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Fly ash-aided phytostabilisation of highly trace element polluted topsoils improves the telluric fungal biomass: A long-term field experiment

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ABSTRACT

Ten years after fly ash (FA) amendments and tree mix plantation (black locust, black alder, sycamore maple, pedunculate oak and white willow), the viability of the telluric microorganisms in a highly trace element (TE) polluted topsoil was studied. Previous to tree plantation, three experimental plots were set up in the field: a non-amended plot (R), an amended plot with silico-aluminous fly ash (F1) and an amended plot with sulfo-calcic fly ash (F2). The arbuscular mycorrhizal fungi (AMF), saprophytic fungal and bacterial biomasses were quantified by the measure of specific lipid markers (phospholipid fatty acids (PLFA) and ergosterol). The highest AMF root colonization was of 18% in the sub-plot of the plot (F1). The highest PLFA C16:1ω5 amount (2 nmol g^{-1} soil), reported as a marker of the AMF biomass, was recorded in the fly ash amended topsoil compared to the control (R). This result was in accordance with the highest number of AMF spores isolated from the sub-plot of the plot F1. Saprophytic and ectomycorrhizal fungal biomasses were estimated by measuring the PLFA C18:2ω6,9 and ergosterol amounts in the topsoil. Similarly to the PLFA C16:1ω5 amounts, the highest PLFA C18:2ω6,9 amounts (4.7 nmol g^{-1} soil) were observed in the fly ash amended topsoil compared to the control. However, no significant effect of fly ash amendments was observed on both Gram-positive and Gram-negative specific PLFA. This study demonstrated the usefulness of FA amendments in the assisted phytostabilisation of TE polluted topsoil through the enhancement of fungal population viability.

1. Introduction

Human activities (agriculture, coal combustion, industrial, and mining) are responsible for a significant accumulation of trace elements (TE) in the environment (Nriagu, 1996; Rai, 2009). These elements can follow different tracks: mobilization and accumulation in the soil carry away by running water during soil leaching, and absorption by plants and animals (Austruy et al., 2013). In soil, high TE concentrations cause a decrease in telluric microorganism number, their diversity and their biological activities (Khan et al., 2010; Wang et al., 2007). Since changes in microbial populations and/or metabolic activities can be detected even before changes in soil physico-chemical parameters, they may provide an early indicator of soil degradation or improvement (Pankhurst et al., 1995). Soil microbes, especially mycorrhizal and saprophytic fungi, beneficial bacteria such as Rhizobium and plant growth promoting rhizobacteria (PGPR), play a fundamental role in the soil activities (Miransari and Mackenzie, 2011). Arbuscular mycorrhizal fungi (AMF) promote plant growth, facilitate nutrient uptake and improve plant tolerance to adverse conditions (Kaya et al., 2003; Labidi et al., 2012). AMF can immobilize TE by secreting compounds such as glomalin which binds TE in the soil (Gonzalez-Chavez et al., 2004). Moreover, AMF can also use other

Abreviations: AMF, arbuscular mycorrhizal fungi; TE, trace elements; FA, fly ash; R, non-amended plot (reference); F1, plot amended with the silico-aluminous fly ash; F2, plot amended with the sulfo-calcic fly ash.

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mechanisms to limit the transfer of TE to their host plants such as precipitation in polyphosphate granules in the soil, adsorption to fungal cell walls, and chelation of metals inside the fungus (Gaur and Adholeya, 2004). On the other hand, saprophytic fungi (decomposers) have a fundamental function in soil and plant litter nutrient cycling in terrestrial ecosystems (Hättenschwiler et al., 2005). Concerning soil bacteria, especially PGPR and Rhizobium, they enhanced plant growth and crop production due to (1) their ability to alleviate the adverse effects of soil stresses on plants (Jalili et al., 2009), (2) the secretion of phytohormones (Bianco and Defez, 2009) and (3) the solubilization of minerals in the soil (Zabihi et al., 2010).

