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Toward a New Way for the Valorization of Miscanthus Biomass Produced on Metal-Contaminated Soils Part 1: Mesocosm and Field Experiments

Marie Hechelski [1,2], Brice Louvel [1,2], Pierrick Dufrénoy [3,4], Alina Ghinet [3,5,6] and Christophe Waterlot [3,*]

1 Laboratoire de Génie Civil et géo-Environnement, ULR 4515—LGCgE, Université Lille, F-59000 Lille, France; marie.hechelski@yncrea.fr (M.H.); brice.louvel@yncrea.fr (B.L.)
2 Health and Environment, Laboratory of Environment, Junia, F-59000 Lille, France
3 UCLille, Health & Environment Department, Sustainable Chemistry Team, Laboratoire de Chimie Durable et Santé, JUNIA, Hautes Etudes d'Ingénieur (HEI), 13 rue de Toul, F-59046 Lille, France; Pierrick.dufrenoy@yncrea.fr (P.D.); alina.ghinet@yncrea.fr (A.G.)
4 Laboratoire de Chimie, Lille Inflammation Research International Center, Normandie Université, UNILEHAVRE, FR 3038 CNRS, URCOM, EA 3221, UFR Sciences & Technologies, 25 rue Philipe Lebon, F-76063 Le Havre CEDEX, France
5 Institut Pasteur de Lille, Université de Lille, Inserm, CHU Lille, U1167-RID-AGE—Facteurs de Risque et Déterminants Moléculaires des Maladies Liées au Vieillissement, F-59000 Lille, France
6 Faculty of Chemistry, 'Alexandru Ioan Cuza' University of Iasi, Bd. Carol I nr. 11, 700506 Iasi, Romania
* Correspondence: christophe.waterlot@yncrea.fr; Tel.: +33-3-28-38-48-01

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Abstract: The effects of P-fertilizers (mono- and di-calcium phosphates) on the bioavailability of metals and nutrients in leaves and stems of *Miscanthus* × *giganteus* were studied in mesocosm and field experiments in order to propose a new way for the valorization of miscanthus biomass. The concentration of potentially toxic elements was generally higher in stems than in leaves. Although P-fertilizers were added to contaminated soils under sustainable conditions (from 0.022% to 0.026% *w*/*w*), the average of leaf and stem biomass generally increased in the presence of P-fertilizers due to the changes in the speciation of phosphorus. Leaves of the investigated miscanthus may be of great interest as a catalyst in organic chemistry, since the Ca concentration was up to 9000 mg kg^{-1} DW. Stems represent a potential biomass that can be used as renewable resource of Lewis acids, currently used in organic syntheses (the sum of Zn, Cu, Mn, Fe, Mg, Si and Al was near 1000 mg kg^{-1} DW). The percentage of Cd and Pb in leaves and stems of miscanthus did not significantly change with P-fertilizers. Depending on the mesocosm and field experiments, it ranged from 0.004% to 0.016% and from 0.009% and 0.034% for Cd in leaves and stems, respectively, and from 0.004% to 0.015% and from 0.009% and 0.033% for Pb in leaves and stems, respectively.

Keywords: miscanthus; metal; fertilizer; soil management

1. Introduction

Soil pollution by metals remains a major environmental problem that affects biodiversity and human health. Many strategies have been proposed to remediate and improve contaminated soil quality over the last few decades. Most remediation techniques (e.g., incineration, soil replacement, soil washing, soil containment, electro remediation) are often inadequate for large contaminated areas, expensive and have negative consequences for soil functionality, which is very close to soil quality (e.g., physical, chemical and biological properties) [1]. In the ecosystem service (ES) community, this means that the "natural capital stocks" of soils decrease, and consequently "soil security" is not preserved. The integrative framework term "soil security" was proposed by Koch et al. [2] and McBratney [3] who defined this term as "the maintenance and improvement of the global soil resource to produce food, fibre and freshwater, contribute to energy and climate sustainability, and to maintain the biodiversity and the overall protection of the ecosystem".

Soft, simple and less intrusive techniques have emerged for the surrounding environment. Gathered under the name of Gentle Remediation Options (GROs) [4,5], these types of remediation are based on the use of bacteria and/or amendments associated or not with plants. These techniques are more time consuming than those cited previously but are less expensive and easier to apply on a large scale. When soils contaminated by metals are considered, the GRO techniques include phytoextraction/aided phytoextraction and phytostabilization/aided phytostabilization. The latter approach is based on the immobilization of metals by means of mineral and/or organic amendments with plants that can be tolerant or excluders. Synergistic effects of amendments and plants can lead to a reduction in metal mobility and phytoavailability due to changes in soil and rhizosphere (rhizospheric acids, fungi, bacteria) properties [6,7].

The production of plant biomass, not dedicated to human consumption, in marginal and contaminated agricultural areas has been recently suggested as a potential solution for the rehabilitation and reconversion of contaminated soils [8]. Modifications of the plant biomass to produce fibers, oil, biofuels, mulch, animal bedding and biochars are topical and of particular interest to manufacturers and researchers because these advances are part of the circular economy and design processes [9–14]. For instance, miscanthus is a promising example of biomass for which there are still new applications to discover.

Miscanthus × *giganteus* was defined as a metals excluder in many studies and was described as a good alternative in the production of non-food biomass on contaminated soils [15]. Indeed, this rhizomatous perennial plant accumulates metals in the roots and rhizomes, limiting their transfer in stems and leaves, and produces biomass ranging from 15 to 22 tons ha/yr on arable land [16]. Resistant to low and high temperatures, this species has a lifespan of 15 to 30 years and was selected in the reconversion of contaminated agricultural soils due to its low fertilizer requirements and to its beneficial agronomic effects. However, Christian et al. [17] reported fertilizer rates of about 100 kg K ha^{-1} yr^{-1} and 7 kg P ha^{-1} yr^{-1} to replenish soil reserves. Phosphorus is also one of the most suitable amendments that can immobilize metals [18] or mobilize under acid conditions [19].

In 2010, an in situ rehabilitation project (PHYTENER) was initiated with the aim of (i) developing a phytostabilization process of metals and (ii) producing plant biomass for energy applications. Indirect planting of rhizomes from a perennial grass (*Miscanthus* × *giganteus*) was conducted on a 0.8 ha agricultural plot (named M700) with different modalities (i.e., density from 15,000 up to 20,000 plants ha^{-1}) [20]. Rhizomes of miscanthus (hybrid plant) were supplied from Novabiom (formerly Bical Biomass). The experimental agricultural plot (M700; latitude: 50°26′15.9″ N; longitude: 3°01′04.9″ E) was located at Evin-Malmaison in the north of France at about 1 km north from the former lead smelter (Metaleurop Nord) (Figure 1). M700 is a silty loam and alkaline soil, poorly carbonated (0.4%) and with low organic matter content (2.7%) [20,21]. The study of Nsanganwimana et al. [22] showed that concentrations of Pb, Zn, Ca and Na were higher in leaves than in stems in summer and autumn, whereas concentrations of N, P, K and Mg decreased. Consequently, for best practices in our phytomanagement, we decided to harvest the stems of miscanthus after the senescent period and to leave the leaves of miscanthus on the soil. Recently, impact of leaf decomposition and the consequences for metal behavior and the succeeding culture was studied [23]. If no persistent impacts of the leaf litter on the soil–miscanthus system was highlighted, the authors showed a significant increase of Cd and Pb $CaCl_2$-extractable concentrations. This result means that the availability of Cd and Pb increases in time when contaminated areas are harvested late (after senescence). Consequently, the current study aimed at evaluating the sustainable use of phosphates on (i) the leaf and stem biomass of miscanthus and (ii) the bioavailability of metals and nutrients in order to propose a potential new valorization method of the aerial organs of miscanthus.

