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Multi-parameter assessment of soil quality under M*iscanthus x giganteus* crop at marginal sites in Île-de-France

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ABSTRACT

In the context of increasing biomass cultivation for energy production in Europe, the objective of this study was to carry out a multi-parameter assessment of soil quality under *Miscanthus x giganteus* crop at marginal sites. Chemical (pH, organic carbon, total nitrogen, C:N, metal trace elements), biological (microbial biomass, earthworm communities) and physical (aggregate stability, bulk density) soil properties were evaluated 5 years after planting Miscanthus on a polluted (CH) and unpolluted (MG) marginal sites and compared with adjacent undisturbed meadow areas and Miscanthus crop cultivated on an arable field (BF). The effect of Miscanthus on soil quality was site dependent and related to soil properties (texture), metal trace element (MTE) contamination and previous land use. At MG, where Miscanthus was cultivated on a previously undisturbed meadow, results suggested a negative effect of Miscanthus on soil biological properties with a lower earthworm abundance and biomass, and a worst functional and species structure in the Miscanthus than in the undisturbed meadow, despite a good yield. Results under Miscanthus at BF were similar in terms of soil quality to Miscanthus at MG and higher than under Miscanthus at the polluted site (CH). At CH, results suggested that Miscanthus may reduce the mobility of MTE, however further longer term studies, at a range of sites are needed to conclude about the impact of Miscanthus on soil quality at contaminated and/or uncontaminated sites.

1. Introduction

To reduce the use of energy from non-renewable sources, the European Union is seeking to increase the proportion of biomass used in energy production [1]. This has led to an increase in the use of agricultural land for bioenergy crop cultivation, which is, at a large-scale, considered unsustainable due to competition with food production. A potential solution is to grow energy crops on inaccessible and/or degraded (e.g. polluted) marginal lands [2] which are not economically profitable for traditional agriculture [3]. A limited number of studies have assessed the impact of using marginal land for cultivating energy crops and two studies have suggested that it can have a negative impact on biodiversity [4,5]. However, a wider assessment of the environmental and ecological impacts of energy crop production is required to enhance sustainability of the sector [6,7].

Soil quality, which is commonly defined as “the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” [8–10], is influenced by natural and human-induced processes [11] predominantly related to land use, climate and geological substrate [9]. Soil degradation has led to erosion of 12.7% and critical compaction of 23% of European soils and increased intensity in land use has led to a decrease in soil biodiversity including, for example, a decrease in species richness of earthworms, springtails and mites [12]. Soil provides many ecosystem services, which are strongly related to its quality [9]. A reduction in the provision of ecosystem services can negatively affect water and air quality, and climate change [13] and may increase risks to human health, especially when the soil is polluted [9] or when food safety is threatened [13]. Soil quality degradation may also have large-scale socio-economic impacts and therefore requires monitoring and promotion of sustainable management practices.

Several studies have highlighted the importance of evaluating a combination of physical, chemical and biological parameters to obtain a holistic view of soil quality [14,15]. Chemical and physical indicators of soil quality are well established [16] and include organic matter, pH, available phosphorus, contamination, water storage, bulk density and aggregate stability [9]. More recently, studies have also high-

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lighted the importance of biological parameters as soil organisms contribute directly to many ecosystem services [15] and may respond more rapidly than physico-chemical parameters to natural and anthropogenic impacts [16]. The importance and relevance of earthworms and microorganisms as bioindicators is also now widely recognised [17–19].

Miscanthus x giganteus (hereafter called Miscanthus) production has a broad range of environmental benefits [20] as it has low fertilizer and weed control requirements, long growth periods (10–25 years) and no-till cultivation [21]. Several studies have also highlighted the potential ecosystem services provided by Miscanthus [22–24] including the ability to increase soil organic carbon stocks [25] and decrease bio-availability of metals through accumulation in the rhizosphere in polluted sites [26]. Therefore, Miscanthus cultivation is considered suitable for inaccessible and/or degraded marginal lands [24,27,28]. Das et al. [29] suggested that water stable aggregates were higher under Miscanthus than under other perennial crops and it may also increase microbial diversity and activity [30]. In addition, Felten and Emmerling [31] observed an increase in the diversity of earthworm communities in Miscanthus compared with annual crops but these positive effects were not observed when compared to meadow [31,32]. Furthermore, Hedde and co-workers [33–35] have shown that transition from food to Miscanthus crop on a polluted site increased abundance and diversity of soil invertebrates.

Previous Miscanthus-based studies have focused on a restricted number of soil quality indicators considered separately. However, there is increased importance being placed on using a multi-parameter approach to accurately assess an overview of soil chemical, physical and biological quality [36]. Our study, part of the "Biomass For the Future" project (BFF, ANR 11-BTBR-0006), sought to assess the effect of Miscanthus on soil quality of two marginal sites, using a multi-parameter approach comparing chemical (pH, bioavailability of inorganic pollutants), physical (bulk density, aggregate stability), and biological (microbial biomass, earthworm communities) soil properties in Miscanthus fields with adjacent undisturbed meadow. A Miscanthus crop on an arable field was also utilised as a reference site.

2. Material and methods

2.1. Study sites

Two marginal lands, Marne et Gondoire (MG) and Chanteloup (CH), and an agricultural site (Bioferme (BF)), were investigated in this study (Table 1).

Table 1
GPS coordinates, elevation (m), annual 30-year average rainfall, soil type, soil texture, clay, silt, sand, organic C content (C_{org}) and pH from 2013 at BF (Bioferme), MG (Marne et Gondoire) and CH (Chanteloup) sites.

Site	Unit	BF	MG	CH
GPS coordinates	()	48°21'8.08″N; 3°1'24.98″E	48°50'57.96″N; 2°39'43.62″E	48°57'44.86″N; 2°2'8.03″E
Elevation	(m)	75	95	45
Annual 30-year average rainfall	(mm)	677	694	638
Soil type [a]	()	Cambisol	Luvisol	Fluvisol
Texture	()	silty clay loam	silty clay loam	loamy sand
Clay	(%)	27	28	7
Silt	(%)	56	56	8
Sand	(%)	17	17	85
C_{org} (0-30 cm)	(g kg^{-1})	10.0	11.2	38.9
pH_{H2O}	()	8.11	8.25	7.84

[a] According to the world reference base for soil resources [39].

