between 2014 and 2019, whereas more diverse trends were observed across sites in L2. The changes in L2 were similar between the two treatments for a given site, except at the Bioferme and Marne et Gondoire sites where the SOC concentration decreased in Gr and increased in Sp.

There was a relatively large inter-site variability of SOC concentration for both layers. The coefficients of variation were rather similar between years, treatments and layers, ranging from 22% (for Sp in L1 in 2019) to 37% (for Gr in L2 in 2019).

3.3 | Effect of treatments on the evolution of SOC stocks

In 2019, a significant increase in SOC stocks ($p < .001$) compared to 2014 was observed in the L1 layer for Gr and Sp treatments, with no significant difference between treatments (Figure 5a). In 2019, average SOC stocks amounted 11.7 t/ha for Gr and Sp, compared to 9.3 and 9 t/ha in 2014 for Gr and Sp respectively. This corresponded to an increase of 2.4 and 2.7 t/ha between 2014 and 2019 for Gr and Sp respectively.

For the L1-2 layer, there was no significant difference between the SOC stock in 2019 and the SOC stock in 2014 ($p = .06$), nor between the two treatments ($p = .89$; Figure 5b). Average SOC stock of Gr was 52.1 and 57.1 t/ha in 2014 and 2019, respectively, while average SOC of Sp was 51.9 t/ha in 2014 and 58.1 t/ha in 2019 (Figure 5b).

In the L1 layer, the SOC stock increased between 2014 and 2019 at all sites for both treatments (Figure 6a). In the L1-2 layer, the SOC stock either increased or decreased, depending on the site, and likewise between the two treatments (Figure 6b). There was, however, an exception at the Bioferme site where the SOC stock in L1-2 decreased in Gr but increased in Sp. As for SOC concentration, the SOC stock showed a fairly significant inter-site variability that was similar for both treatments: the coefficient of variation across sites was on average 28% in L1 and 31% in L1-2.

3.4 | Effect of treatments on annual SOC storage rate

The annual rate of SOC storage in the L1 soil layer was not significantly different between treatments: 0.48 and 0.54 t ha^{-1} $year^{-1}$ on average, respectively, for Gr and Sp (Figure 7a). There was a wider variability between sites for Gr than for Sp: the SOC variation rate ranged from 0.05 to 1.25 t ha^{-1} $year^{-1}$ in Gr and from 0.24 to 0.79 t ha^{-1} $year^{-1}$

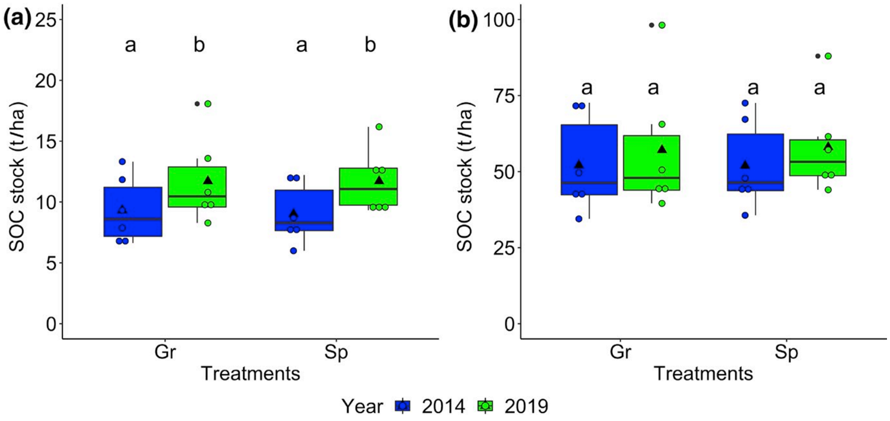


FIGURE 5 Soil organic carbon (SOC) stocks for each treatment and year in the layers L1 (a) and L1-2 (b). Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds. The triangle represents the average value, the bold black horizontal lines the median value, and the fine black vertical lines the 25th and 75th percentiles of SOC. Blue dots and green dots are the individual measurement values. Boxplots with the same letter are not significantly different at the 5% threshold

