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Short communication

Use of the MicroResp™ method to assess Pollution-Induced Community Tolerance in the context of metal soil contamination

Annette Bérard[a,b,*], Christophe Mazzia[c], Valérie Sappin-Didier[d], Line Capowiez[a,b], Yvan Capowiez[e]

[a] INRA, UMR1114 EMMAH, F-84914 Avignon, France
[b] UAPV, UMR1114 EMMAH, F-84914 Avignon, France
[c] UMR 7263 IMBE UAPV 301 rue Baruch de Spinoza, BP 21239, F-84916 Avignon, France
[d] UMR INRA/Bordeaux Sciences Agro 1220 TCEM, BP 81, F-33883 Villenave d'Ornon, France
[e] UR 1115 INRA PSH, F-84914 Avignon, France

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ABSTRACT

Microorganisms are known indicators of soil health, and there are already several microbial tools for assessing substance ecotoxicity, but there is still a need for practical new tools that are ecologically relevant in soil ecosystems. We developed a protocol based on the substrate-induced respiration of a soil community using the MicroResp™ technique as part of a Pollution-Induced Community Tolerance (PICT) approach. We tested the technique in a long-term field experiment studying the effects of sewage treatment plant discharge with high Cd and Ni contents on plants and soil. We found that MicroResp™ can be used in PICT-bioassays to assess heavy metal (Cd) impact to soil microbial communities. Dose–response curves for soil Cd and soil microbial glucose mineralization were obtained on microrespirometric ecotoxicological bioassays with Cd, making it possible to calculate half maximal effective concentration (EC_{50}). EC_{50} values were positively correlated with Cd concentrations in soil plots. A community-level physiological profile based on mineralization of different carbon substrates was established for each soil sample. Basal respiration and microbial active biomass were estimated, and the metabolic quotient qCO_2 was calculated. These ecotoxicological and ecophysiological biomarkers converge to suggest that metal gradient is associated with sludge-impacted soil microbial communities in terms of active biomass, catabolic structure, and allocation of carbon for energy requirements versus growth in response to Cd-induced tolerance. To the best of our knowledge, this is the first study to investigate micro-SIR in a contaminated soil system as a tool for measuring microbial physiological traits and Pollution-Induced Community Tolerance.

1. Introduction

Anthropogenic activities such as the disposal of industrial and domestic waste have resulted in metal contaminants that steadily accumulate in soils adversely affect microbial subsoil biomass, its activities and its diversity (Giller et al., 1998; Brookes, 1995). Soil microbial communities present great diversity, and soil microbial community activities play a critical role in soil organic matter decomposition, carbon sequestration and nutrient cycling (Six et al., 2006). Pollution-induced changes in microbial communities and soil metabolic functioning warrant deeper investigation, as the loss of microbial functions is an indicator of a decrease in soil quality (Chapman et al., 2007).

There is thus a vital need for methods characterizing the health of a given ecosystem suspected to be exposed to xenobiotic contamination. Such methods should ideally be based on both the physiological responses and biological structure of the potentially exposed communities. Blanck et al. (1988) proposed one such method, called Pollution-Induced Community Tolerance (PICT), based on the assumption that the toxicant exerts a selection pressure if applied at sufficiently high concentration and for a sufficiently long period of time, ultimately leading to a more resistant community composed of various members (species, genotypes or phenotypes), some more and others less sensitive to a given xenobiotic. Structural parameters, with physiological parameters obtained by artificially exposing a community to increasing amounts of toxicant, make it possible to state and compare toxicant contamination levels in sites the communities are collected from

* Corresponding author at: INRA, UMR1114 EMMAH, F-84914 Avignon, France. Tel.: +33 (0) 432722243.
E-mail addresses: annette.berard@paca.inra.fr, annette.berard@avignon.inra.fr (A. Bérard).

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(Blanck, 2002). The PICT concept was mainly developed in aquatic algae studies (Bérard et al., 2002) but has since been applied to terrestrial microbial and algae communities (Bérard et al., 2004; Boivin et al., 2002). Research has developed a wide range of approaches to examining soil microbial community tolerance to heavy metals, and techniques based on measuring thymidine (Tdr) and leucine (Leu) incorporation (bacterial activities) give valuable results (e.g. Díaz-Raviña et al., 2007; Brandt et al., 2010) but these techniques remain difficult to implement and are outside the scope of many labs as they use radiolabeled compounds.

Rutgers et al. (1998) proposed an adapted Biolog™ technique to assess soil bacterial community tolerance to metals, but this microplate technique based on analyzing a bacterial community's use of several carbon sources has major drawbacks: incomplete assessment of the whole microbial community, long incubation times inducing bacterial selection and growth (Preston-Mafham et al., 2002), and the use of artificial culture media with buffers that may have artificial significant effects on speciation and consequently on toxicant bioavailability and toxicity (Barranguet et al., 2003). Witter et al. (2000) developed a respirometric technique based on analyzing substrate-induced respiration (SIR) response (using gas chromatography) to measure metal tolerance in a glucose-metabolizing soil microbial consortium. However, this method remains laborious, especially when assaying many microbial samples simultaneously in a multitude of bottles that each have to be separately processed to measure the amount of CO_2 released (Chapman et al., 2007). Campbell et al. (2003) developed the MicroResp™ technique as an alternative method combining the advantages of the miniaturized Biolog™ technique using the microplate system with the advantages of the SIR approach making it possible to measure CO_2 production during short-term incubations from a whole soil microbial community. The MicroResp™ technique was applied to investigate multiple-carbon-source SIR to characterize community-level physiological profiles (CLPPs) of soils subjected to different stresses and disturbances (e.g. Banning et al., 2012; Ben Sassi et al., 2012; Stres et al., 2010). The MicroResp™ technique was recently modified to apply PICT on river biofilms (Tlili et al., 2011a), but rare few ecotoxicological applications have been investigated in soils (Wakelin et al., 2013; Kaufmann et al., 2006). Very recently Wakelin et al. (2013) applied the MicroResp technique to perform soil dose–response curves based on DO measurements with Copper, but they did not applied their protocol on a soil pollution case, and MicroResp™ has never to our knowledge been applied to study PICT in contaminated soil microbial communities.