MG is close to a highway, on which embankments have been laid and with restricted access. CH is polluted with metal trace elements and organic pollutants [37] and was used during the 20th century for spreading raw sewage from Paris and cultivated as a market garden until 2000.

MG and CH had not been cultivated since the early 2000's and therefore Miscanthus was planted on an established meadow and at BF on a field previously cultivated with barley.

At all sites, an experimental plot of 160 m^2 was planted with Miscanthus rhizomes in 2013 (at a density of 1.5 rhizome m^{-2}). The soil was ploughed before rhizome implantation and during the first year herbicide was applied (Roundup) combined with mechanical weeding. Miscanthus was harvested annually in late winter, leaving stubble and leaf litter in the field. In 2018 (5 years after planting), there was a wide variation in Miscanthus yields between the three sites with a high potential yield of 20.5 t ha^{-1} at MG, a medium potential yield of 14.8 t ha^{-1} at BF and a low potential yield of 2.1 t ha^{-1} at CH [38]. On the marginal sites, undisturbed meadow approximately 10 m away from the Miscanthus plot was sampled as an intra-site control.

2.2. Sampling and analysis

Sampling at CH, MG and BF took place in November 2017. The sampling protocol and procedure followed recommendations from the European programme ENVASSO [16,40] and a previous framework from the national programmes Bioindicator [17] and RMQS Biodiv [41]. In Miscanthus and undisturbed meadow plots three blocks (12 m^2 i.e. 5 m * 2.4 m) were randomly delimited. Within each block, four sampling points were selected in a randomised design to perform the following: (i) earthworm community assessment, (ii) soil sampling for bulk density measurements, and (iii) soil sampling for aggregate stability analysis. In addition, 10 randomly selected soil cores (6.5 cm diameter, 20 cm depth) were taken from each block and combined as a composite sample for chemical and microbial biomass analyses.

2.3. Soil chemical properties

Soil core samples were stored at 4 °C and air dried before analyses were performed. The pH (measured in water), total organic C (g kg^{-1}) and total N (g kg^{-1}) content of soils were determined according to NF ISO 10694 [42] and NF ISO 13878 [43] respectively.

Total metal trace elements (MTE) concentrations (Cr, Co, Ni, Cu, Zn, Cd and Pb) were determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (X Series, Thermo Electron Corporation) performed after nitric acid (HNO_3, 70%) digestion according to EPA method 3051A [44] using a microwave (Ethos EZ, Microwave digestion system, Milestone).

In addition, metal availability in soil samples was determined using an ethylenediaminetetraacetic acid (EDTA, $C_{10}H_{16}N_2O_8$) methodology recommended by MAFF [45]. The same analyses were carried out on 5 samples of standard CRM 483 reference material purchased from the European Commission (Geel, Belgium). After extraction, EDTA-extractable metal concentrations in solution were determined by Optical Emission Spectrometry (ICP-OES, Thermo Scientific, iCAP 7000 Series ICP Spectrophotometer). For each element, the mobility factor adapted from Salbu et al. [46] was calculated on the basis of the following equation:

$$\text{Mobility factor} = \frac{\text{Mobile fraction extracted with EDTA (mg kg}^{-1}\text{)}}{\text{Total fraction extracted with nitric acid (mg kg}^{-1}\text{)}} \times 100$$

The percentage of extractable element was used as a proxy of phytostablization ability of Miscanthus.

2.3.1. Soil biological analysis

2.3.1.1. Microbial biomass Soil microbial biomass carbon content (MBC, mg kg^{-1}) was measured from composite soil samples frozen immediately after collection. MBC was measured using a fumigation-extraction method [47]. MBC was estimated as organic C extracted in fumigated samples minus organic C extracted in non-fumigated samples. Extractable C was measured using a Total Organic Carbon Analyzer (OI Analytical – 1010). An extraction coefficient of 0.45 was used as the method was based on UV-persulfate extraction [48]. The C_{mic}-to-C_{org} ratio was calculated as percentage of MBC content in total organic C following Anderson et al. [49].

2.3.1.2. Earthworm community The earthworm sampling methodology was adapted from the ISO 23611-1 standard [50]. A block of soil (25 x 25 × 25 cm) was extracted and hand-sorted for earthworms. 500 ml of mustard solution (30 g L^{-1} according to The Participatory Earthworm Observatory of EcoBioSoil from Rennes 1 University, France) was applied in the hole and any emerging earthworms retrieved over a 20-min period. Collected earthworms were preserved in 4% formalin solution prior to identification in the laboratory using Bouché‘s key [51], and individual masses recorded. For each sampling point, the number of earthworms sorted manually was added to the number of earthworms extracted with mustard. When identification of juveniles to species level was not possible (approx. 25% of time) individuals were first assigned to genus before allocated to species based on the proportion of adults present at each site. Earthworm abundance (individuals m^{-2}), biomass (g m^{-2}), functional structure based on ecological groups (anecic, endogeic and epigeic) [52] and specific structure (species richness, Shannon diversity index and evenness) were used to describe communities.

2.4. Soil physical properties

Bulk density was measured (g cm^{-3}) according to ISO 11272:2017 [53]. In each block, four horizontal soil cores were collected with a metal corer (diameter 5 cm; height 5 cm) from the side of the earthworm sampling pit at a depth of 0–10 cm.

To assess aggregate stability, four soil samples were collected randomly in each block from a depth of 0–10 cm with a spade and combined. This depth was selected as the upper 10 cm of soil is the most exposed to crusting and erosion by rainfall and is in the range of depths sampled in other studies (e.g. Bottinelli et al. [54] and Kraemer et al. [55]). Aggregate stability was measured using Le Bissonnais' method [56] with calibrated and air-dried aggregate samples (3–5 mm). Slaking due to fast wetting was performed as this test is related to land use [57]. After treatment, the fragmented samples were dried (40 °C) and passed through six different sieves with apertures ranging from 0.05 to 2 mm and the mean weight diameter (MWD) of aggregates calculated as follows:

$$\mathrm{MWD} = \sum w_i * x_i$$

where i corresponds to each fraction collected in each sieve, w_i is the dry weight of the fraction collected relative to the total soil used and x_i (mm) is the mean diameter of the fraction collected.