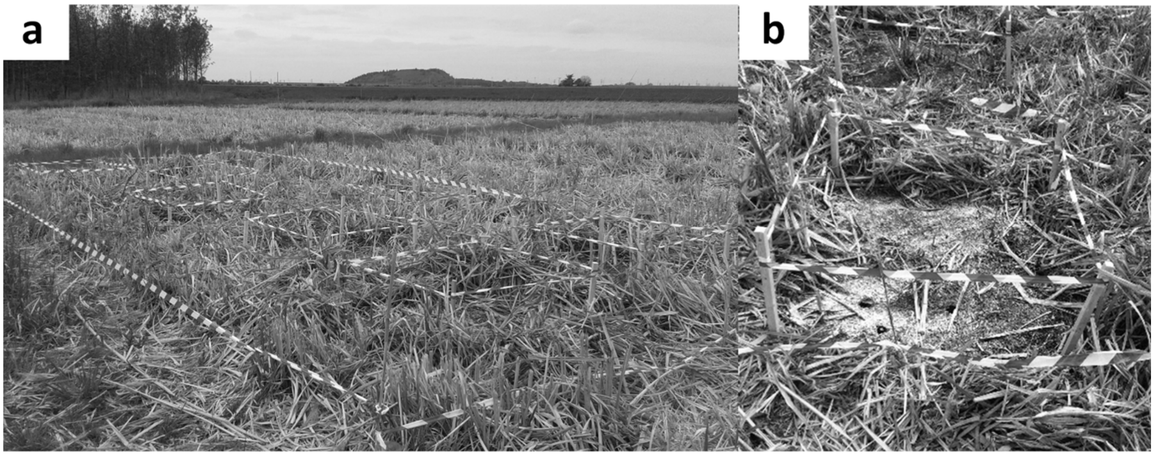

Figure 1. Experimental plots of the field experiments; (**a**) general view; (**b**) view of one treated subplot.

2. Materials and Methods

2.1. Experimental Setup in the Field

The field experiment was started in May 2017 (T = 0), just before the appearance of the aerial parts of miscanthus. Subplots were constituted in a 10 × 4 m quadrat at 85 cm from the border to limit the border effects. Thus, 9 subplots (1 × 1 m) were delimited and randomly placed (Figure 1). Three conditions were studied in triplicates, the first being without phosphate (Ø), the second related to the addition of monocalcium phosphate (MCP) and the third associated with the addition of dicalcium phosphate (DCP), both phosphates being acidic according to the theory described in Chen et al. [24]. Each phosphate-calcic compound was added to the soil surface with respect to the molar ratio P/(Pb + Cd) = 3/5 using $Pb_5(PO_4)_3(F,Cl,OH)$ as reference [25]. Thus, each amended subplot received 74 g MCP (0.022% *w/w*) or 86 g DCP (0.026% *w/w*). No other fertilizer was added, and the rainfall was 203 L m^2 from the beginning to the end. Leaves and stems of miscanthus and soils (0–10 and 10–25 cm) were collected in September 2017 (T = F).

2.2. Experimental Setup in Mesocosms

2.2.1. Soil Sampling

The mesocosm experiments started in May 2017 (T = 0). A total of 400 kg of contaminated soil was collected in February 2017 from M700. Fresh soil was air-dried at room temperature and was sieved to pass through a 10 mm stainless steel sieve. After homogenization, 6 replicates of each treatment (Ø, MCP and DCP) were constituted, and 19 kg of unamended or amended soils was placed in 18 containers. Each amended subplot received 4.37 ± 0.38 g MCP (0.022% *w/w*) or 5.07 ± 0.45 g DCP (0.026% *w/w*). Soils were then watered with tap water to maintain the soil water at 60% field capacity for a two-month contact period (March–May 2017). After this incubation period, soil from each container was homogenized before planting rhizomes.

2.2.2. Rhizomes Preparation for Transplanting and Mesocosm Trial

Fifty healthy rhizomes of *Miscanthus* × *giganteus* from Novabiom (Bical biomass; http://www.novabiom.com/miscanthus/) were planted in April 2017 using universal horticultural compost (N: 160 g/m^3; P_2O_5: 80 g/m^3, pH: 6.5; Gamm vert®). After 4 weeks, the 18 most vigorous plants were selected for the mesocosm experiments, which were set up at the University of Lille (latitude: 50°36′32.6″ N; longitude: 3°08′40.5″ E). After the pre-started phase, the selected rhizomes were planted in the 18 containers (May 2017; T = 0). Stems and leaves of miscanthus were harvested four months after planting (September 2017; T = F), and soils were sampled.

2.3. Soil Analyses

2.3.1. Physicochemical Parameters and Metal Concentrations

Soil pH was measured according to the NF ISO 10390 standard (*v/v* 1:5) [26]. The organic matter was determined by loss on ignition with a muffle furnace (Nabertherm, Controller C6, W-Germany) set at 650 °C for 3 h [27]. The total phosphorus concentrations and the phosphorus fractionation were determined following the procedure described by Waterlot et al. [28]. Briefly, five phosphorus fractions were considered: total phosphorus (TP), non-apatite inorganic phosphorus (NAIP), apatite phosphorus (AP), inorganic phosphorus and organic phosphorus (OP). The concentration of phosphorus in each fraction was determined using a spectrophotometer at 882 nm using the molybdenum blue method [29] and using an electrothermal absorption spectrometer (ETAAS, GFA-EX7, Shimadzu, Tokyo, Japan). Total concentrations of metals (Cd, Pb, Zn, Cu, Fe, Mn, Ca, Mg, Na and K) in soil samples were determined after their digestion using a mixture of nitric acid (69%), hydrogen peroxide (30%) and hydrochloric acid (37%) and a hot block digester (Hotblock™ Environmental Express® SC100, Charleston, SC, USA) according to the USEPA 3050 B method (USEPA, 1996). The concentration of metals was determined by flame atomic absorption spectrometry (FAAS, AA-6800, Shimadzu, Tokyo, Japan). The certified reference soil ERM®-CC141 was used for the quality control.

