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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 = Σ((log (Me_n)/log (background Me_n)/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

on ignition at 350 °C was used to quantify soil SOM (Nelson and Sommers, 1996). Standard methods were used to measure pH(H_2O) (Forster, 1995).

Biological measurements

Measurement of *in situ* soil respiration (CO_2 efflux) is described in detail in Ramsey et al., 2005a. Briefly, a portable Li-6400 (Licor Instruments, Lincoln, Nebraska) (Illeris et al., 2003) infrared gas analyzer fitted with a soil chamber (Licor 6400-09) was used to measure soil respiration. The soil chamber was fitted onto 8 cm diameter soil collars inserted 3–4 cm deep into soil in areas cleared of surface vegetation with scissors.

The standardized ocular macroplot method of Hann and Jensen (1987) was used to collect canopy cover data. Plot size and methods for the description of riparian communities correspond to the procedures used by Hansen et al. (1995). Plots were sampled from July 25 to August 14, 2001. Roots were sieved from the soils using a 0.5 mm sieve. Roots were weighed after drying at 60 °C (Cook et al., 1988). An estimate of microbial biomass was derived from the total microbial phospholipids extracted from the soil (Frostegård and Bååth, 1996). Microbial biomass was also estimated by chloroform fumigation (Horwath and Paul, 1994). Pesticide grade chloroform was used to extract sieved (4 mm) soil for 5 d at 25 °C and then extracted on a shaker for 1 h with 0.5 M K_2SO_4.

Extraction and analysis of microbial phospholipids (PLFA analysis) was conducted according to the protocol of Frostegård et al. (1993), and as previously described (Feris et al., 2003; Ramsey et al., 2005b). Briefly, a buffered chloroform/methanol solution was used to extract phospholipids from 5 g of soil. Phospolipids were then purified and esterified to methyl esters, and quantified by gas chromatography (White and Ringelberg, 1998). Only the 29 PLFAs present in all samples were used in analysis (Bååth et al., 1998a,b). To reduce the effect of biomass differences on the analysis PLFAs were expressed proportionally (mol%). Bacterial biomass was differentiated from microbial biomass by taking the sum of PLFAs thought to be primarily of bacterial origin (a15:0, i16:0, 16:1ω9c, cy17:0, i17:0, 18:1ω9c, and cy19:0) (O'Leary and Wilkinson, 1988; Frostegård and Bååth, 1996; Zelles, 1999). A fatty acid thought to be produced primarily by fungi, 18:2ω6,9c, was used to report fungal biomass (Frostegård et al., 1993; Frostegård and Bååth, 1996). The nomenclature of Frostegård et al. (1993) was used for naming fatty acids.

Statistical analyses

Biological and geochemical parameters were quantified at 30 sites selected using a stratified random sampling procedure to represent a wide range of contamination concentrations. In past studies we used MCI as a general measure of metal contamination because we lacked the statistical tools for analyzing the effects of multiple heavy metals on biological parameters at the same time. We used RDA to separate contributions of each metal to variation in the data. We found that As was responsible for most of the variation due to metals. Thus, we used As in place of MCI in our constrained ordination analyses.

Microbial PLFAs were expressed as molar percentages of individual fatty acids to correct for the influence of biomass. Average values from three sub-samples collected at each site were entered into the ordination analyses. One site was excluded from the analysis due the presence of only one plant species, which skewed the ordination analysis.

Initial detrended correspondence analysis (DCA) of the PLFA biomarker data indicated that a linear ordination method would be appropriate for analysis of the PLFA data (gradient lengths <0.7). The plant community data was transformed into a distance matrix using the Hellinger transformation as previously shown optimal for plant community data containing many blank cells (Legendre and Gallagher, 2001).

Table 1
Rangese of soil geochemical variables, and organic matter and Pearson's product moment coefficients and *P* values of the variables with the MCI ($n = 30$).