2.5. Statistical analysis

For all variables, statistical analyses were performed (using XLSTAT (2014) software) to compare results from undisturbed meadow and Miscanthus plots at MG and CH sites. Comparisons between sites were not conducted due to differences in soil type and contamination level.

For chemical and microbial variables from composite samples and for aggregate stability, the effect of treatment (undisturbed meadow vs Miscanthus) was tested at MG and CH using a non-parametric Mann Whitney *U* test (0.05% significance level) which allows a comparative bi-modal approach for the small sample size (n = 3).

For bulk density and earthworm community (abundance, biomass, species richness, Shannon index and evenness) (n = 12), the normality of sample distributions was tested using the Shapiro-Wilk test. When the assumption of normality was not validated a Mann Whitney *U* test was performed at MG and CH. When normality and equality of variances (Levenes Test) were validated, a Student Two Sample T-Test was performed.

3. Results

3.1. Soil chemical properties

There was no significant difference ($p > 0.05$) and no consistent trend in C_{org}, N_{tot}, C:N ratio and pH between undisturbed meadow and Miscanthus treatments at MG and CH sites (Table 2).

Table 2
Soil quality indicators (mean ± standard deviation) of BF (Bioferme), MG (Marne et Gondoire) and CH (Chanteloup) sites. Superscript letters indicate significant differences ($p < 0.05$) between undisturbed meadow and Miscanthus treatments.

Indicators	unit	BF	MG		CH	
		Miscanthus	*Undisturbed meadow*	*Miscanthus*	*Undisturbed meadow*	*Miscanthus*
Chemical						
C_{org}	(g kg^{-1})	11.9 ± 0.9	17.3^a ± 0.3	12.9^a ± 1.1	25.1^a ± 4.9	33.5^a ± 3.6
N_{tot}	(g kg^{-1})	1.1 ± 0.1	1.6^a ± 0.0	1.3^a ± 0.1	1.7^a ± 0.1	1.7^a ± 0.1
C:N	()	11.03 ± 0.32	10.53^a ± 0.06	10.33^a ± 0.42	15.00^a ± 0.12	19.93^a ± 1.16
pH	()	7.06 ± 0.20	6.92^a ± 0.43	7.38^a ± 0.25	7.52^a ± 0.06	7.51^a ± 0.13
Biological						
Microbial biomass	(mg C kg^{-1} soil)	328 ± 36	527^a ± 68	324^a ± 46	172^a ± 18	120^a ± 3
C_{mic}-to-C_{org}	(%)	2.76 ± 0.22	3.05^a ± 0.35	2.51^a ± 0.33	0.68^a ± 0.06	0.36^a ± 0.05
Earthworm						
Species richness	(species m^{-2})	3.6 ± 1.0	6.1^a ± 1.3	3.2^b ± 1.8	0.1^a ± 0.3	0.5^a ± 0.7
Eveness	()	0.86 ± 0.14	0.85^a ± 0.05	0.77^b ± 0.36	0.00^a ± 0.00	0.08^a ± 0.29
Shannon index	()	1.57 ± 0.42	2.18^a ± 0.27	1.35^b ± 0.80	0.00^a ± 0.00	0.08^a ± 0.29
Physical						
Bulk density	(g cm^{-3})	1.59 ± 0.08	1.39^a ± 0.08	1.51^b ± 0.09	1.33^a ± 0.12	1.38^a ± 0.09
Aggregate stability						
Slaking (MWD)	(mm)	1.25 ± 0.28	0.90^a ± 0.17	0.77^a ± 0.18	0.57^a ± 0.06	0.50^a ± 0.03

3.1.1. Metal trace elements

3.1.1.1. Total metal trace elements No significant difference in total metal trace element concentrations was observed between Miscanthus and undisturbed meadow treatments at MG and CH sites (Table 3). However, at CH total MTE concentrations were between 169% (Cu, 276.6 ± 153.4 vs 102.8 ± 21.3 mg kg^{-1}) and 283% (Cd, 6.9 ± 4.1 vs 1.8 ± 0.5 mg kg^{-1}) higher in Miscanthus than undisturbed meadow with $p = 0.1$ for Cu, Zn, Cd and Pb (Table 4). The results from the CH site were superior to those of BF and MG, where concentrations were in the same order of magnitude.

3.1.1.2. Available metal trace elements and mobility factor There was no significant difference in available MTE concentrations (Table 3) between undisturbed meadow and Miscanthus treatments at MG and CH sites (Table 4) and the results from the BF site were of the same order of magnitude as those from MG (Table 3).Similarly, no significant differences were recorded in mobility factor (Table 3) between undisturbed meadow and Miscanthus treatments at MG and CH sites (Table 4). However, at CH, for all MTEs lower values of mobility factor were observed in the Miscanthus compared to undisturbed meadow: results were between 47% (Cu, 89.3 ± 5.9 vs 47.6 ± 23.8%) to 86% (Cr, 9.5 ± 1.0 vs 1.3 ± 1.2%) lower in the Miscanthus treatment with $p = 0.1$ for all studied MTEs. In Miscanthus plots mobility factors at BF were closer in magnitude to MG than to CH.

3.2. Soil biological properties

3.2.1. Microbial biomass

MG exhibited numerically higher MBC values in undisturbed meadow (527 ± 68 mg C kg^{-1} soil) than under Miscanthus (324 ± 46 mg C kg^{-1}) and this trend was also observed at CH with 172 ± 18 and 120 ± 3 mg C kg^{-1} in undisturbed meadow and Miscanthus treatments respectively ($p = 0.1$). At BF, recorded MBC values (328 ± 36 mg C kg^{-1}) were similar to the MG Miscanthus treatment.

