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Influence of metal contamination in soil on metabolic profiles of *Miscanthus* x *giganteus* belowground parts and associated bacterial communities

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ABSTRACT

Miscanthus x *giganteus* is well known for its ability to grow on metal contaminated soils. However, little is known concerning its metabolic changes including secondary metabolites under metal pressure. These changes might impact the diversity and function of associated bacterial populations. Thus, this study focused on evaluating the modifications of secondary metabolism production of *M.* x *giganteus* belowground parts (i.e. roots and rhizomes), and of rhizosphere bacterial communities under diverse contaminated conditions. Samples of *M.* x *giganteus* roots and rhizomes were collected from 3 sites exhibiting a gradient of metal pollution and extracted with MeOH:H_2O. Secondary metabolic profiles of root and rhizome extracts were analyzed by UHPLC/DAD/ESI-QTOF. The structure and diversity of rhizosphere communities were studied using high-throughput sequencing. The results showed out the modification of the secondary metabolic profiles of *M.* x *giganteus* belowground parts, when they are grown on diversely contaminated soils. Major increased metabolites were identified as 3- and 5-feruloylquinic acid whereas decreased compound was 4-feruloylquinic acid. Metal contamination also led to a shift in rhizosphere bacterial composition and structure as well as the selection of some opportunistic pathogenic genera such as *Pseudomonas* or *Stenotrophomonas* but there was only a weak effect on the bacterial diversity and richness. In the context of a moderate metal contamination in agricultural soil slight changes were seen in the secondary metabolic profiles of *M.* x *giganteus* roots and rhizomes and their associated bacterial communities. Whether the metal-induced changes allow plants to recruit beneficial microbes that favor the adaptation process to this stress need to be further investigated.

1. Introduction

Trace elements as metals and metalloids occur naturally in rocks and soils. At low concentration some are essential for living organisms as micronutrients whereas others like Hg, Cd, and Pb have no known biological importance. However, elevated environmental concentrations are frequently observed as a result of contamination. Anthropogenic mine production, industrial emission, and agricultural practices (such as recycled wastewater and fertilizer uses) are of great concern worldwide as they contribute to trace element dispersion and environmental contamination (Su et al., 2014). In terrestrial ecosystems, as the inputs of metals to soil can be much larger than the outputs, accumulation is occurring and associated to potential loss of property values. However, sustainable agriculture implies that the productivity of soil and the quality of products are being protected. Because of the increasing energetic needs, land bio-energy production *via* cropping of high biomass plants is an alternative way to develop and reclaim agricultural contaminated areas. Such plants are most often perennial

Abbreviations: PAH, polycyclic aromatic hydrocarbon; PGPR, plant growth promoting rhizobacteria; Hg, mercury; Pb, lead; Cd, cadmium; Zn, zinc; Cu, copper; DTPA, diethylenetriaminepentaacetic acid; UHPLC-DAD-ESI/QTOF, ultrahigh performance liquid chromatography with diode array detection coupled to electrospray ionization and quadrupole time-of-flight; UV, ultraviolet; ESI, electrospray ionization; MSMS, tandem mass spectrometry; ESI/MS^2, electrospray ionization tandem mass spectrometry; HRMS, high resolution mass spectrometry; RT, retention time; V, non-contaminated rhizospheric *Miscanthus* x *giganteus* soil from Versailles; D, slightly contaminated rhizospheric *Miscanthus* x *giganteus* soil from Dourges; A21, contaminated rhizosphere *Miscanthus* x *giganteus* soil from Courcelles Lès Lens; HSD, Honest significant difference; PCA, Principal Component Analysis; ANOVA, analysis of variance; OTU, operational taxonomic unit; NMDS, non-multidimensional scaling; ANOSIM, analysis of similarities

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crops. Among them is *Miscanthus* x *giganteus*, a naturally occurring sterile hybrid of *M. sinensis* and *M. sacchariflorus* which has recently raised interest as a biomass crop for non-food utilization due to its excellent productivity, rapid growth and high resistance to disease (Villaverde et al., 2010). In the last 10 years, many works have been published on *Miscanthus* species with a particular consideration for its ability to resist to abiotic stresses i.e. chilling, drought (Purdy et al., 2013) and its biomass production potentiality (Clifton-Brown et al., 2017). Hodgson et al. (2011) described significant genotypic variation in cell wall chemical composition between species and genotypes showing that lignin and cellulose concentration increase with the harvest time while hemicellulose concentration decreases. Recent transcriptomic and proteomic analysis confirmed these observations and showed a decrease in secondary metabolism suggesting more energy available for primary assimilation and biomass production (Straub et al., 2013). One study dealing with exudates composition and its contribution to the biostimulation of PAH degradation, showed the presence of flavonoids (apigenin, isovitexin, catechin) and other polyphenolic compounds e.g. chlorogenic acid and caffeic acid as main secondary metabolites (Técher et al., 2011).

The tolerance of *Miscanthus* spp. to soils contaminated with metals was demonstrated. Among them *M.* x *giganteus* was considered as an interesting candidate for Pb and Zn phytoextraction, favored by the metal accumulation observed and the high biomass produced (Barbosa et al., 2015; Pogrzeba et al., 2013). Regarding accumulation and translocation processes, *Miscanthus* was shown to be able to remove and accumulate metals, but the higher proportion remains in rhizomes and roots (Pidlisnyuk et al., 2014; Wanat et al., 2013).

