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Relative strengths of relationships between plant, microbial, and environmental parameters in heavy-metal contaminated floodplain soil

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ABSTRACT

We used a combination of sampling and statistical approaches to investigate the relative influence of metals, soil acidity, and organic matter on a suite of analogous plant and microbial community parameters in floodplain soils contaminated by mine wastes in the early twentieth century. We compared the sensitivity of plant and microbial communities to environmental variables and to one another using constrained ordination analyses. Environmental factors accounted for a larger percentage of the total variance in microbial communities (56.2%) than plant communities (22.0%). We also investigated biological and geochemical changes that occurred along a short transect (64 cm) that spanned a transition from productive grassland to an area of barren wasteland representing a total functional collapse of the grassland/soil ecosystem. Along this small-scale transect we quantified geochemical parameters and biological parameters in two soil layers, an upper layer (0–10 cm) and a lower layer (10–20 cm). Results from the short transect indicated that soil respiration was not a strong indicator of underlying metal concentrations, but soil acidity was correlated in the upper and lower layers. PLFA profiles changed with distance along the gradient in the upper, but not the lower layer. Implications for remediation of contaminated floodplain soils are discussed.

Introduction

Heavy metal laden mine wastes contaminate river floodplains around the world (Marcus et al., 2001; Macklin et al., 2006). The toxicity associated with heavy metal contamination depends on the metal content of the wastes, as well as on associated soil characteristics such as acidity (pH) and soil organic matter (SOM) concentration, which together in large part determine the bioavailability of metals (Doelman and Haanstra, 1984; Bååth et al., 1998a,b; Lock and Janssen, 2001). Discrimination of the relative contribution of these factors could guide the choice of treatment options such as soil removal, capping, and lime or organic matter amendments that address these variables separately (Adriano et al., 2004). In laboratory studies, separation of the effects of these parameters can be accomplished through experimental manipulations (Speir et al., 1999a,b; Perkiomaki et al., 2003). However, the relevance of laboratory-scale studies to restoration decisions is questionable (Carpenter, 1996). Conversely, in the field, natural variability masks the effects of contaminants and hinders attempts to explore the basic ecological principles underlying the relationship between contaminants and community structure (Arnold and Wilding, 1991; Giller et al., 1998; Ettema and Wardle, 2002; Boivin et al., 2006). For these reasons, there is a need in community ecotoxicology for fresh approaches to discriminate contaminant effects from natural spatiotemporal variation under field conditions (Eijsackers et al., 2008), especially in long-term contaminated systems (Abaye et al., 2005).

In the present study, we used a combination of sampling and statistical approaches to investigate the influence of metals, pH, and SOM on a suite of plant and microbial community parameters in floodplain soils that were contaminated by mine wastes in the early twentieth century. The floodplain supports productive grasslands, wetlands, and scattered barren areas, referred to as

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"slickens". Slickens are areas largely devoid of vegetation and bearing a characteristic surface crust of metal salts due to the wicking and drying of soluble ions from the soil. These areas are of special interest from the standpoint of restoration because the salts blow off them and rainwater runoff carries the salts into surface water, representing a source of acute metal toxicity to fish (Nimick and Moore, 1991). Previously, we observed high metal concentrations in grasslands outside the slickens (twice as high as in slickens) and in functioning grassland systems (in terms of *in situ* soil respiration) close to the perimeter of slickens areas (Ramsey et al., 2005a). Inside the slickens, soil respiration drops to near zero and few if any plants survive. Thus, the transition from grassland to slickens represents an ecosystem collapse over a small spatial scale.

The current study had two objectives: (1) To compare the sensitivity and responses of plant and microbial community parameters to environmental variables and mine waste contamination using constrained ordination analyses. (2) To investigate biological and geochemical changes, which occur in the transition from grassland to slickens. These objectives allowed us to evaluate questions of relevance to the detection and restoration of contaminant effects, as well as to the ecology of contaminated systems. Specifically, (1) Are microbial or plant community parameters more sensitive indicators of contaminant effects? (2) How would we expect plant and microbial communities to respond to various treatments such as amendment with lime or organic material?

In previous work, we found that both increased metals and increased acidity suppressed and constrained variation in soil respiration (Ramsey et al., 2005a). Here, we analyzed two gradients, a floodplain-scale contamination gradient and a small-scale spatial gradient. For the flood-plain scale contamination gradient, study sites were selected using a stratified random sampling approach to acquire a set of sites representing the range of metal concentrations within the contaminated floodplain. Multivariate analyses of abundance variables were used to show community level responses to the contamination. Then we used constrained ordination to describe relationships between plant and microbial community variables, and to determine the amount of plant and microbial community variance that could be explained by metals, pH, and SOM (Cade and Guo, 2000; Ramsey et al., 2005a). Plant and microbial community structure was compared to determine what differences existed in the response of the two communities (Rutgers and Breure, 1999; Winding et al., 2005; Rutgers, 2008).

