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Functional activity and functional gene diversity of a Cu-contaminated soil remediated by aided phytostabilization using compost, dolomitic limestone and a mixed tree stand☆

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ABSTRACT

Trace elements (TEs) availability, biochemical activity and functional gene diversity was studied in a Cu-contaminated soil, revegetated for six years with a mixed stand of willow, black poplar, and false indigo-bush, and amended or not with compost plus dolomitic limestone (OMDL). The OMDL amendment significantly reduced Cu and As availability and soil toxicity, and increased the biochemical activity and microbial functional diversity assessed with the GEOCHIP technique, as compared to the unamended soil (Unt). The OMDL soil showed significantly higher abundance of 25 functional genes involved in decomposition organic compounds, and 11, 3 and 11 functional genes involved in the N, P and S biogeochemical cycles. Functional gene abundance was positively correlated with nutrient contents but negatively correlated with Cu availability and soil toxicity. The abundance of microbial functional genes encoding for resistance to various TEs also increased, possibly due to the microbial proliferation and lower Cu exposure in the presence of high total soil Cu concentration. Genes encoding for antibiotic resistance due to the co-occurrence of TEs and antibiotic resistant genes on genetic mobile elements. Overall, phytomanagement confirmed its potential to restore the biological fertility and diversity of a severely Cu-contaminated soil, but the increase of TEs and antibiotic resistant gene abundances deserve attention in future studies.

1. Introduction

Copper (Cu) is naturally present in all soils with a median concentration of 31.1 mg kg^{-1} (Heijerick et al., 2006; Tóth et al., 2016), but its concentrations in soils under conventional agriculture, vineyards, orchards, or surrounding mining areas, smelters and wood preservation sites can build up to very high concentrations due to anthropic activities, either producing or using Cu-based compounds and materials (Komárek et al., 2010; Mench and Bes, 2009; Ettler, 2016), and Cu sorption by clay minerals and the soil organic matter (SOM) (Quenea et al., 2009; Lagomarsino et al., 2011). Copper is a micronutrient with important physiological activities in all living microorganisms, but Cu excess in soil impacts the soil microbial communities (Sandaa et al., 1999; Lejon et al., 2008) and soil respiration, soil microbial biomass and enzyme activities (Kumpiene et al., 2009). Remediation of TE contaminated soils (TECS) can be carried out by civil engineering technologies such as “dig and dump” operations, thermal stabilization or soil washing, which rapidly reduce the pollutant linkages associated to TEs excess, but are expensive and cause the irreversible loss of soil properties and functions underlying beneficial ecosystem services (Khalid et al., 2017). Phytomanagement is a TECs remediation option carried out by using plants microorganisms and soil organic and inorganic amendments, capable of reducing the TEs bioavailability in soil and improving soil physico-chemical properties and nutrient status, and enhance the soil ecological functions (Raskin et al., 1997). Phytomanagement is effective in reducing soil

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phytotoxicity while preserving the soil resource (Quintela-Sabarís et al., 2017), and can produce income for the local communities (Mench et al., 2010; Ruttens et al., 2011; Van Slycken et al., 2013; Witters et al., 2012), especially when managed as short rotation coppice of biomass producing trees (Van Slycken et al., 2013).

Trace element contamination generally reduce soil microbial biomass and microbial diversity, due to negative selection of TE-sensitive microorganisms (Tyler et al., 1989; D'Ascoli et al., 2006; Azarbad et al., 2016). Moreover, TECS display reduced metabolic processes due to the dominance of less metabolically efficient TE-resistant microorganisms (Mergeay, 2000), or to the direct TE-induced inhibition of soil enzyme activity (Renella et al., 2006). Soils host highly diverse microbial communities involved in the biogeochemical cycles of nutrient in terrestrial ecosystems and for plant nutrition (Torsvik and Øvreås, 2002). Microbial diversity also confer stability and functional resilience to soil microbial activity and ecological functions (Girvan et al., 2005), and changes in the composition assessed by using DNA sequencing technologies of the soil microbial communities can indicate the impact of TEs bioavailability (Daniel, 2005; Singh et al., 2009). However, the assessment of changes in the microbial community composition, is not informative on the potential functional capacity of soils, nor directly related to the soil biochemical activity. The GeoChip technology allows the comparison of functional gene diversity and functional microbial groups across soils, and has been successfully used for studying the functional diversity of agricultural and forest soils under various management and vegetation cover, different climatic regimes and remediated TECS (Epelde et al., 2006; Xue et al., 2013, 2015). While the increase of microbial biomass and enzyme activity in TECS under phytoremediation has been reported (Ascher et al., 2009; Renella et al., 2008), the use of soil chemical and biochemical methods make impossible to understand whether the recovery of soil functionality is contributed by a higher functional gene diversity, preventing long term predictions of phytomanagement on soil quality.