Results for C_{mic}-to-C_{org} ratio exhibited similar trends, there was no significant difference between treatments and values were 21% and 89% higher in undisturbed meadow than Miscanthus plots at MG and CH respectively. At BF the Miscanthus treatment had a C_{mic}-to-C_{org} ratio (2.76 ± 0.22%) that was similar in value to the MG Miscanthus treatment.

3.2.2. Earthworms communities

3.2.2.1. Abundance and biomass At the MG site, earthworm abundance was significantly lower in Miscanthus than in the undisturbed meadow (108 ± 32 vs 397 ± 70 ind m^{-2} respectively, $p < 0.001$) and biomass was also significantly lower (35 ± 14 vs 114 ± 19 g m^{-2} respectively, $p < 0.001$). In contrast, at CH, no significant differences ($p > 0.05$) in earthworm abundance or biomass were recorded (Figs. 1 and 2). However, it is important to note that at CH, a low number of individuals were collected (1 ± 2 and 9 ± 6 ind m^{-2} for undisturbed meadow and Miscanthus treatments respectively). At BF earthworm abundance (129 ± 50 ind m^{-2}) and biomass (48 ± 32 g m^{-2}) were similar to values recorded in the MG Miscanthus treatment.

3.2.2.2. Functional structure At the MG site, abundance of epigeic ($p = 0.0002$), anecic ($p = 0.0019$) and endogeic ($p < 0.0001$) earthworm species were significantly higher in undisturbed meadow treatments (Fig. 1). Earthworm communities were dominated by endogeic species (Fig. 1) representing 79% and 86% of the community in undisturbed meadow and Miscanthus treatments respectively, Miscanthus hosting 70% less of endogeic species than undisturbed meadow (93 ± 56 ind m^{-2} vs 315 ± 107 ind m^{-2}). Anecics only represented 12% (47 ± 30 ind m^{-2}) of earthworm community in undisturbed meadow and 9% (9 ± 14 ind m^{-2}) in Miscanthus and a huge difference was observed between treatments as Miscanthus presents 81% less of anecic than undisturbed meadow. Epigeic values were even lower, representing 9% of earthworm community in undisturbed meadow and 5% in Miscanthus treatments, and again Miscanthus hosting very low abundance (86% less, 5 ± 3 ind m^{-2} vs 36 ± 18 ind m^{-2}).At the CH site, the few individuals collected were predominantly anecics (1 ± 5 ind m^{-2} and 8 ± 11 ind m^{-2} in undisturbed meadow and Miscanthus treatments respectively) with no significant difference between treatments.Functional structure of earthworms under Miscanthus at BF was similar to MG and characterized by dominance of endogeic species which represented 70% (91 ± 41 ind m^{-2}) of the community.

3.2.2.3. Species richness, evenness & Shannon diversity index At the MG site, a total of 9 species (lists of species recorded in each treatment are provided in Table 5 in the supplementary material) were recorded corresponding to a mean of 6.1 ± 1.3 and 3.2 ± 1.8 species m^{-2} (Table 2, $p = 0.001$) in the undisturbed meadow and Miscanthus treatments respectively. The community structures were more or less the same in both treatments: communities were dominated by endogeic species with *Aporrectodea c. caliginosa typica* (36% abundance in both treatments) and *Allolobophora icterica* (30 and 25% abundance in undisturbed meadow and Miscanthus treatments respectively). In the undisturbed meadow treatment, the epigeic species *Lumbricus castaneus* represented 9% of the community and the anecic species *Aporrectodea giardi* represented 8% compared with 4% for both species in the Miscanthus treatment. Evenness ($p = 0.046$) and Shannon index ($p = 0.004$) scores were significantly higher in the undisturbed meadow treatment. For most species, mean abundance was lower under Miscanthus than undisturbed meadow, especially for *A. icterica* (76% lower), *A. c. caliginosa typica* (70% lower), *Allolobophora rosea rosea* (71% lower), *A. giardi* (87% lower) and *L. castaneus* (86% lower), with the exception of *Allolobophora minima* which had a similar density (12 ind m^{-2}) in both treatments.At the CH site, only one species (*Lumbricus terrestris*) was found in undisturbed meadow and two (*L. terrestris* and *L. castaneus*) in Miscanthus treatments providing mean evenness scores of 0 and 0.08 ± 0.29 respectively.A total of seven species, with a mean of 3.6 ± 1.0 species m^{-2}, were found in the BF Miscanthus treatment, which is similar to results for Miscanthus at the MG site. The community at the Miscanthus BF site was dominated by the endogeic *A. icterica* (47%), anecics were represented by *A. giardi* (13%) and *L. castaneus* was the only epigeic species (12%). The evenness index was 0.86 ± 0.14 and the Shannon index had a value of 1.57 ± 0.42 at this site.

3.3. Soil physical properties

3.3.1. Bulk density

Bulk density (Table 2) was significantly higher ($p = 0.004$) in the Miscanthus treatment than in the adjacent undisturbed meadow treatment at MG and no significant difference was observed at the CH site. Comparing the three sites, the highest bulk density value (1.59 g cm^{-3}) was found in the Miscanthus treatment at BF with the lowest values (1.33 and 1.38 g cm^{-3}) recorded in undisturbed meadow and Miscanthus treatments respectively at CH.

3.3.2. Aggregate stability

At MG and CH, no significant differences in aggregate stability were found between undisturbed meadow and Miscanthus treatments (Table 3). However, based on Le Bissonais's classification [56], aggregate stability was medium under the BF Miscanthus treatment (1.25 ± 0.28 mm) and MG undisturbed meadow treatment (0.9 ± 0.17 mm), and low under the MG Miscanthus treatment (0.77 ± 0.18 mm) and both treatments at the CH site.