Amongst the methods used for TE-contaminated soil management, phytoremediation is attractive. This green technology is a cost-effective strategy, not destructive for the soil structure, and applicable on large areas (Ahmadpour et al., 2012). In the case of phytostabilisation, a green cover using TE-tolerant plants is established with the aim to stabilize and reduce the bioavailability of the soil contaminants (Jadia and Fulekar, 2009). Such a vegetation cover minimizes wind dispersion of TE and water migration through the soil due to evaporation (Mench et al., 2010). The phytostabilisation process could be improved by the application of coal fly ashes which decreased the availability of TE in acidic contaminated soils (Gu et al., 2013). Fly ashes are amorphous mixtures of ferroalumino-silicates generated from the combustion of coal at 400–1500 °C (Mattigod et al., 1990). Their incorporation into the top soil can improve chemical, physical and biological properties of soils (Kohli and Goyal, 2010; Ukwattage et al., 2013). They also enhanced the growth of plants (*Solanum melongena* and Manilagrass) cultivated on highly TE contaminated soils (Gond et al., 2013; Xu et al., 2012). However, fly ashes can also contain toxic organic and inorganic compounds which present a limitation for their use as an amendment for soils (Ram and Masto, 2010). Therefore, it is important to test their ecological effects before recommendation.

To the best of our knowledge, few works studied the effect of the long term application of fly ash amendments on the viability of telluric fungal biomass, especially symbiotic groups (AMF and ectomycorrhizal fungi) in TE polluted soils. To assess soil fertility and to monitor the ecological impacts of environmental pollution, several biomarkers are used to quantify the soil microbial biomass. Membrane lipids such as specific phospholipid fatty acids (PLFA) or ergosterol are commonly used to estimate living microbial biomass. AMF are characterized by the presence of 16:1ω5 fatty acid associated to the membrane phospholipids (PLFA) and to neutral lipids (NLFA) (Johansen et al., 1996). Because the PLFA C16:1ω5 is a component of the membrane phospholipids and the NLFA C16:1ω5 is an energy lipid, they are often used to quantify the AMF mycelium and spores biomass, respectively (Olsson et al., 1995). Since 18:2ω6,9 is not a constituent of bacteria (Lechevalier and Lechevalier, 1988) and since it is the dominant fatty acid in most fungi (except AMF), it could be used as a biomass indicator of fungi (Tunlid and White, 1992). Ergosterol, another compound of fungal cell membranes (Weete, 1980), can be used as a fungal biomass indicator (Barajas-Aceves et al., 2002). The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0, C18:1ω7 and cy19:0 were used as an indicator of bacterial biomass in the soil (Frostegård et al., 1996).

The aim of the current study is to examine the long-term effects of two coal fly ash amendments, added ten years ago before tree plantation, on the viability of the telluric microorganisms including: arbuscular mycorrhizal, ectomycorrhizal and saprophytic fungi as well as bacteria in TE (mainly by Pb, Cd and Zn) polluted top soil. The telluric microbial biomass for each of the microorganism group is quantified through the specific lipid marker measurements both in amended and non-amended soils.

2. Experimental

2.1. Experimental site

The experimental site (50°26′N, 3°01′E) with an area of 10,000 m^2 is located at Evin-Malmaison (North of France), on a former agricultural field, 600 m north and downwind of the former smelter Metaleurop Nord. This smelter was one of the major Pb plants in Europe for around 100 years producing significant amounts of dust which lead to an important contamination of the surrounding soils (Douay et al., 2009). Physico-chemical parameters are presented in Table 1. High concentrations of Pb (900–967 mg kg^{-1}), Cd (16.6–18 mg kg^{-1}) and Zn (1211–1127 mg kg^{-1}) were recorded in the experimental topsoil (Lopareva-Pohu et al., 2011). Pb, Cd and Zn concentrations are 20–50 folds higher than regional background values (Sterckeman et al., 2002).