2.3.2. Potential Availability of Metals

The availability of metals was evaluated by means of an organic acids mixture according to the procedure described by Feng et al. [30]. Briefly, 20 mL of 0.01 M low molecular weight organic acids (LMWOAs; malic, formic, citric, lactic and acetic acids in the respective molar ratios 1:1:1:2:4) were added into a 50 mL centrifugation tube containing 2 g of soil sample. The mixture was shaken with a rotary shaker for 16 h at room temperature and centrifuged at 4530 rpm for 20 min. Then, the solution was filtered over an acetate Millipore membrane (0.45 µm porosity) and stored at 4 °C prior to analysis. The concentration of metals was determined by FAAS.

2.3.3. Soil Biology

The determination of microbial biomass was assessed by means of fluorescein diacetate hydrolytic activity (FDAH) quantification using the protocol described in Green et al. [31]. Soil sample (1.00 g sieved at <2 mm) was added to a mixture of FDA (0.5 mL, 10 µg mL^{-1}) and Na_3PO_4 (50 mL, 60 mM, pH 7.6), mixed and incubated at 37 °C for 3 h. Then, 2 mL of acetone was added in order to stop the FDA hydrolysis, and the mixture was centrifuged for 5 min at 4000 rpm. The supernatant was filtered and transferred into a 96-well microplate (200 µL). The determination of FDAH was carried out by spectrophotometry (Multiskan GO-UV/Visible spectrophotometer) at 490 nm.

2.4. Plant Analysis

2.4.1. Growth Parameters

For the mesocosm experiments, the height of the 18 selected plantlets were determined before planting. At the end of the experiment, the highest stems of each miscanthus plant were selected and measured. For the field experiments, no visible plantlet was found at the beginning of the experiment. In contrast, at the end, an average height of three stems was given. The height of each stem was defined as the distance between the base and the last node. At the same time, the diameter of each stem was determined from its base. Leaves and stems were manually separated, thoroughly rinsed with tap water and dried in an oven at 40 °C. The dried biomass of samples was determined after drying at 105 °C until reaching a constant mass.

2.4.2. Concentration of Metals

After air-drying, crushing and sieving at <315 µm, leaves and stems of miscanthus (300 mg) were added to a 50 mL digestion tube. The digestion was conducted in a mixture of nitric acid (69%) and hydrogen peroxide (30%) according to the USEPA 3050 B method [32]. The concentration of metals was determined by FAAS. INCTL-OBTL-5 (Virginia tobacco leaves, $n = 3$) was used as reference material for the quality control.

2.5. Statistical Analysis

Statistical analyses were performed using the XLSTAT 2018 software (Addinsoft, Paris, France). The Shapiro–Wilk test was used to test the normality distribution of the data. ANOVA and Tukey's test were conducted in order to identify significant differences between individual treatments ($p < 0.05$).

3. Results and Discussion

3.1. Soil Characteristics

Clay, silt and sand in the studied soil were 19.5%, 53% and 27.5%, respectively [20]. The cation exchange capacity was 14.9 ± 1.6 $cmol^+ kg^{-1}$, the concentration of phosphorus was 0.16 mg P_2O_5 ± 1.6 cmol kg^{-1}, the mineralization index was C/N = 15.2 ± 0.4 and the total carbonate was below 1 mg kg^{-1} for the nine modalities. The most important physicochemical parameters for the study and the microbial activity of the soil used in mesocosm and field experiments are summarized in Table 1. The soil samples were slightly alkaline (from 7.2 to 7.8), with the lowest in the first 10 cm (from 7.2 to 7.5). The organic matter contents ranged from 4.19 ± 0.98% to 5.17 ± 1.10% and were not significantly different. Interestingly, the biological activities in soil samples used before the mesocosm experiments were almost absent, whereas they were in the range 0.13 to 0.17 mg kg^{-1} in the field experiments. This result may be due to the preparation of soil samples (drying and sieving) before potting. Besides the incubation period needed for the stabilization of phosphates, this period seems to be essential for the development of microbial activities. The concentrations of metals, alkali and alkaline earth metals are summarized in Table 2. Briefly, no significant difference ($p < 0.05$) was found between the concentration of metals measured in non-amended and amended soils for each treatment (mesocosm and field experiments), suggesting a good homogeneity of our soil samples. The concentrations of Cd, Pb and Zn correlated well with those previously reported by Nsanganwimana et al. [20].

Table 1. Physicochemical parameters (pH, organic matter, $CaCO_3$) and microbial biomass of soils under mesocosm (n = 6) and field (n = 3) experiments before and after the experimentation.

		Before the Experimention (T = 0)			After the Experimentation (T = F)		
		pH	OM (%)	FDAH (mg kg^{-1})	pH	OM (%)	FDAH (mg kg^{-1})
Mesocosm experiment	Ø *	7.7 ± 0.0	4.9 ± 0.6	0.03 ± 0.00 [a]	7.9 ± 0.1	4.7 ± 0.2	0.13 ± 0.02 [d]
	MCP **	7.7 ± 0.1	4.3 ± 1.6	0.02 ± 0.00 [a]	7.8 ± 0.1	3.5 ± 0.9	0.14 ± 0.02 [d]
	DCP ***	7.7 ± 0.1	4.4 ± 0.9	0.02 ± 0.00 [a]	7.9 ± 0.1	4.6 ± 1.3	0.16 ± 0.02 [d]
Field experiment (0–10 cm)	Ø *	7.3 ± 0.4	4.5 ± 1.0	0.15 ± 0.04 [bc]	7.4 ± 0.2	4.3 ± 0.3	0.13 ± 0.06 [d]
	MCP **	7.4 ± 0.1	4.7 ± 0.5	0.14 ± 0.02 [bc]	7.2 ± 0.1	4.2 ± 0.6	0.10 ± 0.01 [d]
	DCP ***	7.3 ± 0.1	4.2 ± 1.0	0.13 ± 0.01 [b]	7.3 ± 0.1	4.6 ± 0.2	0.10 ± 0.01 [d]
Field experiment (10–25 cm)	Ø *	7.7 ± 0.1	5.2 ± 1.1	0.15 ± 0.02 [bc]	7.8 ± 0.1	5.2 ± 0.9	0.12 ± 0.05 [d]
	MCP **	7.8 ± 0.1	4.7 ± 0.4	0.16 ± 0.01 [bc]	7.6 ± 0.1	5.7 ± 0.8	0.09 ± 0.01 [e]
	DCP ***	7.7 ± 0.0	5.0 ± 0.7	0.17 ± 0.01 [c]	7.8 ± 0.0	4.5 ± 0.8	0.08 ± 0.00 [e]

* Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate; OM: organic matter; FDAH: fluorescein diacetate hydrolytic activity. Significance levels ($p \leq 0.05$) among treatments are presented with letters ([a–e]).

Table 2. Concentrations of metals in soils under mesocosm (n = 6) and field (n = 3) experiments before the production of plant biomass.