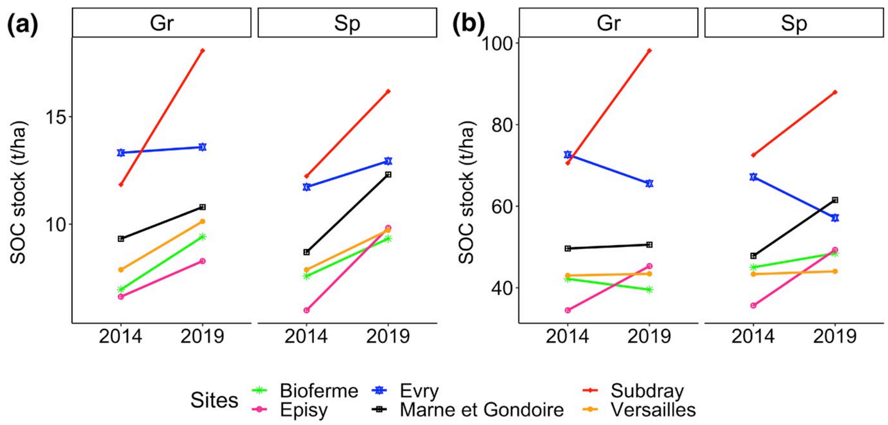


FIGURE 6 Soil organic carbon stock evolution between 2014 and 2019 for each site and each treatment in layers L1 (a) and L1-2 (b). Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds

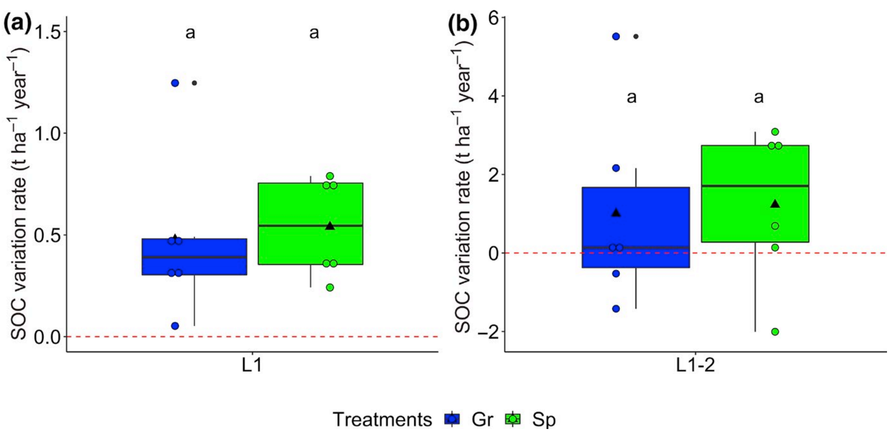


FIGURE 7 Effect of treatments on the annual soil organic carbon (SOC) storage rate for the layers L1 (a) and L1-2 (b). Gr: *Miscanthus* × *giganteus* established from rhizomes. Sp: plantlets of *M. sinensis* K1399 from transplanted seeds. The triangle represents the average value, the bold horizontal lines the median value, and the fine vertical lines the 25th and 75th percentiles of the SOC variation rate. Blue dots and green dots are the individual measurement values. Boxplots with the same letter are not significantly different at the 5% threshold

in Sp. There was not either a significant difference between treatments for the L1-2 soil layer (Figure 7b). The average SOC variation rate was positive for both treatments (1 and 1.2 t ha^{-1} year^{-1}, respectively, for Gr and Sp) but a wide variability was observed: from −1.42 to 5.51 t ha^{-1} year^{-1} for Gr and from −2.01 to 3.08 t ha^{-1} year^{-1} for Sp.

3.5 | Factors explaining initial SOC stock and SOC stock evolution

Clay and silt content (CS) and preceding crop (P) were selected as candidate variables to explain initial SOC stock variability across sites. This analysis was implemented for the layer L1-2. We found a significant effect of the preceding crop on the initial SOC stock in 2014, with a p-value of .04. However, the initial SOC stock was not significantly affected by CS (p = .17; Table 3). The initial SOC stock of a site was therefore linked to the site history: fields with set-aside as the preceding crop had an average SOC stock in 2014 of 56.3 t/ha compared to 43.4 t/ha for sites with an annual crop as the preceding crop.