2.2. Amendments and tree plantation

In spring 2000, the site was divided into three plots about 3000 m^2 each. The first one was not amended and was considered as a reference plot (R). The two other plots were amended with two coal fly ashes provided by Surschiste Ltd. (Mazingarbe, France). Plot F1 was amended with FA1, a silico-aluminous FA Sodeline®, produced from combustion of bituminous coal (Carling thermal power plant, France). The second plot (F2) was amended with FA2, a sulfo-calcic FA Soproline®, produced from the combustion of bituminous from lignite (Gardanne thermal power plant, France). The plots were amended at a rate of 23.3 kg m^{-2}, and then ploughed up to a soil depth of 25 cm. This rate of application was chosen based on the results of a previous study (unpublished results). In winter 2000, the three experimental plots were planted with a tree mix: black locust (*Robinia pseudoacacia* L.), black alder (*Alnus glutinosa* L.), sycamore maple (*Acer pseudoplatanus* L.), pedunculate oak (*Quercus robur* L.), and white willow (*Salix alba* L.) (Lopareva-Pohu et al., 2011).

2.3. Soil and root sampling

Ten years after the start of phytostabilisation experiment, soil samples were taken from a sub-plot (15 m × 12 m) for each experimental plot: R, F1 and F2. Soil was sampled with a gouge in 0–25 cm and 25–50 cm soil depths. For each soil depth, seven soil samples were taken from the sub-plot, in each experimental plot. For root sampling, a pit was dug in each sub-plot, near the

Table 1
Physico-chemical parameters of the soil layer 0–25 cm in the TE polluted plots (Lopareva-Pohu et al., 2011).

		R	F1	F2
pH water		$7.31^{b} \pm 0.20$	$7.95^{a} \pm 0.03$	$7.74^{ab} \pm 0.02$
CEC	$Cmol^{+}\ kg^{-1}$	$14.5^{a} \pm 0.5$	$13.6^{a} \pm 0.2$	$14.6^{a} \pm 0.4$
Clay	$g\ kg^{-1}$	215 ± 5^{a}	196 ± 18^{b}	129 ± 10^{c}
Fine silt	$g\ kg^{-1}$	196 ± 5^{b}	253 ± 27^{a}	267 ± 7^{a}
Coarse silt	$g\ kg^{-1}$	356 ± 14^{a}	328 ± 7^{b}	348 ± 24^{ab}
Fine sand	$g\ kg^{-1}$	202 ± 14^{a}	195 ± 10^{a}	200 ± 17^{a}
Coarse sand	$g\ kg^{-1}$	32 ± 2^{a}	29 ± 5^{a}	56 ± 33^{a}
Pb	$mg\ kg^{-1}$	$926^{a} \pm 29$	$967^{a} \pm 16$	$900^{a} \pm 30$
Cd	$mg\ kg^{-1}$	$16.6^{a} \pm 0.6$	$18.0^{a} \pm 0.3$	$17.8^{a} \pm 0.5$
Zn	$mg\ kg^{-1}$	$1135^{a} \pm 42$	$1211^{a} \pm 26$	$1127^{a} \pm 43$
Corg	$g\ kg^{-1}$	$23.8^{c} \pm 0.7$	$25.9^{b} \pm 0.6$	$29.8^{a} \pm 1.1$
Ntot	$g\ kg^{-1}$	$1.76^{a} \pm 0.06$	$1.68^{a} \pm 0.04$	$1.70^{a} \pm 0.06$
C/N		$13.5^{c} \pm 0.1$	$15.5^{b} \pm 0.1$	$17.5^{a} \pm 0.3$
P_2O_5	$g\ kg^{-1}$	$0.20^{ab} \pm 0.02$	$0.18^{b} \pm 0.01$	$0.24^{a} \pm 0.03$
$CaCO_3$ total	$g\ kg^{-1}$	$1.5^{c} \pm 0.8$	$5.8^{b} \pm 0.9$	$71.6^{a} \pm 6.5$

Data are means ± SE; a, b and c denote significant differences between plots, analysis was carried out separately for each year (Kruskal–Wallis test, $p < 0.05$, $n = 3$).