Variable	Range (mg kg^{-1})	R-MCI	*P*
Arsenic	32–880	0.891	<0.001
Cadmium	3–16	0.559	0.001
Copper	600–7100	0.885	<0.001
Lead	110–1100	0.788	<0.001
Zinc	720–2900	0.782	<0.001
Soil acidity (pH)	4.23–8.25	0.243	0.196
Organic matter (%)	0.9–14.6	0.434	0.017

PLFA and the transformed plant data were analyzed using redundancy analysis (RDA) (Legendre and Legendre, 1998) and distance-based RDA (db-RDA) (Legendre and Anderson, 1999), respectively. RDA and db-RDA are constrained ordination methods that allow for analysis of community structure data in relation to specific variables. We used the forward selection subroutine in CANOCO (ter Braak and Šmilauer, 2002) to determine the relative importance of environmental and community variables to the explanation of the variation in the PLFA and plant community data. Conditional effects, which indicate the order of inclusion, and amounts of variance explained in addition to previously added variables, of each environmental variable in the model were calculated and tested for significance using Monte Carlo permutation tests (499 permutations). Only environmental variables explaining significant amounts of variance ($P < 0.05$) in microbial or plant communities were retained in the models and tested for significance. We also determined and report the variance attributed to each variable independent of other environmental variables (marginal effects). For graphical purposes PLFA RDAs were constructed using the total PLFA profile data from each site, with selected PLFA markers overlaid on the plot as supplementary information in order to present coherent, uncluttered plots. Plant db-RDAs were constructed by using the distance matrix of the plant data as the species input, with the original plant species data overlaid on the plot as supplementary information for the purposes of data presentation.

For the small spatial gradient we had no *a priori* expectation (e.g., linear, unimodal) against which to fit the data, so we chose a locally weighted scatterplot smoothing procedure (Loess), a type of polynomial regression, to describe the shape of the relationship.

Results

Floodplain-scale contamination gradient

The concentrations of the principal toxic metals the metalloid As ranged from 32 mg As kg^{-1}, 3 mg Cd kg^{-1}, 600 mg Cu kg^{-1}, 110 mg Pb kg^{-1}, and 720 mg Zn kg^{-1} to 880 mg As kg^{-1}, 16 mg Cd kg^{-1}, 7100 mg Cu kg^{-1}, 1100 mg Pb kg^{-1}, and 2900 mg Zn kg^{-1} (Table 1). Least squares regression was used to evaluate the relationship between MCI and the metals used in the contamination index as well as the relationship between MCI, pH, and OM. All metals and the metalloid, As, were significantly correlated with MCI (all $P < 0.01$). Soil acidity ranged from 4.2 to 8.3 pH units. MCI and pH were not significantly correlated ($R = 0.243$, $F_{1,29} = 1.76$, $P = 0.196$). OM concentration ranged from 0.9% to 14.6%. MCI and OM were significantly correlated ($R = 0.434$, $F_{1,29} = 6.52$, $P = 0.017$). OM and pH were not significantly correlated ($R = 0.199$, $F_{1,29} = 1.15$, $P = 0.293$).

Fig. 1A and B shows PLFA and plant data vs. environmental variables, presented in RDA plots. Using forward selection, pH was found to account for the largest amount of total variance in the PLFA

Table 2
Relationships between environmental and biological variables.

	pH			SOM			As		
	% Variance	$F_{1,29}$	P	% Variance	$F_{1,29}$	P	% Variance	$F_{1,29}$	P
PLFA peak profile	28.7	10.88	**0.002**	9.6	2.88	**0.046**	17.9	5.87	**0.006**
Plant species coverage	9.7	2.89	**0.006**	5.0	1.43	0.114	7.3	2.13	**0.014**
PLFA Total Biomass	8.9	2.64	0.114	48.7	25.66	**0.002**	1.6	0.43	0.522
Plant Total Biomass	8.5	2.51	**0.013**	15.2	4.84	**0.040**	14.0	4.41	**0.046**
Forb Biomass	21.8	7.53	**0.014**	9.4	2.80	0.112	8.2	2.42	0.136
Graminoid Biomass	5.4	1.56	0.210	17.0	5.51	**0.030**	11.6	3.54	0.058
Bacterial Biomass	0.8	0.23	0.646	4.4	1.26	0.266	8.3	2.46	0.130
Fungal Biomass	0.0	0	0.970	3.1	0.88	0.368	0.0	0.00	0.956

Bold values indicate significant differences at an alpha of 0.05.