4. Discussion

This study sought to assess soil quality under Miscanthus crops following a 5-year establishment phase at three different sites which differed in soil type, contamination levels and previous land use. Although differences between undisturbed meadow and Miscanthus treatments were only significant for earthworm community and bulk density,

Table 3
Total, available and mobility factor (means ± standard deviation) of trace metal element (MTE) content (Cd, Cr, Cu, Ni, Pb and Zn) under Miscanthus treatment at Bioferme (BF) and undisturbed meadow and Miscanthus treatments Marne et Gondoire (MG) and Chanteloup (CH) sites and reference values in Île-de-France. Superscript letters indicate significant differences ($p < 0.05$) between undisturbed meadow and Miscanthus treatments.

Site	*Cr*		*Ni*		*Cu*		*Zn*		*Cd*		*Pb*	
	Undisturbed meadow	*Miscanthus*	*Undisturbed meadow*	*Miscanthus*	*Undisturbed Meadow*	*Miscanthus*	*Undisturbed meadow*	*Miscanthus*	*Undisturbed meadow*	*Miscanthus*	*Undisturbed meadow*	*Miscanthus*
Total MTE (mg kg $^{-1}$)												
BF	-	48.4 ± 0.5	-	34.6 ± 1.1	-	19.2 ± 0.6	-	145.7 ± 50.7	-	0.4 ± 0.0	-	25.01 ± 0.3
MG	45.5^{a} ± 2.9	48.1^{a} ± 2.2	32.1^{a} ± 1.5	34.8^{a} ± 3.4	21.1^{a} ± 1.1	21.8^{a} ± 3.3	157.5^{a} ± 26.9	127.6^{a} ± 34.6	0.3^{a} ± 0.0	0.3^{a} ± 0.0	32.9^{a} ± 4.2	30.7^{a} ± 1.0
CH	36.2^{a} ± 5.6	112.6^{a} ± 77.8	20.1^{a} ± 4.7	54.3^{a} ± 24.7	102.8^{a} ± 21.3	276.6^{a} ± 153.4	303.7^{a} ± 118.2	1028.7^{a} ± 460.9	1.8^{a} ± 0.5	6.9^{a} ± 4.1	161.1^{a} ± 49.6	591.0^{a} ± 484.3
Available MTE (mg kg $^{-1}$)												
BF	-	0.6 ± 0.0	-	5.2 ± 0.1	-	6.2 ± 0.2	-	4.0 ± 0.3	-	0.3 ± 0.0	-	11.3 ± 0.1
MG	0.9^{a} ± 0.1	0.6^{a} ± 0.2	3.5^{a} ± 0.1	3.2^{a} ± 0.45	8.6^{a} ± 0.45	7.6^{a} ± 0.9	6.9^{a} ± 0.4	5.3^{a} ± 0.7	0.3^{a} ± 0.0	0.2^{a} ± 0.1	17.4^{a} ± 2.9	15.4^{a} ± 2.4
CH	3.4^{a} ± 0.2	1.1^{a} ± 0.56	4.0^{a} ± 0.9	3.8^{a} ± 1.2	91.1^{a} ± 13.8	112.5^{a} ± 25.5	171.5^{a} ± 52.2	253.1^{a} ± 48.4	1.8^{a} ± 0.2	2.5^{a} ± 0.4	125.3^{a} ± 38.1	146.3^{a} ± 55.4
Mobility factor (%)												
BF	-	1.3 ± 0.1	-	15.0 ± 0.5	-	32.1 ± 0.1	-	3.0 ± 0.9	-	72.7 ± 2.7	-	45.0 ± 0.6
MG	2.0^{a} ± 0.3	1.2^{a} ± 0.5	10.9^{a} ± 0.2	9.2^{a} ± 0.9	40.8^{a} ± 2.4	35.3^{a} ± 3.1	4.5^{a} ± 0.9	4.3^{a} ± 1.1	89.3^{a} ± 7.8	71.3^{a} ± 18.5	53.0^{a} ± 4.4	50.2^{a} ± 9.2
CH	9.5^{a} ± 1.0	1.3^{a} ± 1.2	20.1^{a} ± 2.9	7.7^{a} ± 3.5	89.3^{a} ± 5.9	47.6^{a} ± 23.8	57.6^{a} ± 4.6	27.6^{a} ± 12.5	102.4^{a} ± 20.2	43.5^{a} ± 19.3	77.9^{a} ± 3.7	33.4^{a} ± 22.9
Suggested reference values of MTE (mg kg $^{-1}$) from Mathieu et al. [59]												
Agricultural soil of Ile de France (P95)	65.2		31.2		28.0		88.0		0.51		53.7	

Table 4
P-values (undisturbed meadow vs Miscanthus) of chemical, biological and physical parameters at MG (Marne & Gondoire) and CH (Chanteloup) sites. Significant effects ($p < 0.05$) are given in bold. MTE: metal trace element.

Chemical	MG	CH
C_{org}	0.077	0.100
N_{tot}	0.100	1.000
C:N	0.658	0.100
pH	0.383	0.663
Total MTE		
Cr	0.100	0.663
Ni	0.100	1.000
Cu	1.000	0.100
Zn	0.383	0.100
Cd	0.383	0.100
Pb	0.663	0.100
Available MTE		
Cr	0.100	0.100
Ni	1.000	1.000
Cu	0.383	0.383
Zn	0.100	0.190
Cd	0.100	0.100
Pb	1.000	0.663
Mobility factor of MTE		
sCr	0.190	0.100
Ni	0.100	0.100
Cu	0.190	0.100
Zn	1.000	0.100
Cd	0.190	0.100
Pb	1.000	0.100
Average	0.190	0.100
Biological		
Microbial biomass	0.100	0.100
C_{mic}-to-C_{org}	0.190	0.100
Earthworm		
Total abundance	**< 0.0001**	0.062
Abundance of epigeic	**< 0.0002**	0.359
Abundance of anecic	**0.002**	0.066
Abundance of endogeic	**< 0.0001**	NA
Total biomass	**< 0.0003**	0.111
Species richness	**0.001**	0.066
Evenness	**0.046**	0.359
Shannon index	**0.004**	0.359
Physical		
Bulk density	**0.004**	0.278
Aggregate stability		
Fast wetting	0.383	0.190

recorded trends were so pronounced and conflicting that it is relevant to discuss them and such discussions are considered of particular merit when recorded p-values are less than or equal to 0.1 as suggested by Webster [58].

