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Assessment of structure and function in metal polluted grasslands using Terrestrial Model Ecosystems

Stefan A.E. Kools [a,b,*], Marie-Elène Y. Boivin [a,c], André W.G. Van Der Wurff [d], Matty P. Berg [a], Cornelis A.M. Van Gestel [a], Nico M. Van Straalen [a]

[a] Vrije Universiteit, Department of Animal Ecology, De Boelelaan 1085 NL-1081 HV Amsterdam, The Netherlands
[b] Grontmij|AquaSense, Amsterdam, P.O. Box 95125, NL-1090 HC Amsterdam, The Netherlands
[c] National Institute for Public Health and the Environment (RIVM), P.O. Box 1 NL-3720 BA Bilthoven, The Netherlands
[d] Wageningen University and Research Center, Laboratory of Nematology, P.O. Box 8123 NL-6700 ES Wageningen, The Netherlands

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ABSTRACT

Ecosystem effects of metal pollution in field situations are hard to predict, since metals occur often in mixtures and links between structural (organisms) and functional endpoints (ecosystem processes) are not always that clear. In grasslands, both structure and functioning was suspected to be affected by a mixture of copper, lead, and zinc. Therefore, the structural and functional variables were studied simultaneously using Terrestrial Model Ecosystems (TMEs). Comparing averages of low- and high-polluted soil, based on total metal concentrations, did not show differences in structural and functional variables. However, nematode community structure (Maturity Index) negatively correlated with metal concentrations. Next to that, multivariate statistics showed that enchytraeid, earthworm and, to lesser extent, nematode diversity decreased with increasing metal concentrations and a lower pH in the soil. Bacterial CFU and nematode biomass were positively related with decomposer activity and nitrate concentrations. Nitrate concentrations were negatively related to ammonium concentrations. Earthworm biomass, CO_2 production and plant yield were not related to metal concentrations. The most metal-sensitive endpoint was enchytraeid biomass. In all analyses, soil pH was a significant factor, indicating direct effects on organisms, or indicating indirect effects by influencing metal availability. In general, structural diversity seemed more positively related to functional endpoints than structural biomass. TMEs proved valuable tools to assess the structure and function in metal polluted field situations. The outcome feeds modeling effort and direct future research.

1. Introduction

Grasslands consists a major biomass, ranging from organisms that are hardly visible (such as bacteria, protozoa, and nematodes) to somewhat larger organisms (such as plants, enchytraeids, and earthworms). These organisms are in general sensitive to stressors and for that reasons often used to evaluate soil pollution. In particular, much is known about their response to metal pollution, also the subject in this paper. For example, bacterial activity and diversity decreased with elevated metal concentrations (Kelly et al., 1999; Sandaa et al., 2001). The nematode community structure is a well-known indicator for metal effects, as longer-lived, larger sized nematodes are generally affected more compared to short-lived, relatively small sized species (Ferris et al., 2001; Georgieva et al., 2002; Yeates, 2003). Moreover, enchytraeids are proposed as indicators for metal stress (Didden and Römbke, 2001) and earthworms tend to avoid and accumulate metals (e.g. Hobbelen et al., 2004).

Indicators for ecosystem functioning are the major ecosystem processes, such as primary production, decomposition rates and carbon and nitrogen cycling, which on their turn may also be affected by metal pollution. For example, the primary production (e.g. seed emergence and plant growth) is sensitive to lead, copper, and zinc, while it has been described that nitrogen cycling is hampered by metal pollution (Lokhorst, 1997).

Next to research on the effects of metals on structure and functioning alone, there is great interest in the literature in the interactions between the structure and the functioning (McCann, 2000). Above-ground structural diversity (plants) is believed beneficial for ecosystem processes (Tilman et al., 2001), less is known about below-ground systems. In soils, bacteria, nematodes, enchytraeids, and earthworms positively influence ecosystem processes (Hedlund and Augustsson, 1995; Bloem et al., 1997;

* Corresponding author. Current address: Grontmij|AquaSense, Amsterdam, P.O. Box 95125, NL-1090 HC Amsterdam, The Netherlands. Fax: +31 20 592 2249.
E-mail address: stefan.kools@grontmij.nl (S.A.E. Kools).