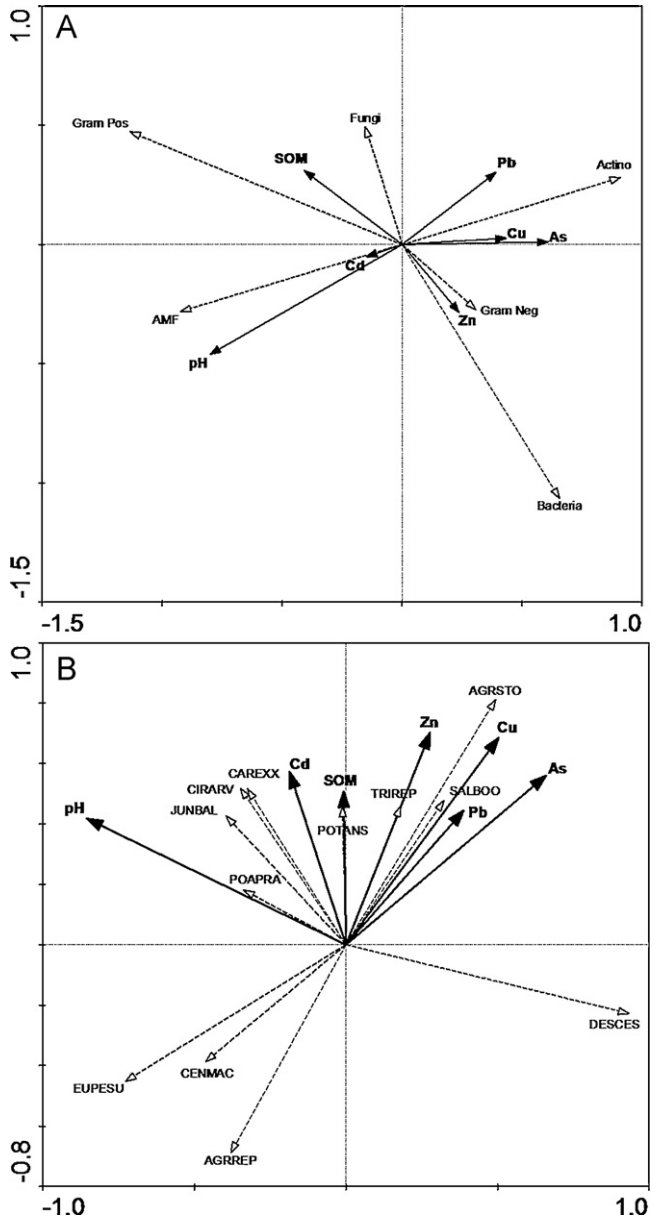

Fig. 1. RDA plots of relationships between environmental variables PLFA profiles (panel A) and plant species abundance (panel B). (A) The *x*-axis accounts for 42.7% of the total variance in the PLFA data and the *y*-axis accounts for 5.2% of the total variance. (B) The *x*-axis accounts for 11.1% of the total plant species variance and the *y*-axis accounts for 6.3% of the total variance. The dotted vectors represent the biological data and the solid vectors represent environmental parameters.

data (28.7%, $P = 0.002$), followed by As (17.9%, $P = 0.006$) and then organic carbon (9.6%, $P = 0.046$) (Table 2). Most of the total variance in the PLFA data and the variance due to the environmental–PLFA relationship were accounted for in the first ordination axis (42.7% and 77.5%, respectively). The second ordination axis accounted for only 5.2% of the total variance and 9.4% of the environmental–PLFA relationship. Both of the axes together accounted for 86.9% of the PLFA variance due to all measured environmental factors. Total PLFA biomass was significantly correlated with SOM (48.7% of variance, $P = 0.002$), but was not correlated with pH or As. Bacterial biomass and fungal biomass were not significantly correlated with any of the three measured environmental variables.