4.1. Soil contamination under miscanthus

In order to assess the level of contamination we used values proposed by Mathieu et al. [59], which correspond to the 95th percentile of ploughed horizons of cultivated soils in the Île-de-France region and provided standardised "normal" concentrations of a given element in agricultural soils. The spatial concordance between this work and our study (Île-de-France) reinforced the relevance of these values compared to those usually used at national scale [60]. This approach is also frequently adopted by French contaminated land managers [61].

At both treatments of the MG site and at the Miscanthus treatment of the BF site, with the exception of Zn and Ni, total MTE concentrations measured in 2017 (Table 2) are below threshold values proposed by Mathieu et al. [59]. Therefore, BF and MG are considered contaminated sites in respect of Ni and Zn concentrations. However, contamination levels could be considered as relatively low, because i) Ni concentrations are close to reference values and ii) values for Ni and Zn do not exceed French threshold values recommended by Villanneau et al. [60]. In addition, although these sites are considered contaminated, they are not classified as polluted because no negative effects of this contamination have been observed on any compartment of the agro-ecosystem [59]. In contrast, at the CH site all MTE concentrations exceed threshold values in the Miscanthus treatment with values between 73% (Cr, 112.6 vs 65.2 mg kg^{-1}) and 1250% (Cd, 6.90 vs 0.51 mg kg^{-1}) higher than the reference values suggested by Mathieu et al. [59] confirming site contamination. Moreover, CH is designated as a polluted site with MTE content considered sufficiently bioavailable to have negative impacts on soil function or land use [59].

At MG, no differences in total MTE between Miscanthus and undisturbed meadow treatments was observed suggesting that at this level of contamination Miscanthus has no detectable effect on mitigating soil MTE contamination. Although there is no significant difference between treatments at the polluted CH site, total MTE levels were 220% higher at the Miscanthus treatment with $p = 0.1$ for Cu, Zn, Cd, Pb. Due to the high variability of the results it is not possible to conclude on the origin of this difference which could be caused by heterogeneous spreading of raw sewage prior to experimental set-up. However, these trends are in accordance with studies that have highlighted the ability of Miscanthus to reduce vertical mobility of MTE and enhanced accumulation in the soil surface horizon [24,26].

The polluted site (CH) also presented the highest MTE EDTA extractable concentration which is consistent with receiving large exogenous input of metals via the spreading of sewage sludge [62–64]. The mobility factor data provides an assessment of the potential of Miscanthus to stabilize MTE. Indeed, all physico-chemical properties being equal, if the mobility factor is lower in the Miscanthus treatment compared to the undisturbed meadow treatment it can be assumed that Miscanthus has a better capacity to stabilize metals than vegetation already present on the site. At the polluted site, although there was no significant difference between treatments, mobility factor results suggest that metals tend to be less extractable in Miscanthus than in adjacent undisturbed meadow areas and therefore less phytoavailable, with $p = 0.1$ for all studied metals (Table 4). This observation is in line with previous studies which have highlighted the ability of Miscanthus to reduce availability of MTE [24] due to accumulation of metals in the rhizosphere [26]. However, it is recognised that phytoavailability is affected by soil characteristics, such as increased organic matter content which can have a negative effect [65]. Iqbal et al. [66] suggested that one of the main parameters inducing changes in metal speciation is incorporation of organic matter [66–68]. Therefore, at the polluted CH site, the lower availability of metals in the Miscanthus treatment may be explained by the combined effect of organic matter addition and phytostabilization.

4.2. Soil biology under miscanthus

4.2.1. Microbial biomass carbon

The MBC values observed in this study were in the range published for meadow by Cluzeau et al. [61] (326.5 and 465.0 mg C kg^{-1} for lower baseline and average respectively) in both treatments at MG and in Miscanthus at BF while at the polluted site (CH), MBC values were lower despite the large amount of C_{org}. It is suggested that these low results are directly related to the high sand content of the soil [69] and the presence of organic pollutants [37] associated with high MTE concentrations inhibiting microbial biomass [70–73]. Indeed, these results are consistent with Adriano [74], who indicated that excessive application of zinc-contaminated sludge can lead to a decrease in microbial activity, and Brookes and McGrath [75], who demonstrated that soils

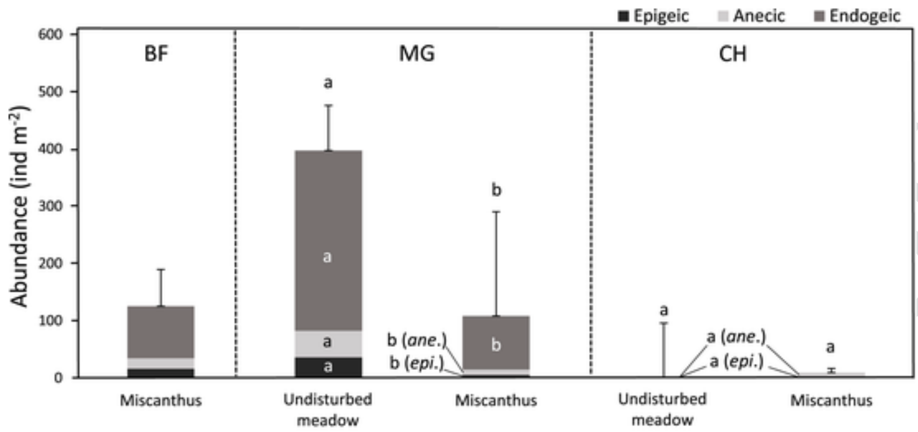

Fig. 1. Total earthworm abundance and abundance of earthworm ecological categories (means and standard deviation) at BF (Bioferme), MG (Marne et Gondoire) and CH (Chanteloup) sites. Different letters indicate significant differences ($p < 0.05$) in total and ecological category between treatments at the same site.

