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The impact of metal pollution on soil faunal and microbial activity in two grassland ecosystems ☆

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ABSTRACT

In this study the influence of metal pollution on soil functional activity was evaluated by means of Bait lamina and BIOLOG® EcoPlates™ assays. The *in situ* bait lamina assay investigates the feeding activity of macrofauna, mesofauna and microarthropods while the BIOLOG® EcoPlate™ assay measures the metabolic fingerprint of a selectively extracted microbial community. Both assays proved sensitive enough to reveal changes in the soil community between the plots nearest to and further away from a metal pollution source. Feeding activity (FA) at the less polluted plots reached percentages of 90% while plots nearer to the source of pollution reached percentages as low as 10%. After 2 and 6 days of incubation average well color development (AWCD) and functional richness (R') were significantly lower at the plots closest to the source of pollution. While the Shannon Wiener diversity index (H') decreased significantly at sites nearer to the source of pollution after 2 days but not after 6 days of incubation. Arsenic, Cu and Pb correlated significantly and negatively with feeding activity and functional indices while the role of changing environmental factors such as moisture percentage could not be ruled out completely. Compared to the Bait lamina method that is used *in situ* and which is therefore more affected by site specific variation, the BIOLOG assay, which excludes confounding factors such as low moisture percentage, may be a more reliable assay to measure soil functional activity.

1. Introduction

Amongst other factors the rate of organic matter decay in soils primarily depends on the biomass and metabolic activity of soil micro- and macrofauna present in the soil and litter layer (Eisenbeis, 2006; Hättenschwiler et al., 2005; Stefanowicz, 2006). In metal-polluted soils, sensitive species are replaced by more resistant ones, which often are not able to perform the same ecological functions (André et al., 2009; Davis et al., 2004; Van Beelen and Doelman, 1997). Disturbances of organic matter decomposition may lead to abnormalities in the turnover of elements, reduced soil porosity, restricted availability of nutrients to plants and reduced ecosystem productivity (Ruiz et al., 2008; Witkamp and Ausmus, 1976). Because of the central role soil fauna plays in the fragmentation of organic matter, measurements of feeding activity can act effectively as an indicator of the integrity of the soil community (Filzek et al., 2004b; Kools et al., 2009).

One screening method to estimate soil faunal feeding activity is the bait lamina assay, which was developed by von Törne (1990). Bait lamina are made of rigid plastic strips each having 16 holes, filled with bait which mimics dead plant material. When placed into the soil, this substrate will be utilized by soil fauna, resulting in a measure of the feeding activity of macro-, meso and micro-organisms in the soil (Kratz, 1998; Jensen et al., 2006; Weeks et al., 2004). This method has been proven to be successful in various ecological studies investigating the effect of fluctuating soil moisture, temperature and climate change on feeding activity (Gongalsky et al., 2008; Larink, 1993; Meyer, 1996; Simpson et al., 2012). In addition the bait lamina assay has also been used in ecotoxicological studies where the impact of metal pollution on feeding activity was investigated (André et al., 2009; Filzek et al., 2004b). The first bait lamina studies acknowledged that it was difficult to disentangle the effects of micro- and macrofauna on feeding activity (Simpson et al., 2012). However, recent studies both in microcosms and in the field have shown that macrofauna (i.e. earthworms) (Förster et al., 2004; van Gestel et al., 2003), mesofauna (i.e. enchytraeids) and microarthropods (i.e. collembolan and ascari) (Helling et al., 1998; Römbke et al., 2006) are the main feeders on bait lamina. Moreover, Gongalsky et al. (2004) suggested that micro-organisms cannot contribute to perforation of bait lamina during the short duration (i.e. 14 days) typically used in such studies. Thus the feeding activity on bait lamina strips alone forms an incomplete picture of soil feeding activity.

☆*Capsule*: Feeding activity and functional diversity influenced by metal pollution.

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Insam (1997) proposed a 96-well microplate with 31 carbon substrates plus control (BIOLOG® EcoPlates™) as a new set of substrates for community level physiological profiling (CLPP) in environmental samples. By applying this method we gain insight into the functioning of the soil microfauna involved in carbon cycling (Schutter and Dick, 2001). Loss in the ability of the microbial biomass to maintain its wide range of functions (e.g. changes in catabolic evenness or uniformity of substrate use) is considered as a warning of decreased soil health (Chapman et al., 2007). However, the principle criticism leveled at this method is that it relies upon the growth of a selectively extracted microbial population, which may not represent the true functioning of the whole soil, including macrofauna (Smalla et al., 1998). Consequently, *in situ* field assessment of soil activity instead of observations based on a cultured selection of microfauna would be preferential.