Fig. 1A shows that Gram-negative bacteria abundance increased with metals, while the opposite trend was found for Gram-positive organisms. AMF correlated positively with pH and SOM, while the marker for actinobacteria shows a negative correlation with pH. Fig. 1B shows the plant vs. environmental variables RDA plot. Using forward selection, pH was found to account for the majority of the variance in species data due to measured environmental factors (9.7%, $P = 0.006$), followed by As (7.3%, $P = 0.014$), and then SOM (5.0%, $P = 0.114$).

The first ordination axis accounts for 11.1% of the total species variance and 17.0% of the plant–environmental relationship, and the second axis accounts for 6.3% of the total variance and 10.2% of the plant–environmental relationship. Both of the axes combined account for 27.2% of the species variance due to measured environmental factors. Total plant biomass is correlated with SOM (15.2%, $P = 0.040$) and As (14.0%, $P = 0.046$), but not with pH. Forb Biomass is correlated with pH (21.8%, $P = 0.014$), but not SOM or As. Graminoid biomass is correlated with SOM (17.0%, $P = 0.030$), but not pH or As. Plant species richness correlated with pH (23.1%, $P = 0.016$), but not with SOM or As.

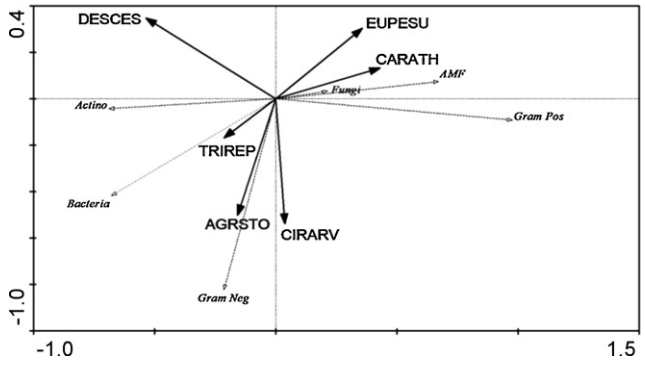

Fig. 2. RDA plot showing relationships between plant species abundance and PLFA profiles. The *x*-axis accounts for 70.8% of the total variance in the PLFA data and the *y*-axis accounts for 11.2% of the total variance. Dotted vectors represent the response variables and solid vectors represent the environmental variables.

Table 3
Pearson's product moment coefficients, *F*, and *P* values of soil geochemical and biological variables with distance along a 64 cm transect from grassland to slickens ($n = 13$). *P*-values significant at $\alpha = 0.05$ are in bold.

	Upper layer			Bottom layer		
	R	$F_{1,12}$	(*P*)	*R*	$F_{1,12}$	(*P*)
Geochemical variables						
Metal contamination index	0.013	0.002	0.967	**−0.579**	**5.54**	**0.038**
Soil acidity (pH)	**−0.764**	**15.4**	**0.002**	**−0.846**	**27.7**	**<0.001**
Organic Matter (%)	−0.237	0.657	0.435	0.451	2.81	0.122
Soil moisture (%)	−0.526	4.21	0.065	0.197	0.443	0.519
Biological variables						
Soil respiration (μmol CO^2 m^{-2} s^{-1})	**−0.978**	**237**	**<0.001**	–	–	–
Microbial community PLFA DCA 1	**0.751**	**14.2**	**0.003**	−0.244	0.7	0.422
Microbial community PLFA DCA 2	**0.621**	**6.89**	**0.024**	−0.181	0.37	0.554
Microbial biomass (nmol PLFA g^{-1})	−0.482	3.34	0.095	−0.364	1.69	0.221
Microbial carbon (μg g^{-1})	**−0.641**	**7.66**	**0.018**	−0.069	0.053	0.823
Bacteria biomass (nmol bacterial PLFAs g^{-1})	−0.395	2.03	0.182	−0.253	0.754	0.404
Fungal biomass (nmol fungal PLFA g^{-1})	**−0.706**	**10.9**	**0.007**	−0.376	1.81	0.205
Root biomass (g g^{-1})	−0.162	0.296	0.597	**−0.637**	**7.49**	**0.019**