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Jongmans et al., 2003; Yeates, 2003). Some authors have suggested that specific functional traits or certain combinations of species may direct ecosystem processes (Heemsbergen et al., 2004); others doubt a strong dependence of ecosystem functioning on biodiversity (Schwartz et al., 2000). In any case, effects of metal pollution on structure and function remain unpredictable. In this paper, we assessed both organism abundance and diversity. We investigated whether a mixture of metals affected organisms and ecosystem functioning in a quasi-field situation. Metal effects have been studied largely in single-species tests and information on the effects of metal pollution in a natural soil setting is scarce. Due to the linkages between functional indicators and community structural characteristics, investigations are more informative in case the effects on structure and functioning are evaluated simultaneously (Van Straalen, 2002). We monitored intact field soils in a laboratory setting. In this way, effects of metal pollution on structure (species diversity and abundance) and on ecosystem processes were investigated simultaneously while visualizing the linkages between structure and function. We expect negative correlations between metal concentrations in the soil and structural characteristics and further tested whether correlations existed between process-related variables and structural variables. Ultimately, we expected that a negative effect of pollution on ecological structure would cause a similar negative effect on the functions. The relations found in this study can facilitate modeling efforts to direct towards more realistic environmental risk assessment and direct new approaches to assess environmental health.

2. Materials and methods

2.1. Study site

The research area, Polder Demmerik, is located 30 km southeast of Amsterdam, The Netherlands (52°13′ North 4°56′ East). This area is designated as a nature reserve and has low densities of sheep and cows. Historically, poor peaty soils were elevated and improved by city waste material from outside this area (Lexmond et al., 1987). This resulted in a layer of approximately 40 cm of clay and sand on top of peat; clearly present with and large fragments of porcelain and stones. The vegetation is quite homogeneous and dominated by rye grass (*Lolium perenne*) and ribwort plantain (*Plantago lanceolata*). We selected two adjacent fields, bordered by ditches and 200 m long and 35 m wide.

2.2. Soil sampling

We mapped the pattern in metal concentrations in two adjacent grassland fields. For this, a grid was marked of 20 rows, each containing five sampling spots (100 spots in total). The rows were at 10 m distances and the sampling spots 5 m apart from each other. Here, we took topsoil samples using a nematode sampler of 3 cm wide (0–10 cm depth; Eijkelkamp, The Netherlands). Total metal concentrations at these spots were determined with a hand-held XRF apparatus (XTAC Analytical, Leiden, The Netherlands). It was know that zinc was highly correlated with Pb and Cu and the XRF-derived data was confirmed by a conventional method (flame Atomic Absorption Spectrometer, unpublished data).

The derived pattern showed that samples in close proximity from each other were not much different in lead, copper, and zinc concentrations. Based on this information, we choose locations in the field that showed the largest range in metal concentrations (Table 1). Here, Terrestrial Model Ecosystems (TMEs) were sampled according to previous TME studies, e.g. Knacker et al. (2004). We sampled in total 80 TMEs as part of a larger experiment. All TMEs were covered with gauze and transported to the laboratory. For this study, we selected 16 columns to be monitored in the laboratory for 6 months, seven TMEs with the lowest and nine with the highest zinc concentrations, based on the XRF-derived pattern.

In addition, total concentrations and organic matter were measured for each TME after incubation (see below). The clay contents were measured on samples that surrounded the location of TMEs, using a method described in Konert and Vandenberghe (1997).