		Metal Concentration (mg kg^{-1})								
		Ø *			**MCP ****			**DCP *****		
Mesocosm experiment	Cd	13.9	±	0.3	14.4	±	1.5	14.7	±	0.7
	Pb	693	±	16	722	±	49	732	±	20
	Zn	1093	±	51	1164	±	80	1219	±	41
	Cu	33	±	2	34	±	2	34	±	1
	Mn	344	±	41	373	±	78	392	±	45
	Fe	11,399	±	612	12,068	±	885	11,908	±	480
	K	1830	±	81	1937	±	185	1925	±	101
	Ca	5197	±	442	5097	±	497	4114	±	1824
	Mg	1466	±	65	1552	±	148	1542	±	81
Field experiment (0–10 cm)	Cd	13.4	±	0.6	13.6	±	0.3	14.3	±	1.5
	Pb	695	±	29	720	±	8	741	±	110
	Zn	1087	±	141	1133	±	65	1065	±	147
	Cu	32	±	1	31	±	0	33	±	5
	Mn	302	±	31	316	±	22	315	±	27
	Fe	12,726	±	308	12,649	±	380	13,561	±	1198
	K	1751	±	255	1676	±	131	1804	±	275
	Ca	4530	±	556	4778	±	394	5160	±	269
	Mg	1643	±	239	1572	±	123	1693	±	258
Field experiment (10–25 cm)	Cd	13.9	±	0.2	13.1	±	1.1	13.2	±	0.6
	Pb	710	±	12	677	±	57	690	±	42
	Zn	1072	±	91	1022	±	19	1049	±	42
	Cu	31	±	1	29	±	3	30	±	1
	Mn	331	±	33	317	±	29	313	±	24
	Fe	12,882	±	692	12,734	±	564	12,703	±	180
	K	1531	±	102	1458	±	78	1538	±	49
	Ca	6031	±	25	4948	±	572	5631	±	434
	Mg	1436	±	96	1368	±	73	1443	±	46

* Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate.

3.2. Change in Soil Physical, Chemical and Biological Parameters of Amended Soils

No significant effect of P-fertilizers was highlighted on soil pH and organic matter contents ($p < 0.05$; Table 1). In contrast, significant differences in the FDAH concentrations were obtained, depending on experiments and treatments. The microbial activities significantly increased over time (from 0.02 ± 0.00 to 0.16 ± 0.02 mg kg^{-1}) in mesocosm experiments, and the activity was slightly improved with DCP (0.16 ± 0.02 instead of 0.13 ± 0.02 mg kg^{-1} for unamended soils). These results may firstly be explained by the humidity and the temperature conditions required for the bacterial growth as well as the presence of miscanthus. Indeed, it has been recently shown that miscanthus has benefits on the functionality of contaminated soils, enhancing the biomass as well as the bacterial and fungal activities [33,34]. Nevertheless, the presence of fungi may change the bioavailability of metals, improving the metal uptake by miscanthus and their translocation [20]. Hromádko et al. [35] showed that *Miscanthus* × *giganteus* roots have a high rate of root exudation compared to other plants (*Oryza sativa*, *Sorghum* × *drummondii*, *Agropyron cristatum*). The root exudates of miscanthus are mainly composed of carbohydrates, organic acids (e.g., succinic, citric and oxalic acids) and amino acids (aspartic and glutamic acids, arginine and alanine). Since carbon is a limiting source for bacteria in soils, the release of assimilated carbon (i.e., carbohydrates) by the roots of miscanthus promotes the dynamics of microbial communities. In this sense, it was highlighted that the production of rutin and quercetin by the roots of miscanthus allow the growth and the stimulation of specific bacteria such as proteobacteria [36,37].

As shown in Table 1, bacterial activities significantly decreased over time in field experiments, especially in amended soils at 10–25 cm depth. If we refer to Shelford's "law of tolerance", an ecological factor plays a limiting factor role for an organism's chances of success in its attempts to colonize an environment [38]. This factor can be limited by the absence or excess of organisms. In our study, it can be assumed that the addition of phosphates to the environment in association with a reduction of biological oxygen demand at a 10–25 cm depth has negative effects on the bacterial biomass and its development. Moreover, Beauregard et al. [39] clearly showed a reduction of soil microbial activity with an increase of the phosphorus flux and soluble phosphorus in soils. These findings are consistent with the total phosphorus concentrations measured in soils from field experiments at a 10–25 cm depth, which were higher than those of 0–10 cm for all modalities (unamended: Ø and amended: MCP or DCP; Figure 2). The significant differences between the bacterial activities in soils from the mesocosm and the field experiments may be explained by the concomitant effects of the soil settling and old age of the roots of miscanthus. Indeed, Nihorimbere et al. [40] demonstrated that the secretion of exudates by young plants is the highest in the first few months of growth, improving the bacterial activities. This phenomenon favored the bacterial activities in mesocosms, whereas old age of the roots of miscanthus and soil settling contributed to the decrease of bacterial activity in soil from field experiments and changes in the physicochemical parameters since the production of miscanthus started 11 years ago [41].

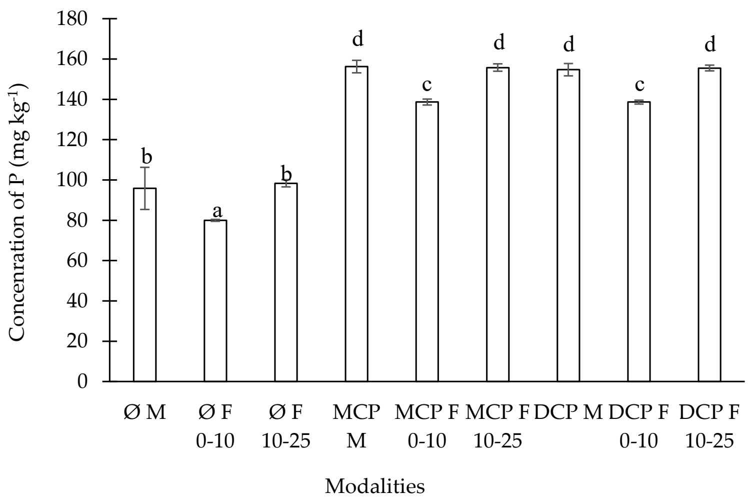

Figure 2. Concentration of total phosphorus (mean ± standard deviation) in soils from mesocosm (M; $n = 6$) and field experiments (F; $n = 3$) before and after treatments. Ø: without phosphate; MCP: monocalcium phosphate; DCP: dicalcium phosphate. Significance levels ($p \leq 0.05$) among treatments are presented with letters (a–d).