Fig. 2 shows an RDA plot of the PFLA data vs. plant species abundance (in lieu of pH, MCI and SOM). The sedge species (CARATH) and leafy spurge (EUPESU) correlate positively with the fungal PLFA markers (total fungi and AMF) and Gram-positive bacteria, and they appear to correlate negatively with total bacterial biomass (Bacteria), actinobacteria and Gram-negative bacteria. Bentgrass (AGRSTO) and the Canada thistle (CIRARV) are positively correlated with Gram-negative bacteria. White clover (TRIREP) is positively correlated with total bacterial biomass. The first ordination axis accounts for 70.8% of the total variance in the PLFA data and the second axis accounts for 11.2% of the total variance. Together, the axes account for 90.4% of the total PLFA–plant variation. Tufted hairgrass (DESCES) was positively correlated with the actinobacteria and negatively correlated with Gram-positive bacteria and AMF. The booth willow (SALBOO) and bentgrass were positively correlated with bacteria. Leafy spurge and spotted knapweed were negatively correlated with bacteria.

Small-scale spatial gradient

The MCI did not vary significantly with distance across the upper layer of the 64 cm transect (Table 3, Fig. 3A). In the lower layer, the MCI declined slightly as the transect entered the slickens. Soil acidity was significantly correlated with distance in both the upper and lower layer of the transect (Fig. 3B). Soil acidity declined at about 40 cm along the transect, the lowest pH values were found in the upper (0–10 cm) layer inside the slickens. Soils in the surface layer of the slickens were more acidic than in the lower layer at all points along the transect. Neither soil organic matter or soil moisture were significantly correlated with distance in the upper or lower layers. Soil moisture was consistently greater in the upper layer than in the lower layer. The reported regression analysis was conducted using distance as the predictor variable. However, in all cases variables that were significantly correlated with distance were also significantly correlated with *in situ* soil respiration with nearly identical slopes and intercepts (data not shown).

Regression analysis indicated that in the upper layer the proportions of several monoenoic fatty acids (16:1ω7, 16:1ω7t, 16:1ω5, 16:1ω9, 15:1, 18:1ω7, 17:1ω7) were positively correlated with distance (data not shown). PLFAs thought to be of actinomycete origin (10-methyl-branched fatty acids) were strongly negatively correlated with distance. Microbial community structure was not significantly related to distance in the lower layer.

Microbial carbon (μg C g $soil^{-1}$), but not microbial biomass (nmol PLFA g $soil^{-1}$) was correlated with distance in the upper layer (Fig. 4B and F). A similarity in the trend lines of microbial biomass and soil organic matter concentration was noted (Figs. 3C and 4B).

Neither biomass estimate was significantly correlated with distance in the bottom layer. Bacterial biomass was not significantly correlated with distance in the upper or lower layer. In the upper layer, the fungal PLFA, 18:2ω6,9, was negatively correlated with distance in absolute terms (Table 3), but increased as a proportion of the total PLFAs detected. Root biomass declined significantly with distance in the lower layer, but not in the upper layer due to an anomalously high sample at 45 cm from the start of the transect.

Discussion

Comparison of sensitivity of plant and microbial communities to MCI, pH and OM

Overall, our the results of the larger-scale field study indicate that microbial community structure was more sensitive to the environmental factors than plant community structure because the measured environmental factors accounted for a larger percentage of the total variance in microbial communities (56.2%) than plant communities (22.0%). Forward selection for RDA analysis of the PLFA data showed that pH had the strongest influence on microbial community structure, a finding consistent with results from controlled experiments that have shown strong influences of pH on PLFA profiles (Hossain and Sugiyama, 2010; Rousk et al., 2010; Ganzert et al., 2011). SOM also had a significant influence over microbial community structure and correlated with an increase in fungal PLFA markers (Table 2), which is consistent with prior observations (Chu et al., 2010). The observed relative increase in monoenoic fatty acids in higher pH soils is consistent with an increase in Gram-negative bacteria (O'Leary and Wilkinson, 1988; Zelles, 1999; Kozdrój, 2008), while the fungal PLFA was highest in soils with high SOM that were also circumneutral, a finding similar to that observed by Kelly et al. (2003). Unlike the results reported in Galbraith et al. (1995), total plant biomass was significantly correlated with As ($P = 0.046$). Microbial biomass was not correlated with As ($P = 0.522$), which is consistent with the results of the soil amendment experiments of Speir et al. (1999a,b).