In order to identify the factors that could contribute to the evolution of the SOC stock between 2014 and 2015, we evaluated the relationship between the SOC stock variation and: (a) the cumulative yield (between 2014 and 2019), used as a

TABLE 3 ANOVA results concerning the effects of candidate factors on initial SOC stock and SOC stock variation

Model	Factor	*p*-value
$\mathrm{SOCin}_i = \mu_Z + \alpha_P P_i + \alpha_{CS} CS_i + e_{SOCin,i}$ [b]	CS	.17
	P	.04*
$\Delta SOC_i = \mu + \alpha SOCin_i + e_i$ [b]	SOCin	.67
$\Delta SOC_i = \mu + \alpha Yield_i + e_i$ [a,b]	Yield	.35[a]
		.43[b]
$\Delta SOC_i = \mu + \alpha CS_i + e_i$ [b]	CS	.89
$\Delta SOC_i = \mu + \alpha P_i + e_i$ [b]	P	.27

Abbreviations: μ, average; CS, clay and silt content; e, residual error; P, preceding crop; SOCin, initial SOC stock; ΔSOC, SOC stock variation between 2014 and 2019.

[a]Relationship over the layer L1.

[b]Relationship over the layer L1-2.

*Significant difference.

proxy of the quantity of aboveground crop residues that could be returned to the soil every year before harvest (leaves that fall in winter) and at harvest (stubbles); (b) soil texture; and (c) initial SOC stock. This analysis was performed for L1-2 and for L1 regarding the cumulative yield (because the upper layer was more susceptible to be affected by aboveground C inputs). Results showed that, in L1 and L1-2, there was no significant relationship ($p = .35$ and .43 for L1 and L1-2 respectively) between the SOC stock variation and the cumulative yield obtained between 2014 and 2019 (Table 3). In L1-2, there was no significant effect of the investigated variables ($p = .89$, .27 and .67, respectively, for CS content, preceding crop and initial SOC) on the SOC variation between 2014 and 2019 (Table 3). The variability in the SOC storage rates observed between sites was therefore not explained by the factors we tested.

4 | DISCUSSION

4.1 | A superficial soil carbon storage

Soil organic carbon stocks increased significantly only in layer L1 (ca. 0–5 cm), with an annual storage of 0.48 ± 0.41 and 0.54 ± 0.25 t ha^{-1} year^{-1} for *M.* × *giganteus* and *M. sinensis* respectively. Our results are consistent with those of Ferchaud et al. (2016) who showed in one site in northern France that SOC storage by *M.* × *giganteus* was significant after 5 years in the 0–5 cm layer but not in the 0–30 cm layer. Zimmermann et al. (2013) also showed a significant increase of the SOC stock in the topsoil (0–10 cm) after 3 years under *M.* × *giganteus* established on former cropland (eight sites in Ireland), but no significant effect in the entire sampled layer (0–30 cm). These results could be explained by the fact that, in the absence of soil tillage, all the aboveground crop residues remain on the soil surface. Large amounts of mulch (i.e. crop residues accumulated on the soil surface) were measured under *M.* × *giganteus* (Dufossé et al., 2014; Ferchaud et al., 2016). Continuous no-tillage generally results in SOC stratification, in contrast with tilled systems (e.g. Luo et al., 2010; Mary et al., 2020). Furthermore, the upper layer can also benefit from belowground carbon inputs (root and rhizome turnover and rhizodeposition). Ferchaud et al. (2016) found that, under *M.* × *giganteus*, the 0–5 cm layer contained 25% of the total belowground biomass (roots and rhizomes) and 31% of the root biomass in the 0–30 cm layer. Clifton-Brown et al. (2007) also quantified approximately four times higher root biomass in the 0–10 cm layer than in the 10–20 cm layer, in a 12-year-old *M.* × *giganteus* plot. Using ^{13}C measurements, several previous studies have confirmed that the upper soil layer concentrates most of the C inputs under miscanthus. For example, Ferchaud et al. (2016) found that 75% of the new C4 SOC originated from the miscanthus crop and, incorporated into the soil profile after 5 years, was localized in the 0–5 cm layer. Similarly, Clifton-Brown et al. (2007) found that 80% of the new C4 SOC accumulated in the 0–30 cm layer was in the 0–10 cm layer.