Based on the results of Waterlot et al. [28], the distribution of phosphorus was established considering inorganic phosphorus as the sum of apatite phosphorus (AP), non-apatite inorganic phosphorus (NAIP) and organic phosphorus (OP). The total phosphorus concentrations were from 95.86 ± 10.46 mg kg^{-1} in unamended soils up to 156.28 ± 3.06 and 154.67 ± 3.03 mg kg^{-1} in soils amended with MCP and DCP, respectively. As shown in Figure 3, these concentrations correlated well with the calculated ones (R^2 = 0.9991). The distribution of phosphorus in soil samples is presented in Figure 4. Phosphorus was mainly accumulated in the NAIP fraction for all modalities (from 58% to 96%), whereas apatite fractions were the least concentrated. Interestingly, the organic fractions were the greatest in amended soils (DCP > MCP > Ø) and higher in field experiments (from 8% to 30%) than in mesocosms (from 3% to 19%).

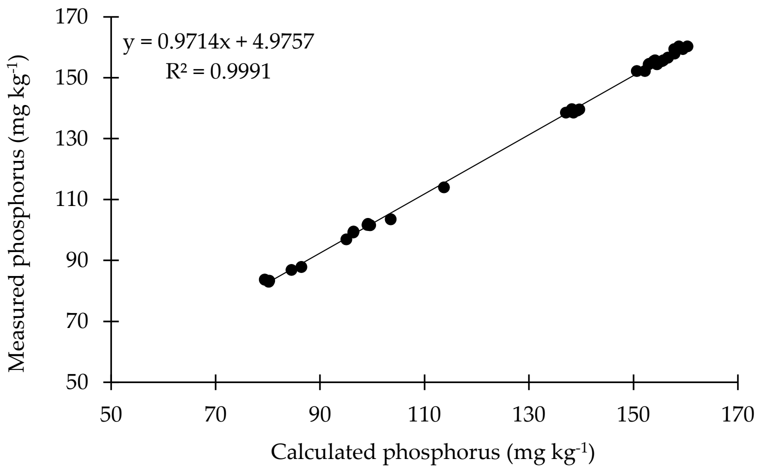

Figure 3. Correlation between the measured P concentrations and the calculated ones (n = 36).

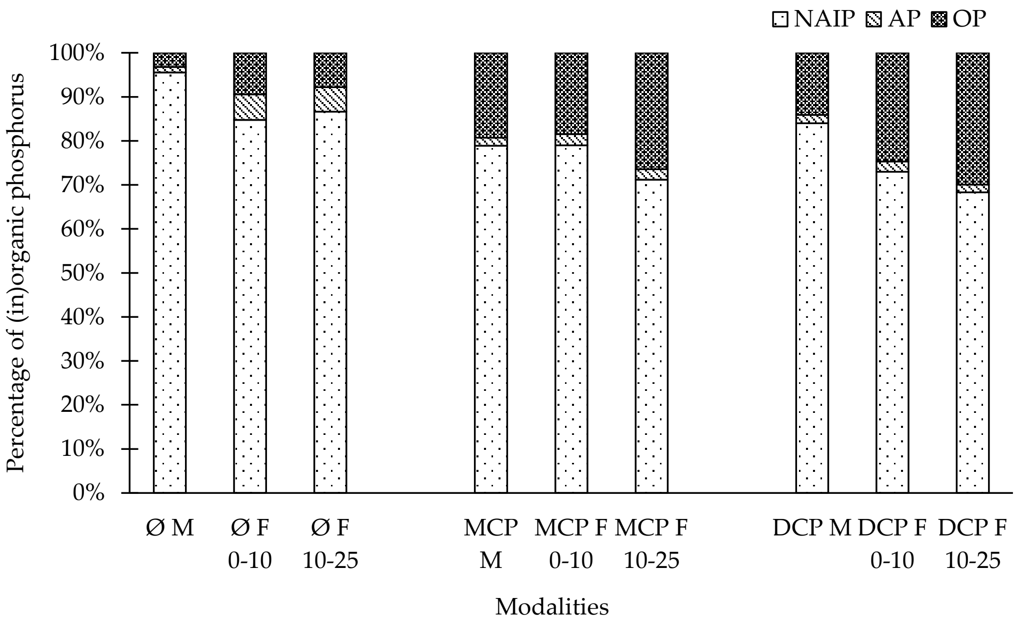

Figure 4. Distribution of phosphorus in soils from mesocosm (M) and field (F) experiments before and after treatments. Ø: without phosphate; MCP: monocalcium phosphate; DCP: dicalcium phosphate.

3.3. Effects of Phosphates on the Extractable Concentrations of Metals in Soil

The concentrations of metals extracted by a mixture of LMWOAs before planting and after the experiments are given in Table 3. Before planting, the concentration of extractable metals was very low compared to the metal concentrations (Table 2), suggesting that metal concentrations in the soils studied are low. For instance, only 13% Cd, 0.8% Pb and 17% Zn were extracted by the mixture. Consequently, significant effects of phosphates were quite absent except an increase of the extractability of Zn and Fe in treated soils from field experiments at a 10–25 cm depth. The same trends were observed after the experiments, since there was almost no significant effect of (i) phosphates, (ii) miscanthus and (iii) miscanthus + phosphates on the extractable metal concentrations. These results confirm the high stability of metals regarding the mixture of LMWOAs, demonstrate the inefficiency of phosphates for metal immobilization under the experimental conditions and reveal the stabilization effect of metals by the belowground parts (rhizomes + roots) of miscanthus. It is worth mentioning some changes in the extractable concentrations of Fe, Zn and K from soils treated with DCP at the end of the experiments. The extractable concentrations of Fe decreased from soils in mesocosm and in field experiments, whereas those of Zn only decreased in the field experiments. These results could be explained by an immobilization of Fe and Zn by DCP or a mobilization of these two elements followed by a sequestration promoted by the organs of miscanthus. Indeed, even if miscanthus is known as an excluder [42], some studies reported high accumulation of metals in the underground organs [43,44]. The concentration of extractable K significantly decreased suggesting an accumulation of this element by the different organs of miscanthus. Nsanganwimana et al. [22] reported in a studied area high concentrations of K in leaves and stems (up to 32 and 75 g kg^{-1} DW, respectively), depending on the sampling period in the same way as for macronutrients [44]. These authors reported that K concentrations in leaves and stems were the highest in the growing period and then decreased until senescence. They explained that K is present in the aerial organs of miscanthus as free cations and constitutes one of the osmotic regulators and enzyme activators.

3.4. Concentration of Metals in the Aerial Parts of Miscanthus × Giganteus

The concentration of metals in leaves and stems of miscanthus from mesocosm and field experiments are summarized in Table 4 for the three modalities (Ø, MCP and DCP). The effects of MCP and DCP on the concentration of metals in leaves of miscanthus from the mesocosm experiments were only limited to Na and Si, since their concentrations in this organ decreased. For the other metals, no significant effect was observed, although the concentrations of Fe, K and Ca in leaves slightly increased. No effect of phosphates on the concentration of metals in leaves of miscanthus grown in field experiments was highlighted. The concentrations of Cd, Pb and Al in the leaves of miscanthus from field experiments were significantly lower than those from mesocosms, whereas the trend was reversed for Cu, Mn, Mg and Si. Phosphates had no significant effect on the concentration of metals in the stems of miscanthus from mesocosms and fields. Significant differences between the data set of stems were recorded for Cd, Pb, Zn, Ca, Mg and Si. Their concentrations were higher in stems from mesocosms than those from fields except for Si. Interestingly, the concentration of this metal was higher in stem from fields than that from mesocosms. This result correlated well with the metal detoxification mechanisms (co-precipitation of metals with Si, structural alterations in different parts of plants, reduction in oxidative stress, compartmentation of metals into metabolically inactive parts, chelation and modification of gene expression) [45]. Finally, the concentrations of Cd and Pb were higher in stems than in leaves, whereas it was the reversed for Ca. In contrast, the Cu concentrations in leaves were systematically higher than those in stems.

