Science of the Total Environment 439 (2012) 211–219

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

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

Soil fertility and plant diversity enhance microbial performance in metal-polluted soils

Anna M. Stefanowicz [a,*], Paweł Kapusta [a], Grażyna Szarek-Łukaszewska [a], Krystyna Grodzińska [a], Maria Niklińska [b], Rolf D. Vogt [c]

[a] W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland
[b] Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland
[c] Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo, Norway

HIGHLIGHTS

- We examined effects of habitat properties on microbial parameters in polluted soil.
- Nutrient content increased microbial activity.
- Plant species richness increased microbial activity and functional richness.
- Toxic effects of trace metals were ameliorated by nutrient content.
- Bacterial and fungal communities were affected by habitat properties differently.

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 13 September 2012
Accepted 13 September 2012
Available online 13 October 2012

Keywords:
Soil microbial communities
Heavy metals
Plant species richness
Plant functional diversity
Soil physicochemical properties

ABSTRACT

This study examined the effects of soil physicochemical properties (including heavy metal pollution) and vegetation parameters on soil basal respiration, microbial biomass, and the activity and functional richness of culturable soil bacteria and fungi. In a zinc and lead mining area (S Poland), 49 sites were selected to represent all common plant communities and comprise the area's diverse soil types. Numerous variables describing habitat properties were reduced by PCA to 7 independent factors, mainly representing subsoil type (metal-rich mining waste vs. sand), soil fertility (exchangeable Ca, Mg and K, total C and N, organic C), plant species richness, phosphorus content, water-soluble heavy metals (Zn, Cd and Pb), clay content and plant functional diversity (based on graminoids, legumes and non-leguminous forbs). Multiple regression analysis including these factors explained much of the variation in most microbial parameters; in the case of microbial respiration and biomass, it was 86% and 71%, respectively. The activity of soil microbes was positively affected mainly by soil fertility and, apparently, by the presence of mining waste in the subsoil. The mining waste contained vast amounts of trace metals (total Zn, Cd and Pb), but it promoted microbial performance due to its inherently high content of macronutrients (total Ca, Mg, K and C). Plant species richness had a relatively strong positive effect on all microbial parameters, except for the fungal component. In contrast, plant functional diversity was practically negligible in its effect on microbes. Other explanatory variables had only a minor positive effect (clay content) or no significant influence (phosphorus content) on microbial communities. The main conclusion from this study is that high nutrient availability and plant species richness positively affected the soil microbes and that this apparently counteracted the toxic effects of metal contamination.

1. Introduction

High concentrations of heavy metals can severely reduce the growth and survival of soil microorganisms and thus adversely affect many ecosystem functions driven by these organisms (Bååth, 1989; Giller et al., 2009; McGrath et al., 1995; Ramsey et al., 2005). Parameters describing the condition of microbial communities, such as microbial respiration and biomass, or functional and structural diversity, are widely used to assess soil health in industrial and urban areas (Avidano et al., 2005; Shukurov et al., 2005; Zhang et al., 2008).

In natural situations, however, biological indicators are influenced by a wider range of environmental factors. Belowground microbiota are particularly sensitive to changes in soil physicochemical properties such as temperature, moisture, pH, nutrient availability or clay content (Balogh et al., 2011; Lauber et al., 2008; Teklay et al., 2010). These factors directly control the abundance and activity of microbes and

* Corresponding author. Tel.: +48 124241770; fax: +48 124219790.
E-mail addresses: a.stefanowicz@botany.pl (A.M. Stefanowicz), p.kapusta@botany.pl (P. Kapusta), g.szarek@botany.pl (G. Szarek-Łukaszewska), k.grodzinska@botany.pl (K. Grodzińska), maria.niklinska@uj.edu.pl (M. Niklińska), r.d.vogt@kjemi.uio.no (R.D. Vogt).

0048-9697/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2012.09.030

indirectly affect them through regulating the bioavailability of the contaminants (Bååth, 1989; Giller et al., 2009; Vig et al., 2003). For example, microbial communities benefit from high contents of organic matter and nutrients in soil. This is due not only to the abundance and quality of food resources but also to the efficient immobilization of hard or type B metals by strong binding to the organic material. Such alleviation of the negative effects of heavy metal pollution on soil biota may be the cause for the weak or absent correlations between trace metal concentrations and microbial activity reported by some authors (Niklińska et al., 2005; Schipper and Lee, 2004).

Recently, much attention has been given to the role of vegetation in shaping soil microbial communities, as this seems to be similarly important as abiotic factors (see e.g. Garbeva et al., 2008; García-Palacios et al., 2011; Liu et al., 2010; Marschner et al., 2004; Sanaullah et al., 2011). Plants supply soil bacteria and fungi with carbon substrates through litter production and root exudates (Grayston et al., 1997; Hooper et al., 2000; Wardle et al., 2004). They can also substantially influence the chemistry of soil solution and thus make local sources of nutrients (or toxicants) available or unavailable for soil biota (Gobran and Clegg, 1996; Hinsinger et al., 2006). Plant species vary in these abilities, so mixtures of them may generate a higher biochemical diversity of belowground ecosystems as compared to monocultures, and in the process may maintain more diverse, presumably better-functioning, microbial communities. Such a mechanism may explain the positive relationships between plant diversity and microbial performance reported by some authors (Eisenhauer et al., 2011; Loranger-Merciris et al., 2006; Stephan et al., 2000; Zak et al., 2003).