The SOC stock did not change significantly in the whole sampled soil layer L1-2 (ca. 0–30 cm) for both genotypes (with an annual SOC stock variation rate of 1.0 ± 2.5 and 1.2 ± 2.0 t ha^{-1} year^{-1} for *M.* × *giganteus* and *M. sinensis* respectively). Our results are consistent with those of Robertson et al. (2017), who found no significant increase in SOC stock in the 0–30 cm soil layer under a 7-year-old *M.* × *giganteus* crop. Rowe et al. (2016) also found no significant difference in SOC stocks (in the 0–30 cm soil layer) between *M.* × *giganteus* and arable control in a network of 11 sites with 3- to 10-year-old plantations. Similar results were obtained by Ferchaud et al. (2016), who found no significant evolution of the SOC stock in the 0–30 cm soil layer. Our results do however differ from those of Dondini et al. (2009) and Dufossé et al. (2014) who found a significant increase of respectively 1.8 and 0.8 t ha^{-1} year^{-1} under *M.* × *giganteus* in the 0–30 cm layer. These results could be explained by the longer duration of these studies (21 years for Dufossé et al. [2014] and 14 years for Dondini et al. [2009]). Yet the duration of cultivation is not the only factor explaining these differences, since Richter et al. (2015) did not find any significant change in SOC stock after 14 years in either the 0–30 or the 0–100 cm soil layer.

Most of the above-mentioned published results have been obtained using a ‘paired plots approach’, i.e. a comparison at a given time of the SOC stocks under a miscanthus plot and a control plot (Olson et al., 2014). Poeplau and Don (2014) demonstrated, using ^{13}C measurements, that this approach can lead to a serious bias when calculating carbon storage under miscanthus cultivation because of preexisting SOC stock differences between the two plots. Using a diachronic approach, that is, comparing SOC stocks in the same plots at several-year intervals, we could have expected less variable results. Our results could therefore be explained by the short duration of our study (5 years), which could not be sufficient to see a significant evolution for the SOC stock in soil layer L1-2 (ca. 0–30 cm). According to Zimmermann et al. (2012), it is indeed difficult to observe a significant SOC stock change for young fields of miscanthus (growing period of <10 years).

4.2 | No genotype effect on soil carbon evolution

The SOC stock evolution after 5 years of growing miscanthus was not significantly different between *M.* × *giganteus* and *M. sinensis*, for the soil layers L1 and L1-2. Our results are consistent with those of Richter et al. (2015) and Gregory et al. (2018) who also found no significant difference in carbon storage between these two genotypes of miscanthus (*M.* × *giganteus* and *M. sinensis*). These results could be explained by similar C inputs (from the crop to the soil) between the two genotypes.

Regarding aboveground C inputs, both *M.* × *giganteus* and *M. sinensis* are affected by pre-harvest leaf loss during winter (Clifton-Brown & Lewandowski, 2002). Straub et al. (2013) and Robertson et al. (2017) showed moreover that the leaves of *M.* × *giganteus* and *M. sinensis* contain the same percentage of carbon (43%–44%). According to Amougou et al. (2012), the fallen leaves represented an average yearly input of 1.4 t ha^{-1} $year^{-1}$ of C (3 t ha^{-1} $year^{-1}$ of DM) under an *M.* × *giganteus* crop with a mean biomass yield of 21 t ha^{-1} $year^{-1}$ of DM. To our knowledge, no similar data have been published for *M. sinensis*. However, results from Richter et al. (2015) showed that after 14 years of cultivation, the quantity of mulch (crop residues present on the soil surface, originating from fallen leaves and harvest losses) was similar under *M.* × *giganteus* (9.3 t/ha of DM) and *M. sinensis* (8.9 t/ha of DM). Furthermore, the leaf area index was also similar for both genotypes in our trial network, despite lower yields for *M. sinensis* than for *M.* × *giganteus* (Ouattara et al., 2020). We can therefore assume that the aboveground C inputs were similar between the two genotypes.

