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LETTER

Relationship between communities and processes; new insights from a field study of a contaminated ecosystem

Philip W. Ramsey,[1]* Matthias C. Rillig,[1] Kevin P. Feris,[2] Nathan S. Gordon,[1] Johnnie N. Moore,[3] William E. Holben[1] and James E. Gannon[1]

[1]Microbial Ecology Program, The University of Montana, Missoula, MT, USA

[2]Biology Department, Boise State University, Boise, ID, USA

[3]Geology Department, The University of Montana, Missoula, MT, USA

**Correspondence: E-mail: philip@hydreion.com*

Abstract

We used a 93-year-old mine waste contamination gradient in alluvial soil to explore the relationship between ecosystem level functioning and community structure in a chronically stressed ecosystem. The sensitivity of broad functional parameters (*in situ* soil respiration, microbial biomass, above and below ground plant biomass) and microbial diversity [phospholipid fatty acid (PLFA) abundance and richness] were compared. Functional responses were linear with respect to contaminants while thresholds were detected in the community structural response to contamination along the gradient. For example, *in situ* soil respiration was negatively and linearly correlated to contamination concentration ($R = -0.783$, $P < 0.01$), but changes in microbial community structure only became evident where contaminant concentrations were greater than 28 times above background levels. Our results suggest that functional redundancy does not prevent depression of ecosystem function in the long-term.

Keywords

Anthropogenic stress, breakpoint regression, chronic stress, ecosystem function, *in situ* soil respiration, long-term stress, microbial community structure, phospholipid fatty acid, segmented linear regression.

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INTRODUCTION

Understanding interactions between ecosystem-level functioning and community structure in contaminated systems will enable us to make better predictions of the consequences of anthropogenic stress (Western 2001; Loreau *et al.* 2002; Wardle *et al.* 2003). Most studies have accessed questions regarding toxicity through experimental amendments of toxins with responses measured over short periods spanning from days to at most a few decades. Yet, ecologically relevant response times to chronic stress may be much longer, on the order of centuries, as new conditions drive changes in diversity (Woodruff 2001). Therefore, a need exists for model systems where predictions derived from short-term experiments can be tested in more realistic settings. Here, we used a 93-year-old mine-waste contamination gradient in alluvial soil as a model to test the hypothesis that community level structural response to toxins leads to the maintenance of ecosystem function under conditions of chronic stress.

Soils have been used to test ecosystem theory because the biological communities in soils are highly diverse (Øvreås 2000). In addition, multi-generational effects can be studied in manageable periods (Wardle *et al.* 2003). A conceptual model of the response of microbial communities to heavy metal contamination has been developed (Salminen & Haimi 1996; Bååth *et al.* 1998) that is similar to the functional redundancy concept advanced by Schwartz *et al.* (2000). It is predicted that when contamination in a soil ecosystem causes toxic stress, functional stability in terms of mass and activity is maintained by community-level replacement of sensitive populations with more tolerant ones. Thus, change in community structure may not be accompanied by impaired soil function or biomass change at low or moderate contamination concentrations because the expansion of tolerant populations compensates for the contraction (in mass and activity) of intolerant populations (Pennanen *et al.* 1996). Depression of soil function is expected at pollutant concentrations where toxic effects overcome the redundancy of functional groups within the soil microbial community (Giller *et al.* 1998).

Six variables related to ecosystem functioning were measured along the contamination gradient. *In situ* soil

respiration (efflux of CO_2 from the soil), an integrated measure of the activity of roots, soil microbes and soil fauna, was used to measure decomposition (Hanson *et al.* 2000). Soil microbial biomass, measured as total microbial carbon and total phospholipids, was measured as an ecosystem property that drives decomposition and regulates the availability of nutrients to plants (Jenkinson & Ladd 1981). We also measured above ground plant biomass, root biomass and litter biomass as related drivers of ecosystem function along the gradient (Wardle 2002).

Phospholipid fatty acid (PLFA) analysis was used to quantify the effects of the mine wastes on soil microbial community structure. Variability in fatty acid structures among phylogenetic groups of prokaryotes and eukaryotes is reflected in the PLFA profile of a soil sample, providing a picture of the structure of the total soil community (see Bååth *et al.* 1998; Øvreås 2000). Principal components analysis of biomass corrected fatty acid concentrations indicates changes in the relative abundance of microbial populations (White & Ringelberg 1998). Further, the number of PLFA peaks in a profile relates to the richness of the microbial community, as the loss or addition of populations with unique or unusual PLFAs is reflected in the loss or addition of detected PLFAs (Federle *et al.* 1986; Fierer *et al.* 2003).

