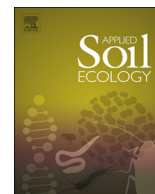




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Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Shift of soil bacterial community and decrease of metals bioavailability after immobilization of a multi-metal contaminated acidic soil by inorganic-organic mixed amendments: A field study

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ARTICLE INFO

Keywords:

Metal bioavailability
In-situ immobilization
 Illumina high-throughput sequencing technology
 Soil bacterial community structure

ABSTRACT

A field study was conducted to evaluate the effects of inorganic-organic mixed amendments on metals bioavailability and soil bacterial community structure by Illumina high-throughput sequencing technology. One year after the application of mixed amendments, the pH of the amended soils were 1.99–3.32 unit higher than the non-remediated soil, and the extractable Cd, Pb, Cu and Zn concentrations reduced by an average of 94.54%, respectively. The application of mixed amendments also remarkably improved lettuce (*Lactuca sativa* L.) growth and inhibited Cd, Pb, Cu and Zn uptake and accumulation in lettuce. The root and shoot Cd, Pb, Cu and Zn decreased by an average of 57.26% and 77.44%, respectively, compared with the control group. In addition, soil respiration and the alpha-diversity of soil bacterial community of the amended soils were significantly increased. With the application of amendments, the relative abundances of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* decreased, while *Acidobacteria* and the unclassified bacteria increased. RDA and regression analyses revealed that soil pH, available P and N, and soil extractable Cd, Pb, Cu and Zn concentrations, which were closely related to the application of mixed amendments, were significantly associated with the shift of bacterial community structure. These results prove that the application of mixed amendments not only decreases the bioavailability of heavy metals, but also simultaneously improves soil respiration and soil bacterial diversity. It suggests that a recovery of soil quality seems attainable by application of organic-inorganic mixed amendments.

1. Introduction

Agricultural soils around metal mines are often seriously contaminated by heavy metals due to the dry-wet deposition of mining dust and irrigation water polluted by acid mine wastewater (Lin et al., 2005; Zhou et al., 2014a; Qu et al., 2016). High concentration of heavy metals in soil can inhibit the growth of plants and threaten human health through dietary of agricultural crops (Li et al., 2014; Lei et al., 2015, 2016). To ensure the yield and quality of crops, sustainable and effective technologies are urgently needed to remediate and manage agricultural soils contaminated with metals. Many physical-, chemical-, and biological-based remediation technologies have been developed and applied to heavy metal contaminated agricultural soils (Wuana and Okieimen, 2011; Bolan et al., 2014). *In situ* immobilization of metals by application of organic and inorganic amendments, such as animal manure, biochar, lime, zeolite and phosphates compounds, is an attractive and promising soil remediation technique because of its simplicity, effectiveness and economic feasibility (Bolan et al., 2003;

Mahabadi et al., 2007; Gupta et al., 2014; Pardo et al., 2014; Sun et al., 2015). Studies have proved that limes (Lee et al., 2009; Cui et al., 2014), zeolite (Mahabadi et al., 2007), phosphate rock and calcium magnesium phosphate (Cao et al., 2004; Li et al., 2008), and pig manure (Zanuzzi et al., 2013) could effectively increase soil pH values and greatly reduce the bioavailability of heavy metals in contaminated soils. However, a single amendment may not be efficient for remediating multi-metal contaminated soils, because some single amendment may be effective in immobilizing one metal while ineffective for another, or even increase the mobility of other metals (Brown et al., 2004; Houben et al., 2012; Zhou et al., 2014b). Thus, application of mixed amendments may be more suitable for remediating multi-metal polluted soils than single amendment (Ruttens et al., 2006; Zhou et al., 2017).

The diversity and activity of soil microbial communities are important and sensitive indexes for monitoring and evaluating soil quality, as microorganisms play an important role in regulating the biogeochemical cycles of nutrients and affecting the growth and health

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<https://doi.org/10.1016/j.apsoil.2018.05.014>

Received 4 January 2018; Received in revised form 17 May 2018; Accepted 19 May 2018
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of plants (van der Heijden et al., 2008). Generally, soil heavy metal pollution can significantly change the microbial structure and composition, and result in decrease of soil microbial diversity and activity (Bååth, 1989; Brookes, 1995; Giller et al., 1998; Pan and Yu, 2011; de Boer et al., 2012). Actually, the impact of heavy metals pollution on soil microorganisms depends more on their availability than their total concentration (Giller et al., 2009). The application of amendments can greatly reduce the bioavailability of heavy metals, and may lead to improved soil microbial activity and diversity in heavy metal contaminated soils (Kaplan et al., 2014). Kelly et al. (2003) reported that the remediated soils showed increase in indicator PLFAs for fungi, actinomycetes and Gram-positive bacteria, compared to non-remediated metal-contaminated soils. A well-functioning microbial community is a prerequisite for the soil ecosystem to adapt to external factors and for ensuring soil fertility (Chodak et al., 2013). Given the importance of soil bacterial community and diversity to the function and health of soil, the soil microbial communities and activity have often been monitored to evaluate the efficiency of remediation of metals contaminated soils, especially for reclamation of mine soils (Epelde et al., 2009, 2014; Kumpiene et al., 2009; Zornoza et al., 2015).

