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Responses of microbial tolerance to heavy metals along a century-old metal ore pollution gradient in a subarctic birch forest☆

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ABSTRACT

Heavy metals are some of the most persistent and potent anthropogenic environmental contaminants. Although heavy metals may compromise microbial communities and soil fertility, it is challenging to causally link microbial responses to heavy metals due to various confounding factors, including correlated soil physicochemistry or nutrient availability. A solution is to investigate whether tolerance to the pollutant has been induced, called Pollution Induced Community Tolerance (PICT). In this study, we investigated soil microbial responses to a century-old gradient of metal ore pollution in an otherwise pristine subarctic birch forest generated by a railway source of iron ore transportation. To do this, we determined microbial biomass, growth, and respiration rates, and bacterial tolerance to Zn and Cu in replicated distance transects (1 m–4 km) perpendicular to the railway. Microbial biomass, growth and respiration rates were stable across the pollution gradient. The microbial community structure could be distinguished between sampled distances, but most of the variation was explained by soil pH differences, and it did not align with distance from the railroad pollution source. Bacterial tolerance to Zn and Cu started from background levels at 4 km distance from the pollution source, and remained at background levels for Cu throughout the gradient. Yet, bacterial tolerance to Zn increased 10-fold 100 m from the railway source. Our results show that the microbial community structure, size and performance remained unaffected by the metal ore exposure, suggesting no impact on ecosystem functioning.

1. Introduction

Contaminants that are persistent in the environment pose potential risks to biota and biological processes world-wide. Given their high diversity (Raes and Bork, 2008; Delmont et al., 2012), microbial communities in soil are some of the most responsive communities to environmental change in terrestrial environments, including that induced by contaminants (Giller et al., 1998; Brandt et al., 2015). Moreover, since microbial communities control the soil biogeochemistry that regulates both plant fertility and the soil-atmosphere C exchange (Brandt et al., 2015; Rousk and Bengtson, 2014), microbial community responses to pollution have far-reaching ecosystem consequences. As such, to evaluate if natural communities in environments have been compromised by pollution, microbial community responses are a powerful set of biomarkers with substantial functional relevance for the entire ecosystem (Brandt et al., 2015; Giller et al., 1999). One of the most persistent and potent group of environmental contaminants derived from human activities are heavy metals (Giller et al, 1998, 2009). However, although heavy metals may compromise soil microbial communities and thus soil fertility, it is challenging to causally link microbial responses specifically to heavy metals due to various confounding factors, including soil pH, organic matter content and soil texture, all of which interact with metals in soil and affect their toxic potential (Berg et al., 2012; Lekfeldt et al., 2014). These complications render measurements of heavy metal concentrations in soil challenging to infer soil microbial toxicity from.

A potential solution to causally linking toxic effects on microbial communities to the exposure to specific pollutants in field conditions is the pollution-induced community tolerance (PICT) approach (Blanck, 2002; Rath and Rousk, 2015; Song et al., 2017).

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Community tolerance is a measurement of the average ability of members of a community to withstand the exposure to a contaminant. Thus, it is a more comprehensive measure than tolerance at the cell level, and often provides a more continuous response (Berg et al., 2012; Blanck, 2002). Any endpoint that can be used to determine microbial tolerance can be used to estimate PICT, such as colony forming units (CFU), respiration rates or nitrification rates, and commonly used targets known to be sensitive measures of bacterial tolerance include bacterial growth rates estimates (Berg et al., 2012; Lekfeldt et al., 2014; Blanck, 2002; Rath and Rousk, 2015; Song et al., 2017). When a community is exposed to a contaminant, organisms that can tolerate the contaminant are favoured, while the more sensitive members of the community will decrease in abundance or grow less active. This selection for more tolerant organisms will shift the community composition towards a community with a higher tolerance (Blanck, 2002; Rath and Rousk, 2015). The changed community tolerance in response to the exposure of a toxicant is called PICT. If tolerance to a specific pollutant has been induced (i.e. PICT can be shown), the compound has had a relevant toxic effect on the studied biota (Brandt et al., 2015; Blanck, 2002). Unlike other assessments of soil microbial communities, including community composition responses and various process rate responses, the determination of PICT can be used to causally link the community response to the presumed toxicant via the observed shift in community trait distributions (Cruz-Paredes et al., 2017). Thus, it is one of the few approaches available to distinguish direct from indirect effects by pollutants on microbial communities (Rath and Rousk, 2015; Cruz-Paredes et al., 2017), which can be especially useful when environments are contaminated by complex mixtures of pollutants with several candidate mechanisms of toxicity (Pennanen et al., 1996; Fernández-Calviño et al., 2011).