A potential drawback of our study design was that we used the same sampling sites for both plant and microbial community sampling. Factors affecting variability in plant and microbial community structure operate at different scales, and thus affect the suitability of sampling regimes for detecting the effects of contaminants (Broos et al., 2005). Initially, we thought that by integrating relatively large soil samples (4 blocks of soil 40 cm × 40 cm × 10 cm deep were combined and then sub-sampled), we would reduce variability due to the effect of natural heterogeneity in microbial community structural parameters.

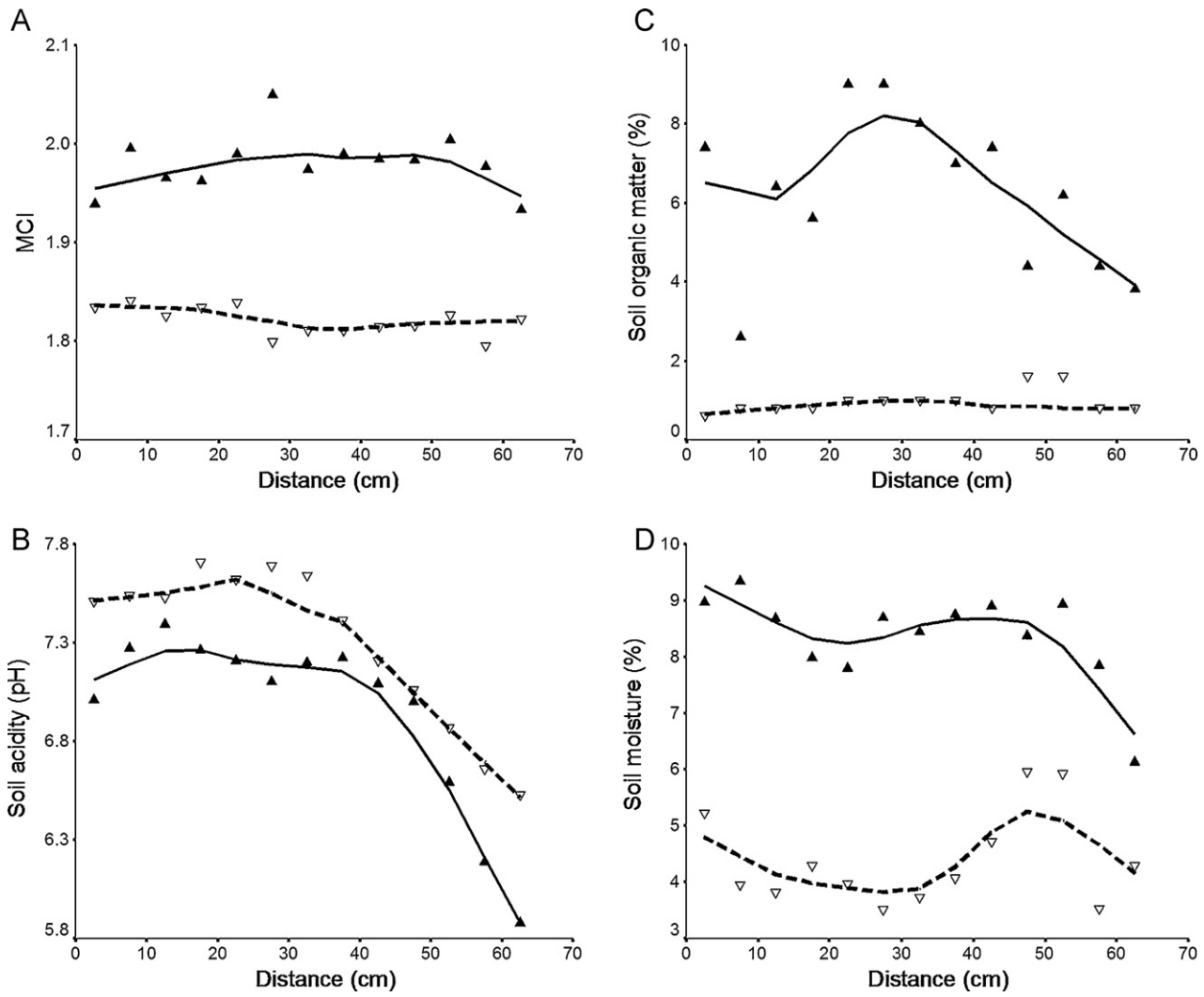

Fig. 3. Soil geochemical variables (MCI, pH, OM, and soil moisture in panels A, B, C, D respectively) with distance along the small-scale transect. Trends are indicated by Loess regressions ($n = 13$, point fit = 50%, iterations = 3). Trends in the upper layer (0–10 cm) are indicated by solid lines. Trends in the lower layer (10–20 cm) are indicated by broken lines. Statistics for linear regressions of the variables with distance are given in Table 3.

A previous study (Ramsey et al., 2005b), in which we used smaller study sites for sampling microbial parameters, yielded tighter correlations between microbial community structure and geochemical parameters. Direct comparisons to the findings of Ramsey et al. (2005a) are obscured by a difference in the range of MCI values in the samples. In Ramsey et al. (2005a,b) the MCI range was 1.1 to 2.3 units, here the MCI ranged from 1.6 to 2.3. Thus, responses here were observed along a shorter metal contamination gradient than the previous study where less-contaminated study sites from a downstream area were included (plant community data for these sites was not collected). Because the downstream sites were not included, the samples analyzed here were all close to or above the thresholds at which responses were detected in previous studies.

Geochemical and biological change along the small-scale spatial gradient

Metal contaminant levels were not correlated with respiration in the upper layer along the transect and were slightly negatively correlated in the lower layer (Table 3, Figs. 3 and 4). The correlation between respiration and the metal contamination index in the lower level is likely to be specious and due to low variance in the metal concentrations rather than a causal relationship. This result indicates that the readily visualized slickens areas are not reliable indicators of high metal concentrations. It is likely that the combined effects of soil acidity and organic matter concentration on metal availability contributed to the observed decline in soil function and microbial carbon along the transect. Microbial biomass increased to 45 cm, then declined sharply as the transect entered the slickens, whereas microbial carbon was more variable but linear in decline. The partial non-linearity of the results are challenging to interpret and highlight the complexity of microbial processes over even very short spatial scales (Broos et al., 2005).

Geochemical factors operating through the soil profile interacted with metal concentrations to affect microbial community structure and function, but the effects on soil function in the lower layer did not appear to be strong. A depth integrated soil profile was needed to rule out the possibility that surface trends were caused by deeper subsurface characteristics. We conclude that the depth of cores in the larger scale study were adequate because analysis of the upper layer alone would have been sufficient to draw inferences regarding the interactions of the geochemistry, and microbial community structural and functional relationships.