In the field, the metal related stress response of soil organisms is difficult to pinpoint because of the large number of environmental and soil related factors that could potentially cause the same response (Stefanowicz et al., 2010). Soil fauna feeding activity and functional diversity are affected by factors such as, plant coverage (i.e. plant species richness; Birkhofer et al., 2011; and grassland versus forest; Stefanowicz et al., 2010, 2012), organic matter content (Bot and Bernites, 2005), clay mineralogy (Chen, 1998), acidity (Chapman et al., 2013; Eggleton et al., 2009), soil moisture and temperature (Gongalsky et al., 2008; Simpson et al., 2012). On the other hand metal bioavailability within the soil compartment is also affected by factors such as soil pH, clay and organic matter content. These factors are known to control metal adsorption/desorption in soils and in turn, may affect metal toxicity to soil fauna. In general metal mobility (Cd, Cu, Pb and Zn) in soils (i.e. soil solution concentrations and free ion activities) decreases with increasing pH and increasing clay and soil organic matter content (Oorts et al., 2006; Sauvé et al., 2000; Takáč et al., 2009). The metalloid As has intermediate properties between metals and nonmetals, tending to form anions instead of cations (Moreno-Jiménez et al., 2009). Inorganic forms of arsenite are considered more mobile and toxic than organic forms (Jedynak et al., 2009). At high pH values As adsorptions will be lower. This is because negatively charged arsenite species are repulsed by negatively charged surface sites which increase As bioaccessibility (Song et al., 2006; Yang et al., 2002). Moreover, As tends to bind to OH-groups of fulvic and humic acids forming ester-like bonds, while clay minerals with large surface areas such as Fe-, Al-, and Mn hydro(oxides) are an important sink for soluble As forms (Song et al., 2006).

Furthermore exposure cannot be expressed similarly for each organism in the soil ecosystem, e.g. soil microbes may be immersed in soil solution films surrounding soil particles, while invertebrates can be partially exposed after dermal (i.e. capable of adsorbing metals) contact with soil solution films (e.g. earthworms). For other invertebrates (e.g. some arthropod species) metal uptake takes place through the ingestion of metal associated with particulate matter, the food or the soil solution (McGeer et al., 2004). Metal bioavailability and the reaction of soil fauna to changing environmental parameters are undeniably interlinked and the effect of one should thus be interpreted in combination with the other.

The general aim of the present work was to assess changes in the soil feeding activity and functional diversity at two sites with different levels of metal pollution, taking into account changing soil physicochemical properties. This was done firstly by applying the *in situ* bait lamina assay which primarily investigates the feeding activity of macrofauna, mesofauna and microarthropods and secondly by using the BIOLOG® EcoPlates™ assays to gain insight into the functioning of the soil microfauna related to carbon cycling.

2. Materials and methods

2.1. Study sites

Sampling took place in the vicinity of the city of Antwerp (Northern Belgium) at two sites with different distance to an historically polluted site where a still active metal refinery is situated today; Fort 8 (F8) (0.3 km) and Hobokense Polder (HOP) (3 km) (Fig. 1). Although both sites share similar grassland habitats, in comparison

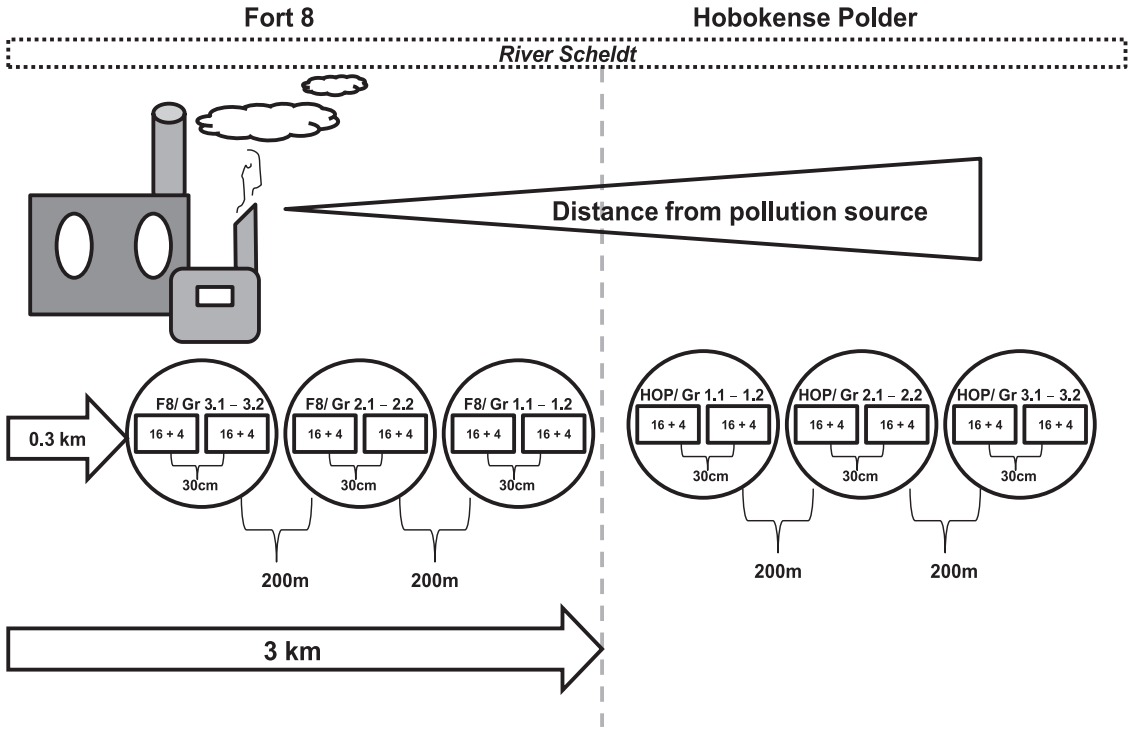

Fig. 1. Schematic presentation of sampling sites (i.e. Fort 8 and Hobokense Polder) and plots in relation to the pollution source. Each one of the subplots contained sixteen plus four control strips (i.e. 16+4) while plots were approximately 200 m apart.