Generally, the criteria for evaluating the efficiency of soil amendments include the assessments of soil pH, metals bioavailability and form, yield and metal accumulation in the edible part of crops (Bolan et al., 2003; Ruttens et al., 2006; Mahabadi et al., 2007; Lee et al., 2009; Cui et al., 2014; Gupta et al., 2014; Zhou et al., 2017). In contrast, few studies have focused on the change of soil bacterial communities induced by amendments. Recently, the development of 16S rRNA pyrosequencing technology can provide unparalleled insight into microbial community structures in complex highly diverse and complex environments such as soils (Roesch et al., 2007; Rastogi et al., 2010; Golebiewski et al., 2014). Particularly, Illumina MiSeq sequencing technology has become a cost-effective and powerful tool in soil microbial ecology studies (Caporaso et al., 2012), and has been used to explore the effects of heavy metal on soil microbial communities by several studies (Chodak et al., 2013; Golebiewski et al., 2014; Hong et al., 2015; Yin et al., 2015). However, to the best of our knowledge, there has been no report to monitor the change of soil bacterial communities and diversity induced by amendments using Illumina MiSeq sequencing technology.

So far, many studies have proven the efficiency of *in situ* immobilization of metals by application of single or mixed amendments using laboratory scale incubation or pot experiments, only a limited number of field studies were conducted to evaluate the feasibility of *in situ* immobilization (Kim et al., 2012; Zanuzzi et al., 2013; Liang et al., 2014). However, under field conditions, many abiotic (such as irrigation, fertilizer, farming methods, and acid rain) and biotic factors (such as the growth and secretion of organic acid of plant root, soil microbial metabolism, and the movement of earthworm) can induce the release of metals adsorbed or deposited by amendments or soil composition to soil solution again, thus the final results may be different from lab data. Our previous studies found that mixed amendments, which include zeolite, limestone, phosphate rock (or calcium magnesium phosphate), and with or without mushroom residue (or pig manure), were very effective for raising soil pH value and reducing the bioavailability of Cd, Pb, Cu and Zn in a multi-metal contaminated acidic soil, and improved vegetable growth under batch and pot experiment conditions (Guo et al., 2015; Cai et al., 2015; Huang et al., 2017). In this study, we aimed at investigating: (i) the effects of application of inorganic and organic mixed amendments on soil pH, metal bioavailability, soil basal respiration, and soil bacterial community of a multi-metal contaminated acidic soil under field conditions; and (ii) the relationship between soil bacterial community composition and soil physicochemical properties (such as pH, nutrients level, and bioavailable heavy metals) which were greatly changed by the application of amendments.

2. Materials and methods

2.1. Site description

The field experiment was located in Shangba country of Shaoguan city, Guangdong province, China (24°32'34.4"N, 113°42'42.1"E). Shangba village is probably the most famous cancer village in China, because more than 3000 local villagers have died from cancer from 1987 to 2005. The tested soil was extreme acidic (pH 4.32) and was moderately contaminated with Cu (1.39 mg/kg), Zn (302.67 mg/kg), Cd (325.08 mg/kg), and Pb (383.67 mg/kg), due to long-term irrigation with water from Hengshi River, which was contaminated by acidic mine drainages (AMD) from the Daobaoshan mine (Fig. 1). The tested soil is Ultisols based on USDA classification.

2.2. Experimental design

Six kinds of mixed amendments, which consist of limestone (analytical reagent grade, L), zeolite (Z), phosphate rock (Pr) (about 29% of available P) or calcium magnesium phosphate (Pcm) (the available concentration P was > 18%), mushroom residue (Mr) or pig manure (M), were used in this field study (Fig. 1, Table A.1). They have been proven to be effective in increasing soil pH, decreasing heavy metals bioavailability and improving plant growth under batch experiment and pot experiment conditions (Guo et al., 2015; Cai et al., 2015; Huang et al., 2017). On April 12, 2014, 21 field plots (2 m × 1 m) were set up and there was a 0.30 m width ditch between two adjacent experimental plots (Fig. 1). The amendments were mixed into a depth of about 0–15 cm soil by raking. The experimental plots were arranged using random block experiment design, and each treatment was done in triplicates.

Lettuce (*Lactuca sativa* L.) was selected as tested crop in this study, because it is a popular vegetable and is grown by the local farmers throughout the year. In addition, lettuce has strong ability to absorb and accumulate heavy metals including Pb, Cd, Zn, Cu, Ni and Cr (Sterrett et al., 1996; Zorrig et al., 2013). For example, Chen et al. (2017) analyzed six metals (Cu, Zn, Pb, Cd, Ni and Cr) in 116 vegetable samples collected from 12 administrative districts of Guangzhou city of Guangdong province, and found that the metals contamination level of lettuce was classified as secure. Lettuce seed was purchased from Guangzhou Academy of Agricultural Sciences. Bunch planting method was employed and 5 seeds were placed in each hold on March 22, 2015. The planting density of lettuce was 20 cm × 20 cm in row spacing. One week later, the lettuce plants were thinned out and kept 1 seedling in each hole, resulting in 32 plants per plot. During the period of plant growth, routine irrigation and weeding were performed until they were harvested on May 22, 2015.

2.3. Soil sampling, pretreatment and analysis

Before the application of amendments, we collected one mixed topsoil sample (0–20 cm depth) from the same experimental block in April 2014, and 8, 10 month (on the day of seeding) and 12 month (on the day of harvesting) after the application of amendments, top soil samples were collected at five randomly selected points in each experimental plot and homogenized to get a mixed soil sample. Each soil sample was placed in a sterile plastic bag and transported to the laboratory immediately. Air-dried soil samples were passed through 20 and 100 mesh sieves, and stored at room temperature for chemical analysis. For the soil samples collected on the day of plant harvesting, soil microbial DNA was extracted from the fresh soil samples immediately back to lab (within 24 h).

Soil pH was measured with a glass electrode meter at a soil: water ratio of 1:2.5 (w:v). Organic matter was analyzed by $K_2Cr_2O_7-H_2SO_4$ oxidation and titration with $FeSO_4$, and alkaline hydrolysis diffusion method was used to determine the NH_4^+ -N concentration. The

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