We hypothesized that reduction of Cu solubility, along with the increased nutrient availability in TECS under aided phytostabilization, could increase not only microbial biomass and biochemical activity, but could also increase the functional gene diversity of soil microbial communities evaluated by the GEOCHIP (He et al., 2007). The increase of functional gene diversity could be used as an indication of the potentials of the aided phytostabilization technique to enhance microbial-driven soil ecosystem services. Because TE impacts on the soil microbial community and functionality are related to their availability, we also hypothesized that the long term effective Cu stabilization could reduce the proportion of metal resistant microorganisms within the soil microbial communities. We tested our hypotheses using the GeoChip technology to study the functional gene diversity in a Cu-contaminated soil after 6 years of aided-phytostabilization using soil amendments and a mixed tree stand. The functional gene diversity was compared with the mobile Cu concentrations evaluated by soil chemical extractions and soil toxicity.

2. Materials and methods

2.1. Site characteristics and soil sampling

Soil samples were collected at an industrial area (6 ha) located in Gironde (SW France, 44°43′N; 0°30′W) used to preserve and store timbers, posts, and utility poles for over a century (Mench and Bes, 2009; Bes et al., 2010). Soil Cu contamination resulted mainly from washing of treated wood. Total topsoil Cu varied from 65 to 2600 mg kg^{-1} on the whole site (Mench and Bes, 2009). Mean value for total topsoil Cu (0–25 cm) at the studied field trial was 1001 ± 279 mg Cu kg^{-1} and values did not significantly differ across the plots. The contaminated soil was of alluvial origin classified as Fluvisol - Eutric Gleysol (WRB, 2006), with a sandy texture and neutral pH value. The aided phytostabilization field trial started in May 2006 at site P1-3, consisting of $1\,m \times 3\,m$ plots. The trial comprised the following four treatments: untreated (Unt), 0.2% w/w dolomitic limestone (DL), containing 30% CaO and 20% MgO combined with carbonates, fineness index < 80%, 0.16 mm, neutralizing power 58 (Prodical Carmeuse, Orthez, France), 5% w/w compost (OM) derived from poultry manure and pine bark chips (ORISOL, Cestas, France), and DL plus OM (OMDL) at the same rates as for the single treatments. Amendments were incorporated into the soil to a depth of 25 cm and the four treatments were randomly replicated in four blocks. the scheme of the treatments and a representative image of the site after 1 and 6 years of experiments are shown in supplementary materials (Figs. S-1, S2). The present study was conducted on soils under the Unt and OMDL treatments 6 years after the soil amendment because the latter proved to be the most successful in increasing the soil microbial biomass, biochemical activity and microbial diversity after two years of treatment (Šimek et al., 2017). The Unt and OMDL soils had a pH value of 7.16 ± 0.12 and 7.33 ± 0.12, respectively, and all plots were managed as short-rotation coppice (SRC) with a mixed stand of willows (*Salix viminalis* L.; *Salix caprea* L.), poplar (*Populus nigra* L.) and false indigo bush (*Amorpha fruticosa* L.). Soil samples made of three 1 kg subsamples per plot were collected from the 0–25 cm soil layer from all plots using a stainless steel spade and kept as independent replicates. Soil samples were immediately transported to the analytical laboratory, sieved (<2 mm) and pre-incubated at 25 °C for 1 week at 50% water holding capacity prior to biochemical determinations whereas soil samples for GEOCHIP analysis were stored at −80 °C prior to DNA extraction. Aliquots of each soil were air dried for chemical analyses.

2.2. Soil chemical and biochemical properties

The method of Walkley and Black (1934) was used to determine the total organic C (TOC) was used, whereas total N was determined using a Perkin Elmer 2400 Series II CHN Elemental Analyzer. Inorganic N (NH_4^+-N and NO_3^--N) was determined by extracting 5 g d.w. soil for 1 h with 1 M KCl (1:5 soil:solution ratio) according to Keeney and Nelson (1982), and analyzed by ion selective electrodes. The available P was measured according to Olsen and Sommers (1982) protocol.

The pseudototal concentrations of As, Cd, Cr, Cu, Mn, Ni, Pb, Zn were measured by microwave-assisted (Milestone 900) acid digestion in a 1:5 HF:HNO_3 solution at 600 W for 24 min using 0.25 g of dry soil. The residue was brought to a final volume of 25 mL with 0.15 M HCl prior to elemental quantification. The extractable TE fraction was determined by soil extractions with 0.05 M ethylenediaminetetraacetic (EDTA) according to Quevauviller (1998), using 10 g of air-dried sieved suspended in 100 mL of EDTA tetrammonium salt at pH 7.00, end-over-end shaken at 120 oscillations per min for 2 h at room temperature. The soil suspensions were filtered through Whatman 42 filter paper and immediately analysed. The exchangeable Cu fraction was determined by soil extractions with 1 M NH_4NO_3 (Pruess, 1998), with the modification that the 1 M NH_4NO_3 solution was buffered at pH 7.00 with concentrated NH_3 (Renella et al., 2004). Twenty grams of soil were suspended in 50 mL of 1 M NH_4NO_3 in polythene bottles end over end shaken at room temperature for 2 h at 20 oscillations per min. The soil suspensions were then filtered through Whatman 42 filter paper, and the extracts were acidified with 0.2 mL HNO_3 prior to elemental analysis. Elemental quantification was conducted by inductively coupled plasma optical