The functional and structural variables were used to test the hypothesis that ecosystem functional properties are maintained through functional redundancy of communities in long-term contaminated systems. The hypothesis would be supported if soil respiration and microbial and plant biomass were less sensitive to contaminant concentrations along the gradient than microbial community structure (Fig. 1a). If this were the case, we would have expected to find thresholds in the functional variables, above which responses could be attributed to contamination effects, but below which the contaminant effects could not be separated from background variability. Instead, we found thresholds in the community structural parameters while the functional parameters were linear with respect to contamination. Our results suggest that low to moderate concentrations of contaminants currently exert slight direct effects on microbial community structure, but evidence of toxic stress is still expressed in terms of depression of ecosystem function (Fig. 1b).

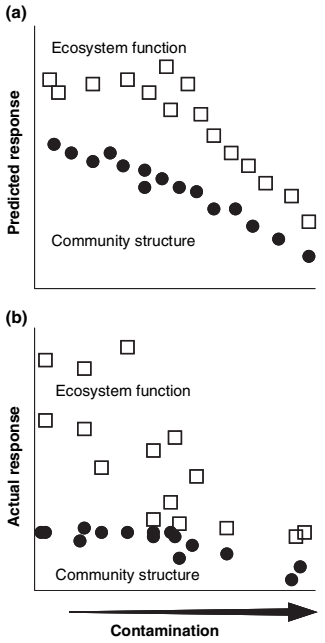

Figure 1 (a) Predicted response in a system where the established organisms have been exposed to the selection pressure presented by a contaminant. Depression of ecosystem level functions (open squares) such as respiration and biomass accumulation will be offset at low to moderate contamination concentrations by more gradual change in community structure (closed circles) as tolerant populations replace more sensitive ones along a contamination gradient. (b) Data collected from a 93 year old contamination gradient in alluvial soil. Soil respiration and microbial community PLFA PC1 data are shown with error bars and statistics in Figs 2a and 3a, respectively. PLFA PC 1 was transformed by the equation: $y = -y$, to better demonstrate the relationship between the data and the model.

MATERIALS AND METHODS

Study site

The study site and the history of the area are described in detail in Ramsey *et al.* (2005) and Moore & Luoma (1990), respectively. Briefly, mine-wastes containing high concentrations of arsenic, cadmium, copper, lead, and zinc were heterogeneously deposited over the study area at Grant-Kohrs Ranch National Historic Site, Montana, around the turn of the century by floods that carried the wastes downstream from mining operations around Butte, Montana. The material transported by the floods, especially a 500-year flood in 1908, raised the level of the valley floor by at least a meter. Subsequent channel down-cutting through the wastes has prohibited floods from topping the channel banks, and flood control and waste containment measures have kept large amounts of new material from being introduced to the system. Historical photographs show that almost all plant life on the valley floor was killed initially by the wastes. Subsequent re-colonization of the valley floor by plants has resulted in a floodplain consisting of grassland with patches of willow. Scattered areas, referred to as slickens, contain high concentrations of contaminants and are largely non-vegetated.

Sampling design

Due to the nature of fluvial deposition, contaminant concentrations vary over a small area, with most areas only

mildly contaminated (Helgen & Moore 1996). Fifteen study sites were selected using a stratified random sampling procedure to represent the range of contaminant concentrations without excess sampling of mildly contaminated areas. Copper concentration values from 100 soil cores taken to a depth of 20 cm in a 200 m grid across the study area were portioned into four sub-ranges. A random number table was used to select three to four cores from within each range. An additional selection criterion that the concentrations of metals other than copper behave broadly similarly was applied to ensure that sites had similar geochemical characteristics. Sampling plots (3 m by 3 m) were established at each selected core location. From each plot, three sets of three soil cores taken to a depth of 10 cm were pooled at the site and taken for geochemical and biological analysis. Soil geochemical measurements were made, 14–18 September 2000. Soil respiration was measured four times during the summer and fall of 2000 on 14–18 September, 26–30 September, 11–16 October, and 2–3 November. Soil biological measurements were made 14–18 September 2000 and 11–16 October 2000. Retrospectively, plant materials were sampled once on 20 July 2004 to evaluate the influence of plants on the other quantified variables.

Geochemical analyses

Total acid soluble metals were recovered from soil samples by US EPA method 3050B. Five grams of dried, homogenized, powdered soil were extracted with 12.5 ml each of trace metal grade HNO_3 and HCl. Samples were refluxed at 95 °C for 1 h, then shaken and allowed to settle overnight before analysis on an ICP (IRIS model; Thermoelemental, Franklin, MA, USA) by the US EPA test method 200.7. The method is considered a near total digest for metals bound to soil surfaces, iron oxides, and organic matter, but major elements bound in silicate minerals are not released. Soil organic matter was analyzed by loss on ignition at 350 °C (Nelson & Sommers 1982). Soil moisture and pH were measured by standard methods (Forster 1995).

