Soil Biology & Biochemistry 86 (2015) 58–66

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Belowground fungal communities in pioneer Scots pine stands growing on heavy metal polluted and non-polluted soils

Michiel Op De Beeck, Joske Ruytinx, Mark M. Smits, Jaco Vangronsveld, Jan V. Colpaert*, François Rineau

Hasselt University, Centre for Environmental Sciences, Environmental Biology Group, Hasselt, Belgium

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form
9 March 2015
Accepted 10 March 2015
Available online 25 March 2015

Keywords:
Metal pollution
Fungal community
Succession
Metabarcoding
454 pyrosequencing

ABSTRACT

The impact of soil metal pollution on plant communities has been studied extensively in the past. However, very little is known about the fungal species that co-occur with these plant communities on metal polluted soils. We characterized the belowground fungal community in a heavy metal polluted and a non-polluted soil using 454 pyrosequencing. The fungal communities at both study sites were shown to consist mainly of the same ectomycorrhizal species, but a consistent shift in the relative abundances of these species was observed, whereas no differences in fungal diversity were found. In metal polluted soil, root tips of young pines were initially largely colonized by stress-tolerant dark Ascomycota that were mostly replaced by metal-tolerant Basidiomycota within 2 years. Compared to older forests, a low belowground fungal diversity was observed in the two pioneer stands.

1. Introduction

Worldwide, vast areas have become contaminated with high concentrations of heavy metals due to pyrometallurgical industry and mining activities. High concentrations of metal ions in soils have been found to have detrimental effects on fungal, plant and bacterial communities. Ernst (1990) for example, observed a decrease in floristic diversity along a metal pollution gradient towards metal smelters. Hence, plant communities thriving on metalliferous soils often consist of well-adapted plant species, some of which may even be endemic to a restricted number of metalliferous sites. For example, the *Violetum calaminariae* association is a plant community occurring exclusively in small areas in Belgium and Germany where metal-rich ores surface (Schwickerath, 1944). In contrast to our vast and long-standing knowledge on plant communities that thrive on metal polluted soils, far less is known about the effects of heavy metal pollution on the diversity and community composition of fungal species that occur in heavy metal polluted soils.

Few authors found evidence that fungal species diversity might be lower in highly polluted soils compared to non-polluted ones (Staudenrausch et al., 2005). Hui et al. (2011), studying ectomycorrhizal (ECM) fungal communities in lead contaminated soils, observed shifts in the composition of the communities, but the heavy metal pollution did not strongly affect fungal diversity. Up to now, most researchers used biomarkers (e.g. phospholipid fatty acids) to investigate differences in fungal community composition amongst polluted and non-polluted soils (e.g. Chodak et al., 2013; Corneo et al., 2013). Although such studies can reveal shifts in fungal community composition, they generally cannot pinpoint the identity of those fungi that are affected by the metal stress. It is expected that fungal species that exhibit heavy metal tolerance and/or resistance mechanisms would have a selective advantage under toxic conditions over more sensitive species.

So far, no fungal species have been described to have a distribution restricted to heavy metal polluted soils. However, a few species have been identified repeatedly from roots of plants growing on severely heavy metal polluted soils. For example, dark pigmented ectomycorrhizal Ascomycota (e.g. Helotiales) are remarkably frequent on roots from woody plants growing on copper polluted sites in Norway (Vrålstad et al., 2000, 2002). Dark septate root endophytes (DSE) belonging to the *Phialophora*/*Cadophora* complex were identified from *Salix caprea* L. roots growing in very toxic soil close to a lead smelter in Slovenia (Likar and Regvar,

* Corresponding author. Agoralaan Building D, 3590 Diepenbeek, Belgium. Tel.: +32 (0) 11 26 83 04; fax: +32 (0) 11 26 83 01.
E-mail address: jan.colpaert@uhasselt.be (J.V. Colpaert).

http://dx.doi.org/10.1016/j.soilbio.2015.03.007
0038-0717/

2009, 2013). The latter authors observed a dominance of root associated Ascomycota in the most polluted plots, whereas there was a greater diversity of Basidiomycota in the less polluted and control plots, suggesting greater stress tolerance of these Ascomyceta in comparison to Basidiomycota. Several DSE fungi were also identified in an ancient Pb–Zn slag heap in Southwest China (Zhang et al., 2013) and Bradley et al. (1981) demonstrated that the ericoid mycorrhizal ascomycete *Rhizoscyphus* (=*Hymenoscyphus*) *ericae* (D.J. Read) W.Y. Zhuang & Korf supported the survival of *Calluna vulgaris* (L.) Hull on acidic copper, zinc and lead polluted soils in the UK. Relatively few basidiomycetes have been reported repeatedly from heavy metal polluted sites, except for *Suillus* and *Pisolithus* species (Colpaert et al., 2004; Adriaensen et al., 2005; Jourand et al., 2010).

Recent developments in molecular biology, such as 454 pyrosequencing (Margulies et al., 2005), now enable us to study which fungal species are able to colonize heavy metal polluted soils in unprecedented detail. Knowledge on the fungal communities from these metal polluted soils is not only important for our general understanding of the functioning of natural ecosystems in stressful environments, this knowledge may also help us in developing strategies to remediate polluted areas (Turnau et al., 2008; Solís-Domínguez et al., 2011). Furthermore, a thorough understanding of the impact of metal pollution on fungal diversity and fungal community dynamics is necessary to understand the faith of fungi and plants after their introduction to metal polluted environments.

Therefore, the aims of the current study were to investigate the effects of heavy metal pollution on fungal community composition and diversity during early colonization of heavy metal polluted soils using a metabarcoding approach.