If we analyze our results with the aim of planning potential applications of aerial parts of miscanthus in the bioeconomy without considering the production of existing resources (e.g., energy, biochars) [15], stems and leaves may constitute two different renewable resources. Considering the three main pollutants (Cd, Pb and Zn), Zn may be of interest, since it can be used as a catalyst in organic chemistry as Lewis acids [46]. Thus, the ratios Zn/Cd and Zn/Pb were calculated in each condition. Values of these ratios ranged from 13 to 64 for all modalities. As shown in Table 5, stems of miscanthus from the mesocosm experiments with DCP present the highest concentrations of Zn, ranging from 85 to 149 mg kg^{-1}. Unfortunately, these concentrations are not sufficient to plan conception of Zn-biosourced catalyst as described in many studies summarized in Hechelski et al. [47]. On the other hand, the concentrations of Cd and Pb were the highest too, making the biomass not suitable for this application taking into account the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation. The first factor that may explain the difference in metal uptake by miscanthus is the geographical components (e.g., location, growing space, climatic factors) [20,21]. For instance, White et al. [46] demonstrated the temperature effects on the retention of metals in unamended soils and soils amended with phosphates. Their conversion rate into metal-orthophosphates is a temperature-dependent transformation as is their reactivity toward metals. The soil humidity and the evaporation rate are two temperature-dependent factors that affect the speciation of metals and thus their behavior. The second factor is the age of the miscanthus plants. Pidlisnyuk et al. [48] showed that the accumulation of Zn, Cu, Fe and Mn in stems was higher than in leaves in the first year of growth, whereas the difference in concentration between leaves and stems tended to decrease after.

Table 3. Extractable metal concentrations in soils from mesocosm (M; $n = 6$) and field (F; $n = 6$) experiments before planting and after harvesting.

		Extractable Metal Concentrations before Planting (mg kg^{-1})			Extractable Metal Concentrations after Harvest (mg kg^{-1})		
		Ø *	MCP **	DCP ***	Ø *	MCP **	DCP ***
Mesocosm experiment	Cd	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.0	1.8 ± 0.0	1.9 ± 0.0
	Pb	5.3 ± 1.0	5.0 ± 0.5	5.0 ± 0.4	6.5 ± 0.5	6.0 ± 0.1	6.7 ± 0.1
	Zn	172 ± 13	153 ± 20	168 ± 24	172 ± 6	163 ± 7	163 ± 22
	Cu	1.1 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0
	Mn	27 ± 3 [a]	27 ± 1 [a]	28 ± 2 [a]	31 ± 1 [b]	32 ± 1 [b]	29 ± 2 [b]
	Fe	191 ± 20 [b]	190 ± 15 [b]	201 ± 20 [b]	196 ± 14 [b]	185 ± 19 [b]	152 ± 20 [a]
	K	8.6 ± 0.4	8.2 ± 0.4	8.2 ± 0.4	6.0 ± 0.4	6.3 ± 0.3	5.4 ± 0.7
	Na	8.7 ± 1.8	7.7 ± 1.7	9.0 ± 0.9	8.7 ± 1.8	7.7 ± 1.7	9.0 ± 0.9
	Ca	85 ± 8 [ab]	77 ± 6 [a]	74 ± 9 [a]	78 ± 4 [a]	86 ± 3 [b]	97 ± 11 [b]
	Mg	1.5 ± 0.1 [a]	1.9 ± 0.6 [ab]	1.6 ± 0.2 [a]	1.8 ± 0.2 [ab]	2.1 ± 0.1 [b]	2.3 ± 0.2 [b]
Field experiment (0–10 cm)	Cd	2.0 ± 0.2	2.0 ± 0.0	1.9 ± 0.3	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
	Pb	7.0 ± 0.5	6.8 ± 1.3	7.1 ± 1.4	5.2 ± 0.7	5.7 ± 0.6	6.1 ± 0.2
	Zn	198 ± 24	218 ± 38	209 ± 52	163 ± 56	204 ± 16	198 ± 9
	Cu	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.1	1.1 ± 0.2
	Mn	42 ± 3	37 ± 4	39 ± 3	29 ± 9	32 ± 2	30 ± 6
	Fe	181 ± 68	190 ± 58	184 ± 15	207 ± 64	163 ± 12	144 ± 7
	K	6.6 ± 3.1	5.4 ± 2.0	6.2 ± 1.2	5.0 ± 2.7	4.5 ± 0.7	5.3 ± 2.1
	Na	1.1 ± 0.2	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
	Ca	65 ± 14 [ab]	63 ± 5 [a]	75 ± 5 [b]	64 ± 9 [ab]	68 ± 2 [ab]	70 ± 8 [ab]
	Mg	1.7 ± 0.3 [b]	1.3 ± 0.0 [a]	1.7 ± 0.3 [b]	1.6 ± 0.2 [b]	1.5 ± 0.0 [b]	1.6 ± 0.3 [b]
Field experiment (10–25 cm)	Cd	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
	Pb	5.1 ± 1.4	6.7 ± 0.8	6.6 ± 0.3	5.5 ± 0.8	5.1 ± 0.5	4.0 ± 0.7
	Zn	124 ± 17 [ac]	176 ± 34 [b]	153 ± 51 [ac]	159 ± 25 [b]	168 ± 39 [b]	103 ± 11 [a]
	Cu	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	1.2 ± 0.1
	Mn	29 ± 2 [b]	29 ± 1 [b]	26 ± 6 [b]	25 ± 1 [b]	25 ± 6 [ab]	19 ± 2 [a]
	Fe	85 ± 14 [a]	126 ± 7 [b]	125 ± 1 [b]	115 ± 3 [b]	127 ± 34 [b]	75 ± 11 [a]
	K	2.9 ± 0.3	2.4 ± 0.4	2.9 ± 0.4	3.0 ± 0.8	3.1 ± 0.3	2.9 ± 0.4
	Na	1.4 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
	Ca	98 ± 6 [b]	83 ± 11 [a]	80 ± 10 [a]	89 ± 6 [b]	82 ± 9 [a]	94 ± 4 [b]
	Mg	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.4	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.1

* Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate. Significance levels ($p \leq 0.05$) among treatments are presented with letters ([a–c]).

Table 4. Concentrations of metals (mean ± standard deviation) in the leaves and stems of miscanthus.