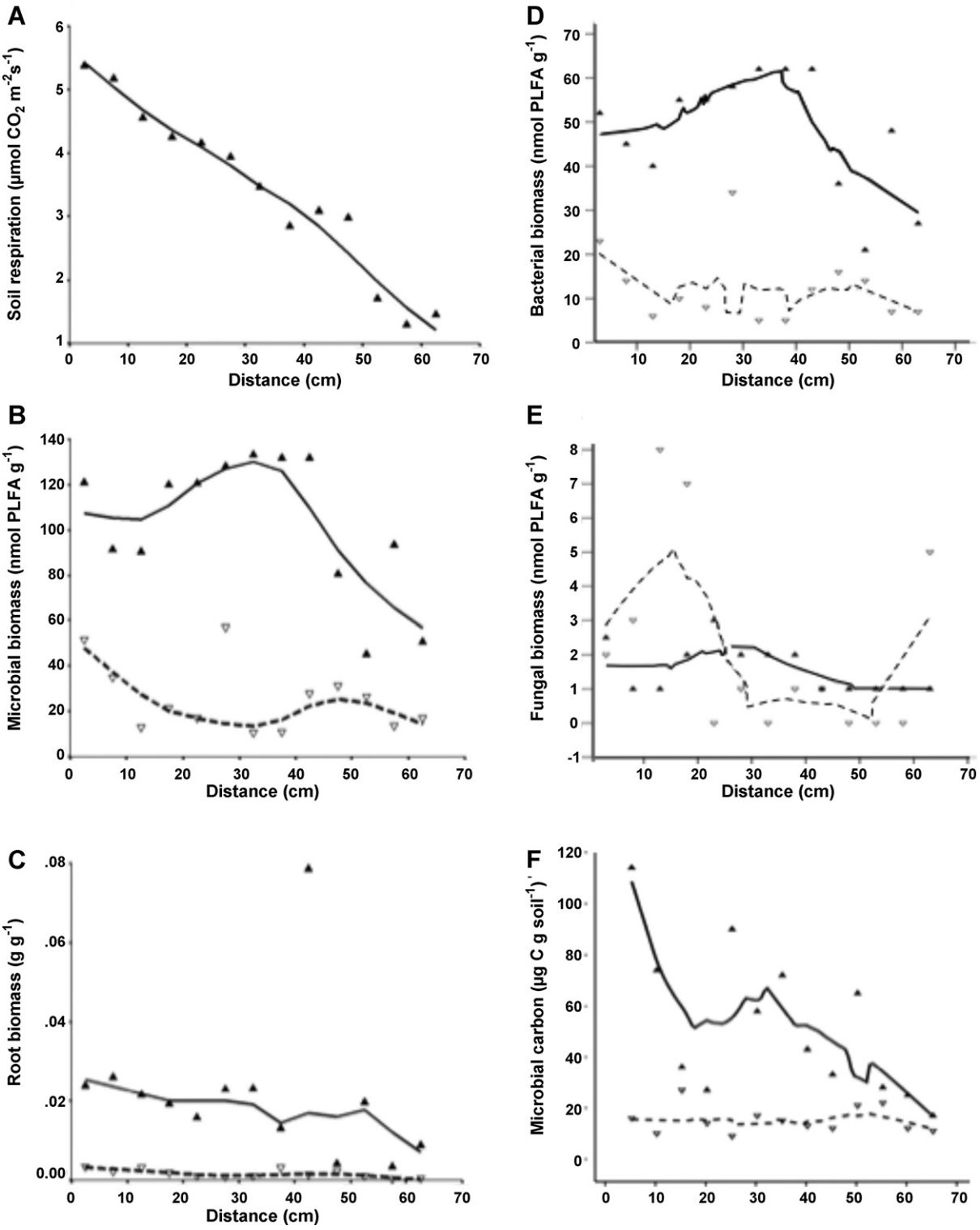

Fig. 4. Soil biological variables (*in situ* soil respiration, microbial, bacterial, fungal and root biomass) with distance along the small-scale transect. Trends are indicated by Loess regressions ($n = 13$, point fit = 50%, iterations = 3). Trends in the upper layer (0–10 cm) are indicated by solid lines. Trends in the lower layer (10–20 cm) are indicated by broken lines. Statistics for linear regressions of the variables with distance are given in Table 3.

Application to mine waste restoration

Multiple correlated variables often complicate the separation of individual effects in contaminated and pristine environments (Speir et al., 1999a,b; Broos et al., 2007). Here, however, the absence of a significant relationship between heavy metal concentration and soil acidity, as well as large variability in metal concentrations, soil acidity, and organic matter concentration, allowed us to make reasonable inferences regarding which factors exert the strongest effects on biotic communities. Natural resource damage assessments that include analysis of limiting factors will facilitate rational decision-making and remediation strategies that make the

most efficient use of resources and increased ability to monitor the success of restoration efforts. For instance, if increased plant species richness were identified as the objective of restoration efforts then neutralization rather than removal of soils could be the most cost effective remediation option because pH, rather than metals or SOM, has the strongest relationship with plant community richness (Table 2). Critchley et al. (2002) also found that soil pH was the strongest driver of plant community richness. Increased plant community richness could then be expected to benefit general ecosystem function (Loreau et al., 2001; Zhang et al., 2007; Farrell et al., 2010).

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