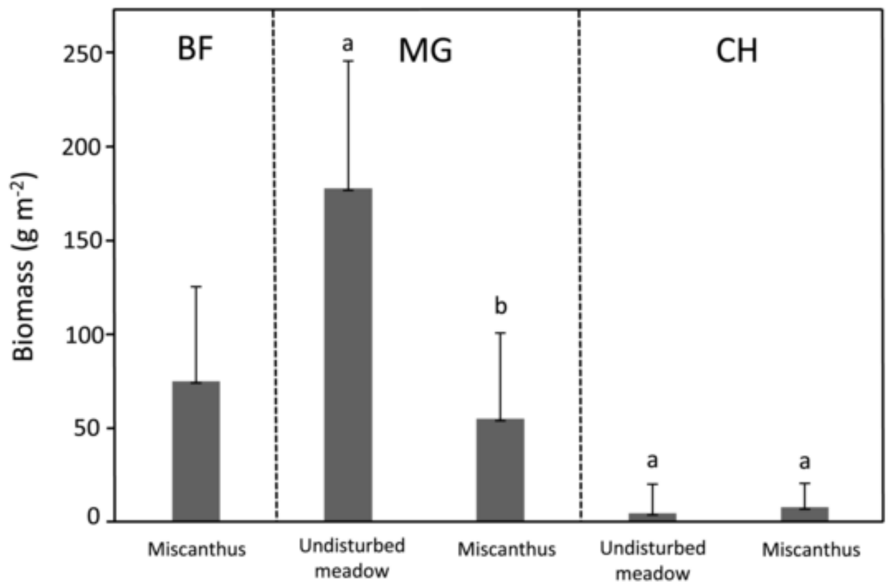

Figure 2. Total earthworm biomass (means and standard deviation) at BF (Bioferme), MG (Marne et Gondoire) and CH (Chanteloup) sites. Different letters indicate significant differences ($p < 0.05$) between treatments at the same site.

amended with sewage sludge contaminated with a range of heavy metals substantially reduced levels of MBC, even twenty years after sludge application.

Regarding the effect of Miscanthus in the two marginal sites, MBC tend to be 40 and 30% lower under Miscanthus soils ($p = 0.1$) compared to the undisturbed meadow, at MG and CH sites respectively. These results are in accordance with Ruf et al. [76] who registered higher MBC results under permanent meadow than under perennial energy crops. In addition, the C_{mic}-to-C_{org} ratio values, which is generally considered an index of microbial activity [77], were either within or above the range of 0.6–2.0% for arable soils [20] for Miscanthus at both sites. This was not the case in Miscanthus at the polluted site which, in addition to having high MTE content, had a relatively high N_{tot} content which is considered one of the main negative influences on C_{mic}-to-C_{org} ratio [77]. Moreover, a positive and significant correlation between MBC results and C_{org} content (Pearson correlation coefficient $r = 0.9200$, $p = 0.0093$) were found at MG which is consistent with other studies [57,78,79], but this link was not recorded at the other two locations.

4.2.2. Earthworms

In both treatments at the polluted site (CH), earthworm abundance, biomass and species richness results were below reference values suggested by Cluzeau et al. [41]. These results are consistent with Hedde et al. [35] who recorded an earthworm abundance of less than 4 ind m^{-2} in Miscanthus at a site with characteristics comparable to CH. These results are explained by the high levels of contamination at the site [80,81] reinforced by the negative effect of a sandy soil [80,82,83]. Moreover, an unbalanced functional structure linked to the absence of endogeic species at CH was previously notified by Pérès et al. [17] and explained by i) the location of endogeics directly in contact with metal pollutants, and (ii) the diet of endogeics which are geophagous and therefore ingest contaminated resources [84]. In some contexts, soil management (fertilization, reduction of tillage action) can moderate the impact of pollution [16], but this was not possible in our study due to the high levels of contamination.

At MG, the undisturbed meadow treatment exhibited earthworm abundance levels close to values recorded by Cluzeau et al. [41] for meadow (350 ind m^{-2}) while Miscanthus had abundance levels below the lower baseline value for meadow of 175 ind m^{-2} [41] but slightly higher than the lower baseline for crop (86 ind m^{-2} [41]). In addition, biomass, functional structure (abundance of epigeic, anecic and endogeic species), and diversity (species richness, Shannon index and evenness) at MG were lower in the Miscanthus treatment. These results are in accordance with literature as it is commonly accepted that cropping has a negative impact on earthworm communities compared to meadow [41]. Our results are also consistent with Felten and Emmerling [31] who reported higher earthworm abundance in a 20 year old fallow site (355 ± 43 ind m^{-2}) than in a 15-year-old Miscanthus crop (132 ± 11 ind m^{-2}).

Earthworm abundance in Miscanthus at BF (previously an annual barley crop) was similar to Miscanthus at MG and was below the lower baseline values for meadow (175 ind m^{-2} [41],) but slightly higher than the lower baseline value for crops (86 ind m^{-2} [41]). Species richness was also below Brittany (France) reference values for both meadow and crops (8.5 and 6 respectively [41]). Soil tillage is recognised as one of the major negative influences on earthworm communities [85,86] and particularly affects deep burrowing species (anecic) [87]. Therefore, soil tillage during establishment of Miscanthus crops may have contributed to reduce earthworm abundance in the Miscanthus treatment, and explains the very low values of anecic species observed under Miscanthus at MG and BF sites. The recovery of earthworm communities is a slow process [88] that explains these results even five years after the establishment of the crop.

It is recognised that plant cover may enhance earthworm populations by reducing evaporation, buffering soil temperature and providing food and protection from predators [20]. However, in our study, the presence of Miscanthus (and associated leaf litter) compared to the presence of grass (meadow) had no positive effect on earthworms. These results could be explained by the low nutritional value and palatability of Miscanthus litter, based on: a) high C:N ratio of Miscanthus residues, which can reach 300 [89], b) the associated time taken to decompose Miscanthus leaf litter and c) the relatively large particle size of the litter [30] which strongly impacts earthworms especially anecic species [90] and may explain significantly lower anecic abundance recorded in Miscanthus treatments at MG.