The aim of this study was to assess SIR, CLPP and PICT using the MicroResp™ method to characterize microbial communities after exposure to environmental concentrations of metals in a long-term field study.

2. Materials and methods

2.1. Soils

Soils were sampled from a long-term sewage sludge field experiment led at the INRA's Couhins experimental farm in Bordeaux, France (Juste and Mench, 1992). Soils are podzols characterized by acid coarse sandy loam developed on colluvial material, and with low organic matter and nutrient content and a Water Hold Capacity (WHC) of 26.9%. The soils sampled had received three different treatments of inorganic fertilizer (former control, C), sewage sludge applied at 10 dry matter (DM) ha^{-1} $year^{-1}$ (SEW10; total sludge loading rate is 50 t DM ha^{-1}), and sewage sludge applied at 100 DM ha^{-1} per 2 years (SEW100; total sludge loading rate is 300 t DM ha^{-1}) between 1976 and 1980 (Table 1). The sludge came from a sewage treatment plant in the Bordeaux conurbation, as a solid, anaerobically digested and heat-dehydrated sludge with high Cd and Ni contents arising from effluents discharged by a battery manufacturer (Cd and Ni batteries). In-soil sludge application was stopped in 1980. The soils were cropped with maize from 1976 to 1996 and with lettuce, potatoes and wheat from 1997 to 2002. The soil has been left unoccupied (laid fallow) since 2002 (for further details of the experimental design, see Juste and Mench, 1992; Sappin-Didier et al., 1997). New reference plots (REF) were chosen in a grassland area at a distance of over 10 m upslope from the polluted plots due to heterogeneity in pollution history (due to a slightly sloping field trial and to local tillage on small 18 m^2 plots inducing control-plot contamination by heavy metals). However, this REF part of the experimental site was never cultivated. This site set-up is a block-design with three replicates of each treatment in the field, except for reference plots which were duplicates (with each pair at least 10 m apart).

2.2. Microbial community parameters

Soils were sampled from the 11 plots at a depth of 0–10 cm, in April 2011. The soils were sieved (0–2 mm mesh), their humidity was gently adjusted with a spray (MilliQ water) to 40% of the water holding capacity (WHC), which is assumed to be into the optimal range of WHC for microbial respiration (Campbell et al., 2003; Ilstedt et al., 2000; Moreno et al., 2002). Soils were then pre-incubated in microcosms for one week at ambient temperature (23 °C ± 2) in dark conditions following the sieving and humidity adjustment disturbances (Bérard et al., 2011).

Community-level physiological profiles (CLPPs) and basal respiration (BR) were measured using the MicroResp™ system (Campbell et al., 2003) consisting in a 96-deep-well microplate (1.2 mL volume) filled with soil and added with water only (BR) or with aqueous carbon substrates (SIR) then sealed to a colorimetric CO_2-trap microplate and incubated in the dark at lab temperature (23 °C ± 2) for 6 h. Mineralization of 7 carbon substrates (30 mg g^{-1} soil water, corresponding to 6.7 mg g^{-1} DW soil) was tested for CLPP: glucose, trehalose, D-cellobiose, glycine, L-alanine, D-(+)-glucosamine and malic acid. The carbon substrates chosen were selected based on their relevance for soil agroecosystems (i.e. plant residues, root exudates, etc.). In each deep well, the carbon substrates (25 µl) were dispensed first and the soil (300 µl at 40% WHC, corresponding to 0.35 g dry soil) was then added using a volumetric dispenser system. The absorbance of the detection microplate was measured at 570 nm (Biotek EL-800 spectrophotometer). The amounts of released CO_2 were calculated (a calibration curve of absorbance versus headspace equilibrium CO_2 concentration (measured by gas chromatography) was fitted to a regression model), and results were expressed in µg C-CO_2 g^{-1} soil h^{-1} (Campbell et al., 2003; Bérard et al., 2011). According to Saul-Tcherkas and Steinberger (2009) and Bérard et al. (2011), the metabolic quotient qCO_2 is the ratio of BR to glucose-induced respiration (GIR, which is assumed proportional to active microbial biomass; Anderson and Domsch, 1978, 1985; Chapman et al., 2007). BR and qCO_2 were considered physiological traits of the microbial communities.

2.3. Microbial tolerance to Cd

The PICT method was applied to Cd contamination using the Microresp™ technique (Campbell et al., 2003) based on a protocol adapted by Tlili et al. (2011a). First, the toxicant was added to the deep-well microplate. A semi-logarithmic series of 7 Cd concentrations (sulphate salt) was freshly prepared in MilliQ water. Final nominal test concentrations in the deep wells ranged from 52.7 to 3372 µg Cd g^{-1} soil (25 µl of metal solution per well, 3 blanks and 3 replicates for each of the 7 concentrations). Soil