One of the primary sources of heavy metal contaminants in natural environments are mining activities (Giller et al., 1998; Tiller, 1989). The world's largest underground iron ore mine is situated in Kiruna, in the north of Sweden. The mine was established over a century ago, and most of its produced iron ore has been transported in open carriages via railway to ports in Narvik, Norway, for shipping. The railway passes through a pristine subarctic environment including the Abisko National park, Northern Sweden, with several open car trains passing daily, creating a semi-continuous deposition of metal ore derived pollution. This industry transport has resulted in a uniform and century-old gradient of exposure to metal ore dust with a well-defined origin from the railway track, and with exposure rates decreasing with distance from the railway into the pristine subarctic birch forest surrounding it. While iron is the dominant metal associated with the mined ore, and has a very limited toxic effect even at relatively high concentrations (e.g. Bruins et al., 2002; Van Anholt et al., 2002), many heavy metals with high toxic potential are also associated with the ore, including Cu and Zn.

In this study, we investigated the responses of the microbial community structure, biomass concentrations, microbial growth rates and respiration along distance transects from the iron ore railway into the pristine subarctic birch forest surrounding it. To link microbial community responses to the exposure to metal ore pollution, we also determined bacterial tolerance to heavy metals (Cu and Zn) along the gradient. We hypothesized that the microbial community structure would shift systematically with distance from the railroad, and that differences would correlate well with differences in bacterial tolerance to Cu and Zn. We also expected rates of microbial growth and respiration to be reduced close to the railroad, and for a relatively higher dominance of fungi, indicated by the fungal:bacterial growth rate ratio, closer to the railroad due to the generally higher tolerance of fungi than bacteria to heavy metals (Rajapaksha et al., 2004).

2. Materials and methods

2.1. Site, transects and soil sampling

The study site was located in Abisko, in the Torneträsk region of Northern Sweden, close to the Abisko Research Station (68°19′N, 18°51′E). The climate in this region is subarctic and as such has a growing season of approx. 3 months lasting from mid-June to early September. The area where the experiment was set up can be characterised as a mix of open birch (*Betula tortuosa*) forest, with the understory dominated by *Empetrum nigrum*, *Vaccinium vitis-idaea* and the mosses *Pleurozium schreberi* and *Hylocomium splendens*. The soil is a Histosol (FAO, 2014), the bedrock is a base-rich schist, and the organic soil horizon at the site is c. 8–25 cm deep. The mean annual air temperature in Abisko is c. 0.2 °C, and the mean annual precipitation is 340 mm (both values are 30-year mean, 1986–2015; Abisko Scientific Research Station, 2016).

The iron ore rail transportation passes through the Abisko National park in a roughly east-to-west trajectory. From the iron ore mine in Kiruna, which is c. 90 km distant from the established transects, around 22 return train departures pass on a daily basis, each is a 750 m long train with 68 open cars containing pelleted iron ore totalling c. 9000 metric tonnes (Fig. S1). In June 2015, we established three parallel transects aligned perpendicular to the direction of the railway in a southerly direction, and c. 100 m apart from each other, which were treated as independent replicates. The first sampled distance was 1 m into the birch forest measured from the gravel bed beneath the railroad track, and further distances included 10, 20, 40, 80, 160, 320, 640, 1280 and c. 4000 m, thus totalling 10 distances per replicate transect. At each distance for each transect, composite soil samples were formed from 3 to 5 randomly positioned cores (5 cm diameter) sampled to a depth of 5 cm and ensuring that only the O-horizon was included. The cores were homogenized and gently passed through a 4 mm grid into plastic bags, and refrigerated (5 °C) within 4 h of field sampling. The samples were subsequently shipped to Lund University (kept at 5 °C), and processed within 10 days of sampling.

2.2. Soil chemistry and characteristics

Soil water content was measured gravimetrically after drying at 105 °C overnight, soil organic matter content was measured as loss on ignition (600 °C overnight). Soil pH was measured in water (1:5, w:V) with a pH electrode following a 2 h extraction. Soil C and N were analysed using the Dumas dry combustion using an elemental analyser (VarioMAX CN, Elementar, Hanau, Germany). Soil Fe, Zn, and Cu concentrations were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Optima 8300, Perkin Elmer, USA) after digestion in HNO_3. The metal availability along the gradient during the studied growing season was assessed with ion exchange resin bags (Skogley and Dobermann, 1996) according to standard procedures (Unibest, 2015). Briefly, the bags were made of nylon stockings and filled with c. 6 g cation exchange resin (Dowex 50W-X8, 16–40 mesh, H^+ form). In June 2015, resin bags were installed at each transect along the gradient at c. 2 cm soil depth in the organic layer. The bags were collected after 2.5 months, in August 2015, thereby covering the peak growing season in subarctic tundra. For the extraction, resin bags were firstly rinsed with double distilled water to remove any soil particles attached to the bags. The resin bags were then extracted 3 times with 15 ml 1 M HCl solution and the extracts were analysed for heavy metal content (Zn, Cu, Fe) using an Atomic Absorption Spectrometer (PerkinElmer PinAAcle 900T) at the University of Copenhagen.