Although chronic pollution or other anthropogenic impacts make industrial and urban wastelands inhospitable habitats for living organisms, they can be colonized by relatively dense vegetation. A relevant example is the mining environ of Olkusz, the study area of this paper, where exploitation of shallow deposits of non-ferrous metals (Ag, Pb, Zn) has been carried out since medieval times (Cabala et al., 2009). Many of the indigenous plant species have developed some tolerance to the heavy metal toxicity (Olko et al., 2008; Wierzbicka and Panufnik, 1998; Wierzbicka and Pielichowska, 2004; Załęcka and Wierzbicka, 2002). They are thereby able to build species-rich communities on the heavily polluted soil. It is possible that the greater plant diversity or density in such habitats may result in an improved microbial performance.

The present work is part of a broader study examining the response of soil microbes to stress induced by the high concentration of trace metals in the soils of the Olkusz ore-bearing region. In the previous paper (Stefanowicz et al., 2010), we reported relatively weak correlations between metal concentrations and microbial parameters despite the high level and steep gradients of the former. We hypothesized that the toxic effect of metals on soil microbiota was ameliorated by the favorable influence of such soil properties as organic matter content and pH since the values of these variables were higher in highly polluted sites. In this study we took into account not only the above mentioned (and other) soil physicochemical properties, but also parameters of the herbaceous vegetation (i.e. species richness, species composition, functional diversity and coverage), as factors potentially affecting microbes. By means of multivariate methods, we identified the main sources of variation in the data studied and estimated their effects on soil basal respiration, microbial biomass, and the activity and functional richness of culturable soil bacteria and fungi.

2. Materials and methods

2.1. Study area and sampling procedure

The study was carried out in the Olkusz ore-bearing region, an area heavily contaminated with trace metals due to centuries of mining and smelting activity (Cabala et al., 2009). The study area was mostly a mixture of post-mining sites and fallow farmland (abandoned in the 1970s after a buffer zone was established around the mine and a metallurgical plant in Bukowno), afforested or spontaneously colonized by grassland vegetation. Within this area, 49 study sites were selected to represent the dominant vegetation types (grassland and pine forest) and substrates (mining waste and sand). They were classified in 6 habitat categories described in more detail by Kapusta et al. (2011) and Stefanowicz et al. (2010).

In May 2009, three soil samples of the top mineral soil horizon were collected from the center of each of the 49 study sites after careful removal of the top soil organic layer. The three samples were bulked into one composite sample. The sampling places were situated around a circular plot (4 m^2) from which the density of particular herbaceous species on the Braun–Blanquet scale (ranging between 1 and 6) as well as the total percent plant cover were estimated during the growing season. Plant species were identified in the field or were sampled for identification in the laboratory, following the nomenclature by Mirek et al. (2002).

2.2. Chemical and biological analyses

The composite fresh soil samples were sieved through 2 mm mesh. Then the samples were split into two parts: one air-dried at room temperature for analysis of physicochemical properties, and the other kept moist at 4 °C in a climate chamber for analysis of microbial parameters. Soil dry weight was measured after drying at 105 °C for 12 h, and organic matter content was determined as weight loss upon ignition at 550 °C. The particle size distribution (sand, silt and clay fractions) was determined by a combination of sieving and sedimentation. Soil suspension pH was measured electrometrically using a pH electrode following the extraction with H_2O at a 1:5 (w:v) ratio. Total and organic C contents were analyzed by dry combustion technique with a LECO SC-144DRPC analyzer (Leco) — organic C was determined after treating the soil samples with 2 N H_2SO_4 and 5% $FeSO_4$ in order to remove carbonates. Total N was determined by a method based on Kjeldahl digestion using a Kjeltec 2300 (FossTecator). Contents of Ca, Mg, K, Zn, Cd and Pb in the soils were measured by flame or graphite furnace atomic absorption spectrometry (Varian 220 FS) after hot $HClO_4$ digestion (metals termed in this paper as "total"). Exchangeable Ca, Mg, K, Zn and Cd were determined by extraction with 0.1 M $BaCl_2$ at pH 7, and water-soluble Zn, Cd and Pb were measured after the extraction with deionized water at a 1:10 (w:v) ratio. The pool of total P was released from the soil by mineralization with $HClO_4$ and available P was extracted using 0.5 M $NaHCO_3$ (Olsen method). The amount of phosphorus in the extracts was determined by the molybdenum blue method using a Hach-Lange DR 3800 spectrophotometer.

Soil basal respiration (BR) and microbial biomass (C_{mic}) as substrate-induced respiration (SIR) were measured by a chemical method with the absorption of CO_2 in 0.2 M NaOH followed by titration of excess hydroxide with 0.1 M HCl. Glucose (10 g kg^{-1} soil dry weight) was added to soil samples 4 h before SIR measurements. For details of BR and SIR measurements see Stefanowicz et al. (2010).

The activity and functional richness of microbial communities were estimated with Biolog GN2 (bacteria) and SFN2 plates (fungi), containing 95 sole carbon substrates for microbes. The procedure started from shaking fresh soil samples (3 g dry weight) in 30 ml 0.9% NaCl for 1 h. Then the extracts were frozen in liquid nitrogen and kept at −70 °C until analysis (Boivin et al., 2007). Thawed soil solutions were diluted 10 times in physiological salt (GN2 plates) or in 0.1% water agar + 0.04% Tween 80 (polyoxyethylene (20) sorbitan monooleate) (SFN2 plates). Adding gelling agents and surfactants allows a uniform dispersion of fungal spores in a solution (Buyer et al., 2001; Kraus et al., 2004). Streptomycin sulfate (1 μg per well) and chlortetracycline (0.5 μg per well) were added to the solution