To further investigate the biological and geochemical changes that occurred in soil of the slickens areas, we excavated and sampled a transect (64 cm) that extended from grassland into slickens. We quantified geochemical parameters (heavy metal concentrations; soil acidity; SOM content; and soil moisture) and biological parameters (respiration; microbial PLFAs; microbial biomass; root biomass) in two soil layers, an upper layer (0–10 cm depth) and a lower layer (10–20 cm depth) across this transect. Analysis of the lower layer allowed us to evaluate whether factors acting deeper in the soil profile were correlated with trends on the surface, a possibility that could have confounded our analysis of the larger contamination gradient where we sampled to a depth of 10 cm.

Methods

Study area description

The study area was the riparian zone of the Clark Fork River as it flows through Grant-Kohrs Ranch National Historic Site, an active cattle ranch in Deer Lodge, Montana, USA. The site has been previously described in detail (Ramsey et al., 2005a,b). Briefly, heavy metal contaminated mine wastes were heterogeneously deposited by a large flood in 1908 that brought material downstream from copper mining operations around Butte, Montana (Moore and Luoma, 1990; Helgen and Moore, 1996). The mining spoils contained complex mixtures of elements in a sulfidic crystal matrix. The oxidation of sulfidic ores in the mine wastes cause soils to be acidic in some contaminated areas, but circumneutral, high metal soils are also present (Ramsey et al., 2005a). Historical photographs indicate that, initially, the wastes killed off almost all vegetation. Flood control measures emplaced in the 1950s as well as channel down-cutting through thick deposits of wastes have prohibited subsequent floods from depositing more material. Natural regeneration of vegetation has lead to a floodplain vegetated by grasslands, with patches of willows and scattered areas of low-pH tailings deposits referred to as slickens that remain largely unvegetated. Rainfall averages 34 cm y^{-1}.

Soil sampling

The study sites used in the flood-plain scale contamination gradient study were previously described as principal study sites in Ramsey et al. (2005b). All study sites were selected from a fenced riparian area paralleling the Clark Fork River. 30 sites were selected by stratified random sampling to acquire a range of metal contaminations without excessive sampling of areas with concentrations near the mean for the area, as described previously (Ramsey et al., 2005a). A stainless steel shovel was used to excavate four blocks of soil (40 cm by 40 cm by 10 cm deep) from the corners of a 1 m by 2 m rectangle oriented with the long axis east to west, centered on the original core location that was used to select study sites. The soil was bulked, homogenized, air-dried, and sieved (4 mm), after which sub-samples were taken for analysis of geochemical and microbiological variables. Results of the mean value of 3 laboratory replicates are reported.

To determine a location for the small-scale spatial gradient study, *in situ* soil respiration was measured along five transects leading from riparian grassland into slickens areas (soil respiration measurements are described below). Transects ranged in length from 64 cm to approximately 10 m. The 64 cm transect was selected for excavation because it displayed the most linear relationship between respiration and distance ($R = 0.978$, $F = 237$, $P < 0.001$). This transect was excavated in blocks (5 cm × 5 cm × 10 cm deep) in two layers, an upper layer (0–10 cm) and a lower layer (10–20 cm). Only the last respiration measure and soil sample were located entirely in the slickens. On the day of excavation, respiration values ranged from 5.5 μmol CO_2 m^{-2} s^{-1} in the riparian grassland to 1.3 μmol CO_2 m^{-2} s^{-1} inside the slickens. Based on previous experience with measurement of soil respiration in the area, the value of 5.5 μmol CO_2 m^{-2} s^{-1} was considered near the maximum that could be found at the study area given the season, soil moisture, and air temperature.

Geochemical analyses

Geochemical analyses are as described previously (Feris et al., 2003; Ramsey et al., 2005a). Briefly, U.S. EPA method 3050B was used to extract "total acid soluble metals." Dried, powdered, soil (5 g) was extracted with 12.5 ml each of trace-metal-grade HNO_3 and HCl. The extracts were diluted to 50 ml, refluxed at 95 °C for 1 h, shaken, and left overnight. An ICP-OES (IRIS model, Thermoelemental, Franklin, MA) was used to quantify metal content of extracts following U.S. EPA method 200.7. Metal concentrations were used to derive an empirical contamination index (MCI) where: $MCI = \Sigma((\log(Me_n)/\log(\text{background } Me_n)/\text{number of metals included in index } (5))$, where n represents As, Cd, Cu, Pb, and Zn. Background concentrations of the metals and metalloid were 10 mg As kg^{-1}, less than 1 mg Cd kg^{-1}, 16 mg Cu kg^{-1}, 17 mg Pb kg^{-1}, and 49 mg Zn kg^{-1} as determined from soil pits. Loss