Available online at www.sciencedirect.com
ScienceDirect
Applied Soil Ecology 34 (2006) 103–113
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Discriminating between effects of metals and natural variables in terrestrial bacterial communities

M.E.Y. Boivin [a,b,*], G.D. Greve [a,b], S.A.E. Kools [b], A.W.G. van der Wurff [c], P. Leeflang [a], E. Smit [a], A.M. Breure [a], M. Rutgers [a], N.M. van Straalen [b]

[a] *National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*
[b] *Vrije Universiteit, Department of Animal Ecology, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*
[c] *Wageningen University and Research Centre, Laboratory of Nematology, P.O. Box 8080, 6700 DD Wageningen, The Netherlands*

Accepted 31 March 2006

Abstract

This study aimed to assess effects of metals on bacterial communities in grassland soil and to discriminate these effects from natural variability in soil properties. Changes in gross parameters of bacterial communities were investigated by the determination of ^{14}C-leucine and ^{3}H-thymidine incorporation rates, CO_2 evolution, biomass indicators and N mineralization rates. ^{14}C-leucine incorporation rate and CO_2 evolution showed correlations with all metals tested as well as with organic matter content. ^{3}H-thymidine incorporation rates, biomass indicators and N mineralization rates did not strongly correlate with any physical or chemical parameters. Further, community-level physiological profiles (CLPP) and polymerase chain reaction (PCR)-amplified denaturing gradient gel electrophoresis (DGGE) of 16S rDNA were performed. Monte-Carlo permutation testing was performed to analyse CLPP and DGGE data to allow for a stringent discrimination between sources of effects. CLPP changes correlated with the Pb concentration and pH in the soil. DGGE changes correlated with Pb and Cu concentrations and organic matter content. Pollution-induced community tolerance (PICT) was not found for any of the metals assessed. A causal relation between the effects observed on bacterial communities and the presence of metals was not established with PICT. The negative outcome of PICT can probably be attributed to indirect effects or to methodological problems. We concluded that the observed shifts in CLPP and DGGE patterns are a strong indication of metals effects on bacterial communities in this grassland. These effects could only be filtered from the total variation by multivariate analyses.

Keywords: Metals; Community-level physiological profile; Denaturing gradient gel electrophoresis; Bacterial communities; Pollution-induced community tolerance

1. Introduction

It is a challenge to assess effects of metals on soil bacterial communities in natural conditions especially when pollution gradients are small. Factors such as organic matter and clay contents, and soil pH play a role in metal bioavailability, therewith modulating effective exposure. Even more important, such soil properties are also directly affecting soil microorganisms. For example, a positive correlation has been found between pH and bacterial biomass (Bååth and Anderson, 2003). Therefore, when pollutant concentrations are correlated with other soil parameters, false positive responses may occur. An alternative for the determination of field effects of

* Corresponding author. Tel.: +31 30 274 4109; fax: +31 30 274 4441.
E-mail address: Marie-Elene.Boivin@RIVM.NL (M.E.Y. Boivin).

0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2006.03.009

pollutants on species composition and abundances is the concept of pollution-induced community tolerance (PICT) which was introduced by Blanck et al. (1988). PICT is considered to be an option for discriminating pollutant effects from confounding factors. When co-tolerance is excluded, tolerance develops only for the pollutant to which the community is exposed (van Beelen et al., 2004; Boivin et al., 2002). Consequently, the observation of PICT would be a strong indication for field effects of pollution.

Microorganisms are important endpoints in investigating effects of pollutants. Microorganisms are primary decomposers of dead organic material, such as dead plant materials, including roots, soil organisms and animal manure. They play key roles in food webs and nutrient cycles (Bouwman et al., 1994; Bloem et al., 1997; Schouten et al., 1997). Effects of metals on bacterial communities have been demonstrated under laboratory conditions (Bååth et al., 1998; Dìaz-Raviña and Bååth, 1996; Doelman et al., 1994), in spiked-fields and under natural conditions (Gong et al., 2000; Kandeler et al., 2000; Kelly and Tate, 1998; Brookes and Mcgrath, 1984; Jordan and Lechevalier, 1975; Davis et al., 2004). Bacterial communities exposed to a high level of metals showed different physiological traits and bacterial species composition as well as a development of tolerance to metals. Consequently, it might be concluded that microorganisms are generally sensitive to metal pollution.

This research was focused on the determination of effects of metals on bacterial communities in a grassland soil of the Demmerik, a polder in the central western part of The Netherlands. Traditionally, the region was used for an extensive form of cattle grazing (dairy farms). From the 16th century to the 1950s, the poor soil quality, worsened by oxidation of peat was improved by applying a mixture of city waste, dredged sludge, manure and dune sand. Nowadays, the peat is covered by a completely anthropogenic soil layer of 15–50 cm (Bosveld et al., 2000). This unique layer of soil is man-made over a period of about 500 years. The soil has a high organic matter content as well as sand particles and contains elevated concentrations of metals, mainly Zn, Cu and Pb (Bosveld et al., 2000). Furthermore, Intervention Values for Pb (655 mg/kg DW; corrected for soil type) and Cu (253 mg/kg DW; corrected for soil type) are exceeded at many locations (Swartjes, 1999).

Our research aimed to assess whether metals in these polluted grasslands affect the genetic and physiological structure of bacterial communities and to discriminate metals effects from the natural variation in soil properties.

2. Materials and methods

2.1. Field characteristics and sampling

Ninety soil samples of 2 kg were taken from the upper 10 cm surface layer in the Demmerikse Polder in October 2001 and 100 samples in March 2003. The extensive samplings were intended to cover a sufficient number of samples at the extremes of the metal concentration range. All 90 and 100 samples were separated in four parts, respectively, in 2001 and in 2003. The first part was used to identify samples with high and low concentrations of metals. These samples were all dried at 100 °C overnight, ground and analysed using a hand-held spectrum analyser (X-ray fluorescence (XRF) spectrum analyser, XTAC Analytical, Leiden, The Netherlands). From the 90 samples taken in 2001, 30 were selected: 15 samples showing the lowest Zn, Pb and Cu concentrations and 15 samples showing the highest Zn, Pb and Cu concentrations. From the 100 samples sampled in 2003, 18 were selected: nine samples showing the lowest Zn, Pb and Cu concentrations and nine samples showing the highest Zn, Pb and Cu concentrations. Then, the second part of the selected samples was used to measure soil moisture, clay, soil pH, total organic matter, total metal concentrations and metal concentrations after removal of the calcium chloride exchangeable fraction. The third part of the samples was used for community-level physiological profiling (CLPP) and pollution-induced community tolerance analysis. The fourth part of the samples was used for denaturing gradient gel electrophoresis (DGGE). For practical reasons, DGGE analysis was omitted in the analysis of the samples from 2003.

2.2. Preparation of the samples

All selected soil samples were sieved using a 4 mm mesh sieve. For all biotic analyses, the samples were brought to a humidity corresponding to 50% of the water holding capacity and incubated for two weeks in the dark at 10 °C. For metal concentration analyses, soil samples were dried overnight at 100 °C and subsequently ground using a mortar.

2.3. Abiotic analyses

2.3.1. Water holding capacity

Empty rings with gauze of 100 μm mesh size were weighed. The rings were filled with soil and weighed again. Then, they were placed in a plastic container and saturated with tap water. Subsequently, the samples