2. Materials and methods

2.1. Study sites and sampling

Fungal communities were sampled in two pioneer Scots pine forests (*Pinus sylvestris* L.) growing on sandy soils in the Campine region in Belgium. The first fungal community was sampled in a metal polluted site in Lommel-Maatheide (LM: 51° 14′ 10″ N, 5° 15′ 50″ E). $Ca(NO_3)_2$ extractable soil Zn and Cd concentrations in this site range from 1 to 197 μg g^{-1} dry weight (d. wt) Zn and <0.1–1.56 μg g^{-1} d. wt Cd. The second site is situated in Hechtel-Eksel (HE: 51° 7′ 33″ N, 5° 22′ 22″ E). This site is hardly polluted by pyrometallurgical activities and is used as a reference site in this study. $Ca(NO_3)_2$ extractable soil Zn concentrations in this site range from 3 to 13 μg g^{-1} d. wt and Cd concentrations were below the detection limit of 0.1 μg g^{-1} d. wt. Mosses and lichens form the main accompanying primary pioneer vegetation at both sites. The pioneer grass species *Corynephorus canescens* Horst occurs sparsely throughout both study sites and in HE, a few *C. vulgaris* shrubs are present as well. The soil at both study sites is a dry sandy soil without a litter layer, poor in organic matter and slightly acidic. The average soil organic matter (OM) content in HE was 0.7% ± 0.1% (standard error) and the average pH (measured in KCl-extracts) was 4.5 ± 0.02. In LM, the average OM content was 0.8% ± 0.1 and the average pH was 4.6 ± 0.07. More detailed information on measured environmental variables in LM and HE can be found in Fig. S1. The region has an average annual rainfall of 800 mm and the average annual temperature is 10 °C (Royal Meteorological Institute, Ukkel, Belgium).

The pioneer forest in LM is growing on a site where polluted topsoil was removed in 2004. This disturbance introduced heterogeneity in the newly exposed soil and resulted in large differences in metal concentrations over short distances. To estimate the overall metal exposure of individual pine trees in LM, soil samples and last-year pine needles from 22 one-year old (in 2009) trees were collected for Zn and Cd analysis. Finally, a subset of 10 trees from LM containing between 200 and 400 μg Zn g^{-1} d. wt in needles were selected for the fungal community analysis. Needles of these trees were not chlorotic, though the critical leaf tissue concentrations affecting growth in most plants ranges from 200 to 300 μg Zn g^{-1} d. wt (Påhlsson, 1989). Needle Zn concentrations are a good measure for the actual Zn exposure of individual trees (Colpaert et al., 2004).

The 10 LM trees were compared to 10 one-year old trees from HE, containing 20–90 μg Zn g^{-1} d. wt in needles (Fig. S1). Selected pine trees were at least 20 m apart from each other. The pioneer forest in HE is growing on a stabilized sand dune.

For the characterization of fungal communities, soil and root tip samples were collected at both sites in November 2009 and in November 2011. Soil samples were collected with a soil corer with a diameter of 1 cm at a depth of 0 cm–20 cm. For each tree, five samples were collected according to the cardinal directions at different distances from the stem. These included samples collected immediately next to stems and at a distance of 25 cm, 50 cm, 75 cm and 100 cm from stems (Fig. S2). Samples were pooled for each of these distances and mixed, resulting in a total of five pooled samples for each tree with each sample representing a certain distance from the stem. Additionally, roots from selected pine trees were collected in both sampling years. Two long roots were unearthed per tree from the stem base up to the growth tip of the roots. In the lab, roots were washed with tap water to remove most adhering soil. For each tree, all visible short root tips were collected from the entire length of the long roots, pooled and homogenized. Root tip samples were stored at −80 °C until DNA extraction. Soil samples for fungal community analysis were homogenized, sieved with a 2 mm sieve to remove small stones, roots, and other debris, and stored at −80 °C until DNA extraction. Samples for physical and chemical soil characterization were collected next to each tree with a soil corer with a diameter of 10 cm at a depth of 0–20 cm. These samples were dried at ambient temperature for two weeks before physical and chemical analyses were conducted. Collected pine needles were dried for 2 weeks at 60 °C before being analysed for their metal content.

2.2. Soil physical and chemical characterization

pH was measured in both a water extract (10 g soil extracted with 25 ml distilled water) and a KCl extract (10 g soil extracted with 25 ml 1 M KCl) of soil samples. Conductivity was measured on the water extracts. Soil organic matter content (OM) was analysed with the Walkley and Black method (Walkley and Black, 1934). Cation exchange capacity (CEC) was measured according to Rhoades' method (Rhoades, 1982). Exchangeable cations were extracted using 0.1 M $Ca(NO_3)_2$ (25 ml for 5 g soil). Dried pine needles were digested with nitric acid (65%) and hydrochloric acid (37%) at 120 °C. Concentrations of zinc (Zn), cadmium (Cd), iron (Fe), magnesium (Mg), potassium (K), copper (Cu) and manganese (Mn) were measured with inductively-coupled plasma– optical emission spectroscopy (ICP–OES) in samples obtained from calcium nitrate soil extracts and in pine needle digests. Calcium (Ca) was only measured in pine needle digests.

2.3. Characterization of the fungal communities

To characterize the fungal communities in soil and root tip samples, DNA was extracted using the UltraClean soil DNA isolation kit (MoBio, Carlsbad, CA, USA) from approximately 250 mg of soil or root tips according to the manufacturer's instructions. DNA was extracted in quadruplicate from each sample and replicated