	Metals	Concentration of Metals in the Leaves of Miscanthus (mg kg^{-1})			Concentration of Metals in the Stems of Miscanthus (mg kg^{-1})		
		Ø *	MCP **	DCP ***	Ø *	MCP **	DCP ***
Mesocosm experiment (*n* = 6)	Cd	2.1 ± 0.6	2.5 ± 0.4	2.6 ± 0.6	4.4 ± 0.8	4.8 ± 1.1	5.2 ± 1.0
	Pb	2.0 ± 0.5	2.4 ± 0.4	2.4 ± 0.6	4.3 ± 0.7	4.7 ± 1.0	5.1 ± 1.0
	Zn	47 ± 4	49 ± 4	51 ± 4	117 ± 19	119 ± 16	139 ± 9
	Cu	5.6 ± 0.2	5.7 ± 0.3	5.7 ± 0.1	4.4 ± 0.2	4.4 ± 0.3	4.4 ± 0.3
	Mn	7.8 ± 1.7	8.8 ± 0.7	9.8 ± 2.0	10.6 ± 2.5 ^{ab}	10.4 ± 1 ^{a}	13.6 ± 2.0 ^{b}
	Fe	44 ± 7	63 ± 20	53 ± 12	42.7 ± 6.5	33.9 ± 7.7	55.5 ± 12.5
	K	7015 ± 673	7561 ± 909	7592 ± 1116	12,080 ± 2518	12,014 ± 744	11,798 ± 1747
	Na	30 ± 8 ^{a}	16 ± 1 ^{b}	15 ± 1 ^{b}	21.8 ± 2.8	20.5 ± 3.7	23.6 ± 4.9
	Ca	7452 ± 1251	7976 ± 528	7946 ± 1033	2313 ± 91	2525 ± 489	2754 ± 637
	Mg	387 ± 71	391 ± 58	417 ± 70	348 ± 24	390 ± 38	404 ± 54
	Si	120 ± 36	82 ± 9	88 ± 34	115 ± 29	133 ± 27	134 ± 24
	Al	20.6 ± 5.5	25.4 ± 9.3	20.8 ± 8.5	9.2 ± 4.6	6.5 ± 2.3	10.3 ± 4.3
Field experiment (*n* = 3)	Cd	1.0 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.5 ± 0.3
	Pb	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.4 ± 0.2
	Zn	43 ± 4 ^{ab}	38 ± 1 ^{a}	43 ± 2 ^{b}	42 ± 3	38 ± 8	38 ± 5
	Cu	7.1 ± 0.3	6.9 ± 0.3	7.0 ± 0.5	5.4 ± 0.7	4.9 ± 0.3	4.9 ± 0.2
	Mn	17 ± 2	17 ± 2	20 ± 4	13 ± 5	9 ± 1	9 ± 0
	Fe	58 ± 4 ^{b}	53 ± 3 ^{ab}	51 ± 2 ^{a}	22 ± 4	25 ± 6	26 ± 6
	K	12,765 ± 943	14,435 ± 1503	13,982 ± 2811	11,904 ± 1179	13,149 ± 491	13,961 ± 1691
	Na	31 ± 3	33 ± 4	35 ± 7	23 ± 5	20 ± 1	21 ± 2
	Ca	6816 ± 879	5715 ± 559	6779 ± 778	1 053 ± 256	1031 ± 56	1100 ± 227
	Mg	582 ± 90	591 ± 141	644 ± 179	259 ± 57	272 ± 20	246 ± 19
	Si	170 ± 27	150 ± 10	171 ± 34	186 ± 26	196 ± 23	157 ± 60
	Al	8.8 ± 1.6	9.2 ± 0.9	9.1 ± 1.5	8.6 ± 2.8	7.0 ± 1.3	6.9 ± 3.6

* Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate. Significance levels ($p \leq 0.05$) among treatments are presented with letters (^{a,b}).

Table 5. Bioconcentration factors (BCFs) of metals in the studied organs of miscanthus produced in mesocosm and field experiments in presence of or without P-fertilizers.

		Bioconcentration Factor BF_{leaves}			Bioconcentration Factor BF_{stems}		
		Ø *	**MCP ****	**DCP *****	**Ø ***	**MCP ****	**DCP *****
Mesocosm experiment	Cd	0.15	0.18	0.18	0.31	0.33	0.36
	Pb	0.00	0.00	0.00	0.01	0.01	0.01
	Zn	0.04	0.04	0.04	0.11	0.10	0.11
	Cu	0.17	0.17	0.17	0.13	0.13	0.13
	Mn	0.02	0.02	0.02	0.03	0.03	0.03
	Fe	0.00	0.01	0.00	0.00	0.00	0.00
	K	3.83	3.90	3.94	6.60	6.20	6.13
	Ca	1.43	1.56	1.93	0.44	0.50	0.67
	Mg	0.26	0.25	0.27	0.24	0.25	0.26
Field experiment (0–10 cm)	Cd	0.07	0.07	0.06	0.11	0.11	0.10
	Pb	0.00	0.00	0.00	0.00	0.00	0.00
	Zn	0.04	0.03	0.04	0.04	0.03	0.04
	Cu	0.23	0.22	0.21	0.17	0.16	0.15
	Mn	0.06	0.05	0.06	0.04	0.03	0.03
	Fe	0.00	0.00	0.00	0.00	0.00	0.00
	K	7.29	8.62	7.75	6.80	7.85	7.74
	Ca	1.50	1.20	1.31	0.23	0.22	0.21
	Mg	0.35	0.38	0.38	0.16	0.17	0.15

* Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate.

In their review, Hechelski et al. [47] explained that Professor Grison's team, who work on the conception and valorization of hyperaccumulating plants as ecocatalysts, highlighted synergistic effects of metals, enhancing the reactivity of catalysts. Based on this fact, mineral salts of Zn, Cu, Mn, Fe, Mg, Si and Al as Lewis acids may be of great interest. The sum of metal concentrations (945 mg kg^{-1} DW) was the highest in leaves of miscanthus grown on soils amended with DCP from fields. This concentration is very close to 1000 mg kg^{-1} DW, the minimal concentration of metals for the conception of ecocatalysts [49]. This result is of great interest in the north of France, since the stems of miscanthus, used in phytomanagement of contaminated soils, are harvested to produce energy after the leaf fall [20,22,32]. Currently, the main problems related to the contaminated leaves of miscanthus at the soil surface are the presence of bioavailable metals and the very low degradability rate of leaves due to the presence of silica and lignin. On the other hand, it was shown that after senescence, Cd and Pb $CaCl_2$-extractable concentrations increased through leaf decomposition [23]. Finally, it is worth mentioning the high average concentration of Ca in the leaves of miscanthus from microcosm and field experiments (up to 7300 mg kg^{-1} DW and 9000 mg kg^{-1}, respectively, depending on the replicates (n = 18) and thus their potential interest in organic synthesis. Indeed, it was shown that calcium-rich plants could also be used to produce ecocatalysts for aldol, C-glycosylation and chemoselective hydrolysis reactions [50].