4.3. Effect of miscanthus on soil structure

4.3.1. Bulk density

At MG, lower bulk density results in the undisturbed meadow treatment may be attributed to higher biological activity, including burrowing by earthworms [91,92]. In addition, the annual use of machinery to harvest Miscanthus may increase compaction [93] in the Miscanthus treatment.

At the polluted sandy site (CH), results for both treatments were lower than the threshold value, which is consistent with a recognition that soils rich in organic matter generally have lower bulk density [94], and no significant difference in bulk density was recorded between treatments.

While some studies reveal a decrease in bulk density after planting Miscanthus [29,95] others show an increase [5], it is therefore difficult to establish with any certainty the effect of planting on this indicator. In the current study, soil bulk density was influenced by the history of the site and the type of land management (agricultural land or marginal land) more than by vegetation cover.

4.3.2. Aggregate stability

Five years of Miscanthus cultivation have not influenced soil aggregate stability at any of the sites. However, trends related to soil type, contamination levels, previous land use and characteristics of undisturbed meadow soil can be observed and discussed.

For sites with similar texture, such as BF and MG, it is organic matter content that mainly influences MWD results [56]. At these sites, values are close to those obtained (with the same method) from soils with the same texture and level of organic matter [89,96,97]. Organic matter promotes the development of microbial biomass, which is positively correlated with aggregate stability [98] through the production of mucilages, polysaccharides [99,100] and hydrophobic molecules [101].

Results tend to be higher under Miscanthus at BF than MG and this may be explained by differences in biotic properties of the soil resulting from historical land use. The change from an annual intensively tilled crop to a perennial no-till and unfertilized Miscanthus crop combined with Miscanthus crop residues with a high C:N ratio [89] and lignin content [102] left on the soil surface may have caused the development of fungi [103]. Previous studies have suggested that mycelial hyphae are correlated to aggregate stability [68] and increase resistance to slaking [97]. Results from the MG site suggest lower aggregate stability under Miscanthus than under non-polluted and non-compacted undisturbed meadow as reported in previous studies [5,20]. Moreover, our results are in accordance with Ruf et al. [76] who reported that 6-year-old perennial energy crops, including *Miscanthus x giganteus*, had aggregate stability values 57% lower than permanent meadows. This result could be attributed to biological soil quality indicators such as earthworm abundance ($p < 0.0001$), earthworm biomass ($p = 0.0002$) [97–100] and MBC ($p = 0.1$) which were lower under Miscanthus at this site.

In the sandy polluted site (CH), aggregate stability values were lower than recorded by Amezketa et al. [104] in soil with a similar texture. C_{org} was 30% higher under Miscanthus but did not contribute to soil aggregation probably because of the low yield potential of this site (2.1 t ha^{-1} in 2018 [38]) resulting in lower root activity and exudates which are known to contribute to aggregate stability [105] and the inhibitory effect of metal concentrations on hyphal length density [106].

4.4. Multi-parameter approach

The assessment of several indicators (biological, physical and chemical) allows an overview of soil quality. Results have suggested that the effect of Miscanthus on soil quality is site dependent, even at a regional scale and is related to the type of soil (texture, soil properties), the level of trace element contamination and previous land use.

In a previous meadow location with a silty clay loam texture and low levels of contamination (MG), undisturbed meadow has a better soil quality than under Miscanthus with results suggesting that soil quality is negatively impacted by Miscanthus cultivation (lower microbial biomass, reduced earthworm community, lower aggregate stability). These results are in accordance with Felten and Emmerling [31] and Ruf et al. [107] who recorded higher biological quality and soil quality under uncultivated areas compared to Miscanthus. However, Miscanthus reached a high yield (20.5 t ha^{-1} in 2018 [38]) at this site which may be accounted for by high mineral nitrogen availability [38] from grass returned to the soil and associated net nitrogen mineralization. In comparison, after an annual crop without any additional nitrogen fertilization (BF), Miscanthus achieved a lower yield in 2018 (14.8 t ha^{-1} [38]) even if still higher than the minimum reference value (10 t ha^{-1}) suggested by Lewandowski et al. [21]. However, growing unfertilized Miscanthus after a cereal crop would have induced lower soil mineral nitrogen levels at the agricultural site (BF) which in turn would have encouraged the development of fungi and aggregate stability after the fast wetting test [97,108].

Additional studies employing a wider range of sites with similar soil properties are required to confirm the trends that have been observed. However, increasing the number of sites and samples while maintaining viable financial costs may only be achieved by decreasing the number of indicators studied. Since our results show similar trends between treatments and sites for microbial biomass and earthworm abundance and biomass, it is proposed that one of these two biological indicators could be removed from future assessments.

In polluted soil (CH), Miscanthus achieved a low yield in 2018 [38] and therefore has limited financial benefit. Moreover, Miscanthus did not improve microbial biomass, earthworm community and soil structure. However, while our results do not allow for any conclusion on the phytostabilising effect of Miscanthus or on the decrease in the availability of pollutants, result do suggest trends in a reduction in mobility and availability of metals which is consistent with the literature [24,26]. On a polluted site with a sandy texture, even if Miscanthus has no positive effect on soil quality at the plot level, at the scale of the Chanteloup plain phytostabilization could lead to a decrease in metal mobility [109] and reduce the transfer of contaminants into groundwater and the nearby river Seine. Further studies are needed to confirm if vertical mobility of heavy metals is decreased in the Miscanthus rhizosphere and stratified sampling looking at different depths is proposed [110] as well as adapting sampling to account for the heterogeneity of the site.

5. Conclusion

By measuring biological, physical and chemical soil quality indicators, complemented by crop yield at three locations, our work provides a first overview of the influence of Miscanthus on soil quality in different contexts. It highlights that soil quality under Miscanthus is dependent on initial site conditions and history and suggests that maintenance/improvement of soil quality is an important factor in achieving sustainable cultivation of Miscanthus. Further studies should assess soil quality under Miscanthus at other sites, in other contexts and with a longer time scale in order to further determine on which types of marginal land Miscanthus is environmentally as well as economically sustainable.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2020.105793.

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