Contamination index

An empirical Contamination Index (CI) was used as a single predictor variable to quantify the amount of mine waste contamination at each site (Feris *et al.* 2003). The index was derived by taking the sum of the concentrations of the principal toxic contaminants contained in the wastes (As, Cd, Cu, Pb, and Zn) divided by the respective background values of each metal and then dividing the sum by the number of metals included ($n = 5$). The index, therefore, expresses contamination concentration in terms of multiples of the background metal concentration. Background values for the contaminants were determined from depth profiles in soil pits excavated to the historic floodplain surface. The background values 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} (Ramsey *et al.* 2005).

Soil respiration

A Li-6400 (Licor Instruments, Lincoln, NE, USA) infrared gas analyzer with a 6400–09 soil chamber was used to quantify soil respiration *in situ*. The chamber was mounted on 80 cm diameter PVC collars installed 3 cm deep into areas cleared of surface vegetation with scissors. The Li-6400 uses soda lime to draw-down CO_2 concentration in the chamber to a prescribed concentration below the ambient CO_2 concentration and then measures the time taken for CO_2 evolution from the soil surface to refill the chamber to a prescribed concentration above the ambient CO_2 concentration. Soil respiration (μmol CO_2 m^{-2} s^{-1}) is calculated from six cycles of CO_2 draw-down and refill. Soil temperature, an important co-variate of respiration, is a concomitant measure made by the Li-6400 system.

Microbial biomass

Microbial biomass was estimated by the chloroform fumigation method of Horwath & Paul (1994), and as the total moles of microbial phospholipids detected in each sample (Frostegård & Bååth 1996). Soil samples for chloroform fumigation were passed through a 4 mm sieve, then fumigated with pesticide grade chloroform for 5 days at 25 °C and then extracted on a shaker for 1 h with 0.5 M K_2SO_4.

Phospholipid fatty acid analysis

Phospholipids were extracted and analysed according to the protocol of Frostegård *et al.* (1993), and as previously described in (Feris *et al.* 2003). PLFAs from 5 g of soil were dissolved into a buffered chloroform/methanol solution, purified, and then quantified by gas chromatography (White & Ringelberg 1998). A principal components analysis of microbial PLFAs was used to quantify community structural differences (Bååth *et al.* 1998; Wardle *et al.* 2003). The 30 most abundant PLFAs present in each sample were used in the principal components analysis (Bååth *et al.* 1998). To remove the effect of biomass differences on the analysis, principal components analysis was performed with individual PLFA values expressed as a proportion of the total moles of PLFA in a sample. Microbial community richness was reported as total number of fatty acid peaks identified in each chromatogram (Fierer *et al.* 2003). Fatty acids are

named using the nomenclature described in Frostegård *et al.* (1993).

Changes in the abundance of specific populations of microbes were inferred from changes in the abundance of specific fatty acids that have been used as signature lipids as follows. Gram-negative bacteria were tracked with monoenoic and cyclic fatty acids (18:1ω9*c*, 16:1ω9*c*, *cy*17:0, and *cy*19:0) (O'Leary & Wilkinson 1988; Zelles *et al.* 1992). Several branched fatty acids (a15:0, i16:0, and i17:0) were used as markers for Gram-positive bacteria (O'Leary & Wilkinson 1988; Zelles *et al.* 1992). 10*me*16:0, 10*me*17:0, and 10*me*18:0 were used as indicators of Actinomycetes (Kroppenstedt 1985). The fatty acid 18:2ω6,9*c* was used to indicate the abundance of fungi (Frostegård *et al.* 1993, 1996).

Plant biomass measurements

Litter biomass, root biomass and above-ground plant biomass measurements were made in three 0.25 m^2 plots within each study site. Above-ground biomass was measured by clipping all standing vegetation from the plots, drying at 60 °C, and weighing. A 2.5 cm diameter soil corer was used to collect three 10 cm deep cores from each plot for root biomass measurement. Cores from each plot were compiled in the field for three measurements per site. Roots were sieved from the soils using a 0.5 mm sieve, dried at 60 °C, and weighed (Cook *et al.* 1988). Litter biomass measurements were obtained by raking all the litter from the plots after clipping for the standing biomass measurement.