3.5. Effects of Phosphates on the Biomass of Miscanthus and the Bioconcentration Factor

Significant differences between leaf and stem biomass from mesocosm and field experiments are reported in Figure 5. As described in many studies, after the second year, macronutrients are stocked with rhizomes after senescence, contributing to the development of plants [51–53]. Consequently, the length of miscanthus stems was significantly higher in field than in mesocosm experiments (Figure 6). In contrast, no causal relationship may be established between the diameter of stems and the age of the plantation. As reported in Figures 5 and 6, significant effects on leaf and stem biomass and on length and diameter of stems were highlighted between the mesocosm and field experiments. Although phosphates were added in small amounts to soils (from 0.022% to 0.026% *w*/*w*), the average biomass of leaves from fields increased with MCP and DCP as well as the average biomass of stems with MCP

(Figure 5). According to our results, the main factor that contributes to the increase of the stem biomass seems to be the length (Figure 6) and the diameter of stems from soil treated with MCP (Figure 6). Interestingly, differences between the biomass from the aerial parts of miscanthus from mesocosm and field experiments may be correlated to the organic phosphorus in soils. As shown in the previous section, organic phosphorus contents in soils from field were higher than those in mesocosm (Figure 4), and addition of inorganic phosphates under sustainable conditions increased the fraction of organic phosphorus. The contribution of organic phosphorus to plant nutrition was estimated to represent 30% to 50% of the total assimilated phosphorus in soils [54] and in some cases, up to 80% [55].

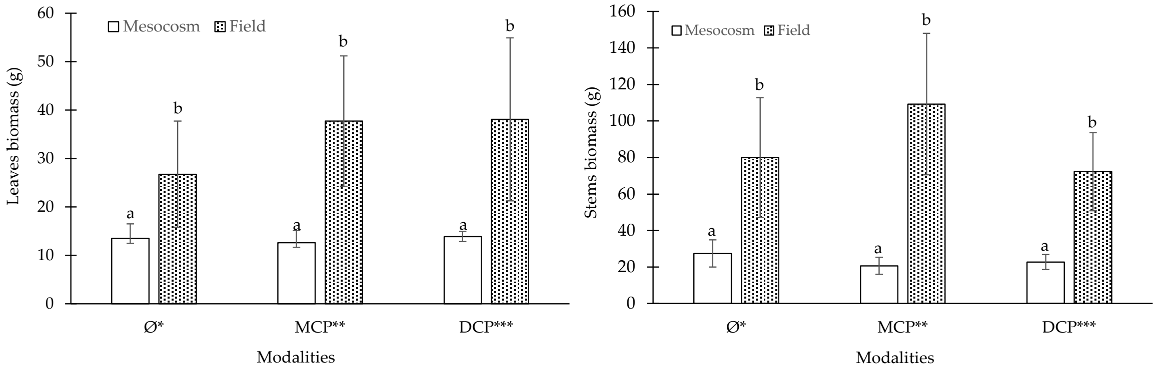

Figure 5. Biomass of leaves and stems of miscanthus from mesocosm (M; $n = 6$) and field (F; $n = 3$). * Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate. Letters (a) and (b) denote significant differences ($p < 0.05$).

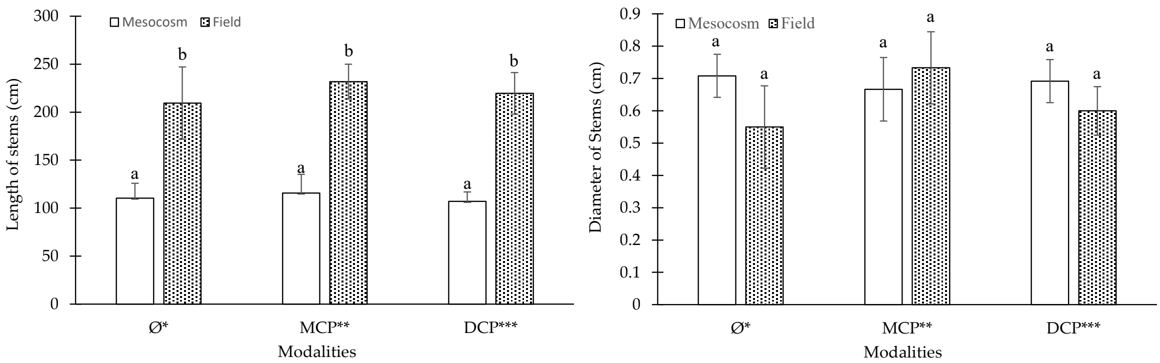

Figure 6. Length and diameter of miscanthus stems from mesocosm (M; $n = 6$) and field (F; $n = 3$). * Ø: without phosphate; ** MCP: monocalcium phosphate; *** DCP: dicalcium phosphate. Letters (a) and (b) denote significant differences ($p < 0.05$).

The average bioconcentration factors (BCFs) of Cd, Pb, Zn, Cu, Mn, Fe, K and Ca in the leaves and stems of miscanthus are summarized in Table 5. The BCFs allowed the accumulation of metals in the mesocosm experiment to be ranked as follows: K > Ca > Mg > Cu > Cd > Zn > Mn > Fe > Pb in leaves and K > Ca > Cd > Mg > Cu > Zn > Mn > Pb > Fe for each modality. Results from field experiments showed that these orders are: K > Ca > Mg > Cu > Cd > Mn > Zn > Fe > Pb in leaves and K > Ca > Mg > Cu > Cd > Zn = Mn > Fe = Pb in stems. These results correlated well with those of Nsanganwimana et al. for Cd, Pb and Zn [22]. On the other hand, the bioconcentration factors are generally less than 1 suggesting that metal transfer from the soil to miscanthus is limited. This result confirms that *Miscanthus* × *giganteus* is an excluder species suitable for phytostabilization [15,42]. However, it is worth mentioning that K-BCF and Ca-BCF in leaves were greater than 1. As shown in Table 5, DCP enhanced the transfer of Ca from the soil to the leaves of miscanthus and positively influenced the biomass of leaves. Consequently, potential utilization of leaves of miscanthus to catalyze organic reactions could be investigated in the future.

4. Conclusions

Sustainable amounts of P-fertilizers (MCP and DCP) were added to soil to evaluate their effects on (i) the accumulation of metals and nutrients in leaves and stems of miscanthus, (ii) the biomass of the aerial parts (leaves and stems) and (iii) the length and the diameter of stems. Organic phosphorus increased overtime, and biomass production was favored in presence of P-fertilizers. Significant effects of phosphates were registered on LMWOA-extractable concentrations of Zn, Fe and K and led to an increase of the sum metal concentrations (Zn, Cu, Mn, Fe, Mg, Si and Al) in stems of miscanthus making them suitable in organic synthesis. Otherwise, very high concentrations of Ca were measured in the leaves of miscanthus, which can be used as a renewable resource of this essential element in organic synthesis or in other applications.

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