Tolerance to abiotic stress in plants can be enhanced by plant growth promoting rhizobacteria (PGPR). An endophytic bacterium, *Pseudomonas koreensis* AGB-1, isolated from *M. sinensis* roots was metal-tolerant and exhibited plant growth promoting traits (Babu et al., 2015). Similarly, various nitrogen fixing bacteria were found associated to *Miscanthus* spp. enabling its colonization of areas with low nitrogen contents (Eckert et al., 2001; Kirchhof et al., 2001; Miyamoto et al., 2004). Although few studies investigated the impact of metal contamination on plant metabolism, plants are known to modify their production of secondary metabolites to adapt under metallic stress (Singh et al., 2016; Michalet et al., 2017). Recently, Thijs and coworkers hypothesized that pollution-induced changes in root exudation, might select for microbial populations bearing beneficial traits for plants (e.g. degradative capacities), allowing a better adaptation under contaminant stress (Thijs et al., 2016). It is actually well known that plants, *via* their root exudates, can shape the structure and function of rhizosphere microbial community (Hartmann et al., 2008; Michalet et al., 2013).

Thus the present study focuses on evaluating the effect of soil metal contamination (i) on secondary metabolism of plants by comparing the secondary metabolite profiles of roots and rhizomes of *M.* x *giganteus* grown on contaminated or not contaminated soils, in order to evaluate the effect of metal pollution on secondary metabolism of plants, and (ii) on the structure of *M.* x *giganteus* rhizosphere bacterial communities. Metabolic profiling of roots and rhizomes of *M.* x *giganteus* was based on UV-absorbing compounds, thus targeting more specifically phenolics and other aromatic compounds. Shifts in bacterial community structure were evaluated using a metabarcoding approach.

2. Materials and methods

2.1. Site, plant and soil sampling

M. x *giganteus* plant and soil samples were collected in March 2013 both from a non-contaminated site and two contaminated sites chosen in order to exhibit a gradient of metal pollution. The contaminated sites are located in the North of France surrounding the former smelter Metaleurop, a lead and zinc production plant that has been working for the whole 20th century until 2003. Surface horizons of the agricultural soils around this source of dust emission were contaminated mainly by Pb, Zn and Cd (Douay et al., 2008; Sterckeman et al., 2006), and following the wind direction it is possible to find soils exhibiting a gradient of metal pollution in a small area around the former smelter. We thus chose one site located near the town of Courcelles Lès Lens (called thereafter A21; geographical coordinates N: 50°25′5.67″; E: 3°1′7.06″). This site is heavily contaminated by Cd, Pb and Zn, respectively 38, 15 and 8 times the value of the local pedo-geochemical background as determined by Sterckeman et al. (2006). The second site is located nearby, in the town of Dourges (called thereafter D, geographical coordinates N: 50°26′16.19″; E: 2°59′5.92″) with a metal content only 2 to 3 times the geochemical background in Cd, Pb and Zn. (Table 1). Because of extended atmospheric contamination, it was not possible to find a non-contaminated site with the same type of soil nearby, so that the non-contaminated taken for control samples was from an experimental site in Versailles (called thereafter V, geographical coordinates N: 48°48′23.12″; E: 2°3′56.52″). The three sites located in the northern part of France benefit from an oceanic climate with mean rainfall around 660 mm and a mean annual temperature between 11 °C and 12.5 °C. *M.* x *giganteus* was planted either in 2007 (contaminated sites) or in 2009 (non-contaminated site) so that plants were between 4 and 6 years old at the time of sampling. From each site, five individuals of *M.* x *giganteus* were selected and roots and rhizomes of each collected were further considered as replicates. The plants were collected at the same period of the vegetation cycle (i.e. end of vegetation).

Rhizosphere soil samples from each of the five plants at each site were collected and stored frozen at −40 °C for further bacterial metabarcoding analysis.

At each site soil was collected from the upper layer (0–15 cm) for physico-chemical characteristic measurement performed at the Laboratory of soil analysis (INRA Arras, France) using standard methods. Furthermore, the pool of metals extracted by DTPA was determined according to Lindsay and Norvell (1978) procedure, and used to mimic soil metal bioavailability for plants instead of total soil metal content. Soil characteristics in Table 1 show that the three soils exhibit the same texture and mostly differed on their metal, organic matter and carbonate contents. Metal extractability with DTPA used here to mimic the availability of soil trace elements to plants confirms the metal gradient from the non-contaminated V site, to the most contaminated A21 one.

Table 1
Physical and chemical characteristics of the different soil samples.

Properties	Courcelles Lès Lens (A21)	Dourges (D)	Versailles (V)
Clay (%)	23	25	19
Fine silt (%)	18	28	22
Coarse silt (%)	32	42	35
Fine sand (%)	24	4	21
Coarse sand (%)	3	1	3
$CaCO_3$ ($g.kg^{-1}$)	3.5	44	< 1
P_2O_5 ($g.kg^{-1}$)	1.6	1.8	1.5
Organic Carbon (C) ($g.kg^{-1}$)	16.7	25.5	9.7
Total Nitrogen (N) ($g.kg^{-1}$)	1.3	1.7	0.9
C/N	12.5	14.5	10.9
pH	7.8	8.3	6.5
Zinc (Zn) ($mg.kg^{-1}$)	320	127	71
Lead (Pb) ($mg.kg^{-1}$)	211	62	47
Cadmium (Cd) ($mg.kg^{-1}$)	4.1	1.1	0.2
Extractable DTPA-Zn (mg/kg)	39.80	10.25	7.04
Extractable DTPA-Pb (mg/kg)	63.88	19.05	6.49
Extractable DTPA-Cd (mg/kg)	2.00	0.58	0.11