Regarding belowground C inputs, it is possible to compare the belowground biomass (rhizomes and roots) of the two genotypes. Root biomass or length density may be a good indicator for belowground C inputs. Gregory et al. (2018) showed that there was a strong relationship (R^2 = .79) between SOC derived from miscanthus, and root density. This relationship (R^2 = .66) was also described by Richter et al. (2015). The root length density of *M. sinensis* and *M.* × *giganteus* was not significantly different in two field experiments in Rothamsted, U.K. (Gregory et al., 2018; Richter et al., 2015). Christensen et al. (2016) also found similar root C in the 0–20 cm layer for both genotypes (1.3 t ha^{-1} $year^{-1}$ of C). However, rhizome biomass was found to be lower for *M. sinensis* than for *M.* × *giganteus* (Christensen et al., 2016; Richter et al., 2015). Although it is known that some of this belowground biomass dies every year and decomposes into the soil, there is no information regarding a possible genotype effect on this turnover.

4.3 | SOC stock variability across sites

The initial SOC stock in the 0–30 cm layer was significantly higher for sites with a set-aside history (56.3 t/ha) than for sites where arable annual crops (43.4 t/ha) were the preceding crops. Our results are consistent with those of Zimmermann et al. (2012) who showed that initial value of SOC stock was greater under set-aside land (79.1 t/ha) compared to tilled soil (62.8 t/ha). Zimmermann et al. (2014) and Cai et al. (2016) also showed a significantly higher value of initial SOC stock on set-aside land compared to arable land. These results could be explained by higher carbon inputs under set-aside land, where the whole aboveground biomass was returned to the soil, than under annual crops. However, although land use history influenced the initial SOC stock, it did not significantly affect the SOC stock variation under miscanthus.

We found no effect of texture (i.e. clay and CS) on initial C stocks and on C storage, although many studies clearly illustrate that finely textured soils store more SOC than do coarsely textured soils (e.g. Cai et al., 2016; Meersmans, Martin, De Ridder, et al., 2012; Tan et al., 2004). Our results could be explained by the fact that the relationship between CS and SOC stock depended on the land use. For example, at the French national scale, there is a poor spatial correlation between texture and SOC stocks because land use is also affected by soil texture (Meersmans, Martin, Lacarce, et al., 2012). Cai et al. (2016) also showed that land use had a direct effect on the ability of fine particles (CS) to store carbon. Therefore, a relevant approach would be to assess the relationship between CS and SOC initial stock and storage separately for sites with set-aside land as the preceding crop, and for sites with annual crops as the preceding crop. Indeed, land use history (or preceding crop) often determines the initial SOC level, which decides whether a piece of land can become a carbon 'sink' or 'source' under bioenergy cropping (Anderson-Teixeira et al., 2009; Qin et al., 2016; Zimmermann et al., 2012). However, we did not have enough data to perform this analysis in our study.

5 | CONCLUSION

Our study aimed to evaluate within a multilocal trial network the evolution of SOC stocks between 2014 and 2019 under *M.* × *giganteus* and *M. sinensis* crops in contrasting sites and for different land use histories. Our results showed that SOC stocks significantly increased for both genotypes (0.48 ± 0.41 and 0.54 ± 0.25 t ha^{-1} $year^{-1}$ for *M.* × *giganteus* and *M. sinensis* respectively) in the L1 layer (ca. 0–5 cm), although no significant change was detected in the layer L1-2 (ca. 0–30 cm). As the carbon storage capacity of these two genotypes was similar, the choice of genotype for growing miscanthus should be based on other traits, such as the ability to cope with a given agroclimatic context.

There is a need for more experimental research on a longer term and using adequate protocols (diachronic approach, ESM stock measurements) to better evaluate the potential of miscanthus to store SOC. Furthermore, quantitative assessments of the annual C inputs (leaf fall, root and rhizome turnover, etc.) under different miscanthus genotypes could help to orient plant-breeding schemes towards genotypes with an enhanced ability to sequester C.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Malick S. Ouattara https://orcid.org/0000-0002-5546-6508
Fabien Ferchaud https://orcid.org/0000-0002-2078-3570

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