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Microbial properties and attributes of ecological relevance for soil quality monitoring during a chemical stabilization field study

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ABSTRACT

Chemical stabilization is a soil remediation technique based on the incorporation of organic and/or inorganic amendments to metal contaminated soil in order to decrease metal bioavailability and improve soil quality. Consequently, the establishment of follow-up monitoring programmes is essential to ensure the long-term effectiveness of chemical stabilization in terms of both metal bioavailability reduction and soil quality improvement. In this study, three doses (20, 40 and 80 t ha^{-1}) of a lime-treated sewage sludge, that meets legal standards regarding metal contents, were added to a metalliferous mine soil and a variety of physicochemical and microbial indicators of soil quality were measured over time (immediately before treatment application and one and six months after such application). Soil $CaCl_2$-extractable and plant metal concentrations were also measured. We carried out a complementary interpretation of soil microbial properties through their grouping within a set of ecosystem attributes of ecological relevance: vigour, organization, stability, suppressiveness and redundancy. Sewage sludge addition led to an increase in soil pH, but this beneficial effect was transient. The addition of sewage sludge had a more pronounced effect on parameters used here to estimate soil vigour (dehydrogenase activity, basal and substrate-induced respiration). On the contrary, the addition of sewage sludge did not significantly alter the composition of soil microbial communities, as reflected by PCR-DGGE data. Chemical stabilization was only partly successful: it did improve soil quality but the expected reduction in soil metal bioavailability (as reflected by the values of $CaCl_2$-extractable metal concentration) was clearly observed only for Cd (not for Pb or Zn); however, SL addition led to a significant reduction in shoot metal concentration for the three metals under study. The assessment of soil quality at the attribute level has proven useful for the interpretation of the effect of chemical stabilization on soil functioning.

1. Introduction

Mine soils are frequently unfavourable environments for living organisms due to high levels of bioavailable metals, acidity, lack of organic matter (OM) and associated nutrients, and poor substrate structure (Tordoff et al., 2000; Wong, 2003). The incorporation of organic and/or inorganic amendments to metal contaminated soils, followed by a chemical stabilization period, aims to reduce metal bioavailability and improve soil quality through the increase of soil pH, OM content, nutrient content and water-holding capacity (Alvarenga et al., 2009a,b). Then, the establishment of follow-up monitoring programmes is essential to ensure the long-term effectiveness of chemical stabilization.

The main goal of any soil remediation technology is to remove the contaminant(s) from the site or to render them harmless but restoring the soil quality, i.e. the capacity of soil to perform its functions (Doran and Parkin, 1994; Karlen et al., 2003). Soil microbial properties are well-known valuable indicators of soil quality; in particular, microbial properties have often been used to assess the recovery of soil quality during phytoremediation of metal contaminated soils (Epelde et al., 2008, 2009a, 2010a; Kumpiene et al., 2009). Soil microorganisms have key functions in many vital soil processes, such as OM decomposition and nutrient cycling, and are responsible, in a great extent, for the functioning of soil ecosystems (Anderson, 2003; Lin et al., 2004; Nielsen and Winding, 2002; Schloter et al., 2003).

Soil microbial properties, together with soil physical and chemical parameters, are being increasingly used as bioindicators of soil quality (Bastida et al., 2006; Karlen et al., 1994; Puglisi et al., 2006). With the objective of facilitating the interpretation of soil microbial properties in terms of soil quality through the utilization of less context-dependent (more universal indicators), Garbisu et al. (2011) recently proposed to link the concept of soil quality to that

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of ecosystem health through grouping of soil microbial properties within a set of ecosystem attributes of ecological relevance, such as vigour (a measure of the activity, metabolism or primary productivity of a system), *organization* (which may be assessed in terms of the diversity of components and their degree of mutual dependence), *stability* (a system's ability to maintain its structure and pattern of behaviour in the presence of stress), *suppressiveness* (the capacity of a given soil to maintain disease severity or incidence at a low level, despite the presence of a pathogen, a susceptible host plant, and climatic conditions favourable for disease development) and *redundancy* (the number of, for instance, species per functional group). These five attributes overlap with each other to a certain extent, but are at the same time complementary (that is why, when possible, all five attributes should be measured).

The main aim of this work was to validate this approach in a chemical stabilization field study. To this aim, the abovementioned five attributes were quantified in a field experiment carried out in a metalliferous mine soil heavily contaminated with cadmium (Cd), lead (Pb) and zinc (Zn), where increasing doses of a lime-treated sewage sludge were applied. The evolution over time of a variety of soil physicochemical and microbial properties was monitored. We hypothesized that grouping soil microbial properties within ecosystem attributes of ecological relevance could facilitate the interpretation of the effect of chemical stabilization on soil functioning.

2. Materials and methods

2.1. Site description

The studied area is located in an abandoned Pb–Zn mine (Coto Txomin) in the Western Biscay district, Basque-Cantabrian Basin, northern Spain (43°13′ N, 3°26′ W), within the temperate Atlantic Region of the Iberian Peninsula. The climate is temperate and wet, with no dry season or extreme seasonal changes. Mean annual rainfall in the nearest town (Lanestosa) is about 1400 mm y^{-1}. Mean annual temperature can vary from 11 to 15 °C, with a mean value of 18 °C in July and 5.5 °C in January. For a more detailed description of the experimental site, see Barrutia et al. (2011).