Table 1
Topsoil (0–10 cm) properties (average ± SD) from grassland in the Polder Demmerik, Ronde Venen

Variable	Class LOW ($n = 7$)	Class HIGH ($n = 9$)	*T*-test, *p*-value	Sign.
pH ($CaCl_2$)	5.8 ± 0.5	5.4 ± 0.2	0.06	NS
Moisture (%WHC)	35 ± 14	28 ± 7.0	0.16	NS
% Carbon	**30 ± 5.0**	25 ± 1.0	0.04	*
% Nitrogen	2.2 ± 0.2	2.1 ± 0.1	0.09	NS
Cu_{tot}[b] (mg/kg DW)	130 ± 49	161 ± 31	0.15	NS
Cu_{ex}[a] (mg/kg DW)	0.43 ± 0.23	**0.77 ± 0.16**	< 0.01	**
Pb_{tot}[b] (mg/kg DW)	536 ± 199	**745 ± 91**	0.03	*
Pb_{ex}[a] (mg/kg DW)	1.20 ± 1.5	**4.01 ± 2.9**	0.03	*
Zn_{tot}[b] (mg/kg DW)	269 ± 80	383 ± 122	0.05	*
Zn_{ex}[a] (mg/kg DW)	6.56 ± 6.5	**16.9 ± 5.4**	< 0.01	**
Cd_{ex}[a] (mg/kg DW)	0.03 ± 0.02	**0.06 ± 0.02**	0.01	**

The Netherlands after 6 months laboratory incubation in Terrestrial Model Ecosystems, typeface bold are higher values (significance levels indicates by asterisks; * = 0.05, ** = 0.01, NS = not significant: *T*-test).
[a] In 0.01 M $CaCl_2$ solution (exchangeable metal concentration).
[b] In acid solution (total metal concentration).

2.3. TME incubation

In the laboratory, the cover gauze was removed. The TMEs were installed in a climate room with a standardized day/night cycle at random positions using a frame that exposed the soil surface to the controlled air conditions of 20 °C and 60% relative air humidity (RH) during 14 h artificial daylight. During darkness, the air temperature was 16 °C and RH increased to approximately 80%. The frame separated the lower part of the TMEs from the rest of the room and allowed the soil temperature to be kept between 10 and 14 °C for 24 h. All TMEs received 375 mL artificial rainwater twice a week, summing up to 570 mm rain during the incubation. The artificial rainwater was prepared as described in previous TME studies (Koolhaas et al., 2004).

2.4. Soil chemistry analysis

After 6 months of incubation, all TMEs were destructively sampled. First, an enchytraeid sample was taken from the center of the column, as described below. The remaining topsoil (0–10 cm) was removed and mixed and distributed over three subsamples, for bacterial analysis, nematode analysis and soil chemistry analysis. The 0.01 M $CaCl_2$ exchangeable concentrations of cadmium, copper, lead, and zinc were determined using flame AAS (Atomic Absorption Spectrometry, model 1100B, Bodenseewerk Perkin Elmer, Überlingen, Germany).

Nitrate and ammonium concentrations in the 0.01 M $CaCl_2$ extracts were measured colorimetrically by means of an autoanalyzer (Skalar model SA 400 Erkelenz, Germany).

Previously weighed wet soil was dried for 3 days at 50 °C to determine water contents. The dry soil was used to measure carbon and nitrogen contents on an elemental analyzer (Interscience model 1106, Breda, The Netherlands). Another sample of the dried soil was used to measure the total metal concentrations. One gram was digested in 2 mL demineralized H_2O, 6 mL HCl (37% pro analysis grade (p.a.), Baker, Philipsburg, NJ, USA and 2 mL HNO_3 (65%, p.a., Riedel-deHaën, Seelze, Germany) in a microwave (model MDS 81 D, CEM Microwave Technology Ltd., Buckingham, UK). On these extracts, the copper, lead, and zinc concentrations were determined using flame AAS (model 1100B, Bodenseewerk Perkin Elmer, Überlingen, Germany). San Ioaqin soil (NIST, USA) was included as a certified reference; metal concentrations were within 20% deviation of the certified reference values.

2.5. Bacterial colony forming units (CFU)

Due to constraints in time and resources, seven TMEs in a large range in metal concentrations were sampled to determine CFU. First, the soil moisture level was set at 50% of the water holding capacity and the samples were incubated for 2 weeks at 10 °C in the dark. Then, an equivalent of 25 g dry weight was mixed with 250 mL BisTris buffer (10 mM, pH 7), blended for 10 min and centrifuged for 10 min