Within the mine, an experimental area of approximately 10 m^2 was chosen for this study, owing to its flat surface, lack of vegetation and uniform soil physicochemical properties. The soil has the following physicochemical properties: 14.3 mg Cd kg^{-1} dry weight (DW) soil, 13,683 mg Pb kg^{-1} DW soil, 26,810 mg Zn kg^{-1} DW soil, sandy loam texture, an OM content of 4.2%, a pH of 6.7, and an ammonium content of 0.13 mg N-NH_4^+ kg^{-1} DW soil. Total concentration of metals was determined using an Atomic Absorption Spectrometer (Spectra AA-250 plus, Varian, Australia) following aqua regia digestion (McGrath and Cunliffe, 1985).

2.2. Experimental setup

Different doses of a lime-treated sewage sludge (20, 40 and 80 t sewage sludge ha^{-1}) were applied to 1 m^2 size plots ($n = 3$) within the 10 m^2 experimental area, following a randomized block design with a 1 m space between plots. Non-amended plots ($n = 3$) were included in the experiment as controls. The physicochemical properties of the sewage sludge were the following: 25.4% dry matter, pH = 8.1, 64.4% OM, 1.38% total nitrogen (on a fresh weight basis), and 2.4, 45, 2310, 347, 68, 1.02 and 138 mg kg^{-1} DW of cadmium, lead, zinc, copper, nickel, mercury and chromium, respectively. Regarding its metal content, the sewage sludge meets the requirements of the Spanish Royal Decree 1310/1990, which regulates the use of sludge from sewage treatment plants in the agricultural sector. Microbiological tests were also performed to look for potential pathogens in the sewage sludge: *Salmonella* sp. and *Ascaris* sp. were absent, and the number of *Escherichia coli* cells was below the threshold established by the Spanish Royal Decree 824/2005 for fertilizers. In any case, the sewage sludge was treated with lime (6% CaO) in order to minimize its bacterial load and, thus, reduce the risk of incorporating potential pathogens into the environment: prior to its incorporation to the soil, the sewage sludge was grounded with a plant debris brush cutter; afterwards, 6% CaO was spread over it and then the mixture was thoroughly homogenized in a mixer. Soil was sampled (upper 0–10 cm) in all the experimental plots (within each plot, a composite soil sample from six randomly selected places was obtained) immediately before the application of lime-treated sewage sludge (SL; initial sampling), and one and six months after such application (these sampling times correspond to February, March and August 2012, respectively). In the last sampling (August), the natural vegetation present in an area of 0.5 m^2, randomly selected within each plot, was cut down to about 1 cm above the soil for metal analysis.

2.3. Soil physicochemical parameters

For the analysis of physicochemical parameters, soils were sieved to <2 mm and air-dried until constant weight. For the estimation of metal bioavailability, $CaCl_2$-extractable (0.01 M) Cd, Pb and Zn fractions were obtained following Houba et al. (2000) and then analysed using an Atomic Absorption Spectrometer. Soil pH was measured with a pH-metre in a soil suspension with deionized water (1:2.5, w:v). Water-soluble organic carbon (WSOC) was extracted and measured according to Epelde et al. (2010b). Ammonium (N-NH_4^+) was extracted using 1 M KCl and then determined following Nelson (1983).

2.4. Plant parameters

In the last sampling, harvested plants were washed thoroughly with deionized water and gently dried with paper towels. Fresh weights were recorded and, subsequently, shoots were oven-dried at 70 °C for 48 h to calculate dry weights. Subsamples (0.2 g) of dried shoot tissue were digested with a mixture of HNO_3/$HClO_4$ (Zhao et al., 1994) and, finally, Cd, Pb and Zn were determined using an Atomic Absorption Spectrometer (Spectra AA-250 plus, Varian, Australia).

2.5. Soil microbial parameters

For microbial parameters, soils were sieved to <2 mm and stored fresh at 4 °C for a maximum of two months until analysis (subsamples for molecular analysis were stored at −20 °C). Except for the soil stability test (see below), three biological replicates were used in every analysis. Dehydrogenase activity was determined as described in Epelde et al. (2009a). Basal and substrate-induced respiration were measured following ISO 16072 Norm (2002) and ISO 17155 Norm (2002), respectively. Microbial biomass carbon was determined following Vance et al. (1987). ATP content was measured according to Webster et al. (1984) and Ciardi and Nannipieri (1990): ATP was extracted by ultrasonication of 0.5 g of soil in 7.5 mL of an acid medium (20 mL of 3.33 M phosphoric acid, 20 mL of dimethyl sulfoxide, 20 mL of 10 M urea, 20 mL of 100 mM EDTA, 4 mL of 18.75 mM adenosine, 5 mL of 10% benzalkonium chloride and 11 mL of distilled water), followed by centrifugation at 14,000 rpm for 1 min. Subsequently, 200 μL of the supernatant were neutralized to pH 7.8 ± 0.2 by the addition of 0.3 M Trizma (for each set of analyses, the volume of Trizma required to neutralize the supernatant was previously calculated; it was approximately 5.8 times the volume of the supernatant), followed again by centrifugation at 14,000 rpm for 1 min, to clean the