Data analysis

No attempt to isolate the effects of individual contaminants was made, as the intent was to quantify ecosystem and community level effects of the introduced contamination, rather than to isolate effects of individual contaminants. SPSS v. 10, SegReg v. 1, and Excel for Microsoft Windows XP was used for statistical analyses. SegReg (http://www.waterlog.info/segreg.htm) was used to conduct segmented linear regression to identify breakpoint concentrations at which contaminant effects on quantified variables became detectable (Neter *et al.* 1996). The regression program uses an algorithm that tests multiple data fits to locate the best breakpoint. The programme was used to identify portions of response variable distributions that were flat followed by sloped portions. Break points suggested by the SegReg program were evaluated in SPSS by performing regression analysis on the response variable distributions lying to the left and the right of the suggested breakpoint. Slopes to the right of the breakpoint suggested by the SegReg program were found to be insignificantly different from zero in all cases.

RESULTS

Concentrations of the principal contaminants (As, Cd, Cu, Pb, and Zn) ranged from 26 mg As kg^{-1}, 1.3 mg Cd kg^{-1}, 110 mg Cu kg^{-1}, 30 mg Pb kg^{-1}, and 86 mg Zn kg^{-1} at the least contaminated sampling location to 450 mg As kg^{-1}, 8.1 mg Cd kg^{-1}, 4600 mg Cu kg^{-1}, 400 mg Pb kg^{-1}, and 2300 mg Zn kg^{-1} at the most contaminated sampling locations. The contamination concentrations represent a range of approximately four to 77 times above background levels as indicated by the CI range of 4.33–77.1 (mean = 36.2, SE = 5.76). Important mediators of metal bioavailability and toxicity (soil acidity, organic matter and moisture) were correlated with the CI (Table 1). Soil acidity ranged from pH 4.9–8.3 (mean = pH 6.96, SE = 0.252) and was correlated with the CI ($R = -0.80$, $P < 0.001$). Soil moisture ranged from 8.6% to 50% (mean = 20.5%, SE = 3.0) and was correlated with the CI (R = −0.55, $F = 5.5$, $P = 0.035$). Soil organic matter ranged from 3.1% to 16.1% (mean = 8.8%, SE = 1.3) and was correlated with CI ($R = -0.66$, $F = 9.8$, $P = 0.008$).

The relationship between soil respiration and the CI was monitored at four times spanning a period from late summer to late fall during a dry year (Table 2). Respiration values ranged from high values near 10 μmol CO_2 m^{-2} s^{-1} in the least contaminated sites (four times background metal levels; 364 mg total contaminants kg^{-1}) to less than 1 μmol CO_2 m^{-2} s^{-1} in the most contaminated sites (77 times background metal levels; 7470 mg total contaminants kg^{-1}). The correlation and slope of the relationship between soil respiration and CI was highest at the first measurement period (14–18 September) when soil temperatures were the warmest ($R = -0.783$, $F = 21$, $P = 0.001$, Slope = −0.15) (Fig. 2a). Soil temperatures declined from a mean of 14.6 °C, SE = 0.038, to 3.03 °C, SE = 0.018, between the first and last respiration measurement period, as the soil respiration values declined from an average for all sites of 5.23 μmol CO_2 m^{-2} s^{-1} (1.06 SE) to 0.81 μmol CO_2 m^{-2} s^{-1} (0.17 SE). The linear relationship remained

Table 1 Ranges of soil geochemical variables, organic matter, and soil moisture and Pearson's product moment coefficients of the variables with the CI ($n = 15$, all $P < 0.05$)

Variable	Range	R-MCI
As (mg kg^{-1})	(2.6E+1 to 4.5E+2)	0.77
Cd (mg kg^{-1})	(1.3E+0 to 8.1E+0)	0.52
Cu (mg kg^{-1})	(1.4E+2 to 4.6E+3)	0.99
Pb (mg kg^{-1})	(4.1E+1 to 4.0E+2)	0.76
Zn (mg kg^{-1})	(1.1E+2 to 2.3E+3)	0.79
Acidity (pH)	(4.89–8.28)	−0.80
Organic matter (%)	(3.07–16.1)	−0.66
Moisture (%)	(8.25–50.1)	−0.55

Table 2 Average soil temperature and soil respiration across four measurement periods ($n = 6$ measurements at $n = 15$ plots), correlation coefficients, F-values, slopes, and y-intercepts of soil respiration with CI

Date	Soil temperature, average °C (SE)	Respiration, μmol CO_2 m^{-2} s^{-1} (SE)	Pearson's R (P-value)	F	Slope	Intercept
14–18 September	14.6 (0.038)	5.23 (1.06)	−0.783 (0.001)	21	−0.15	10.5
26–30 September	10.1 (0.034)	2.32 (0.47)	−0.737 (0.002)	15	−0.06	4.50
11–16 October	7.54 (0.013)	1.97 (0.34)	−0.697 (0.004)	12	−0.04	3.48
2–3 November	3.03 (0.018)	0.81 (0.17)	−0.580 (0.023)	6.6	−0.02	1.43

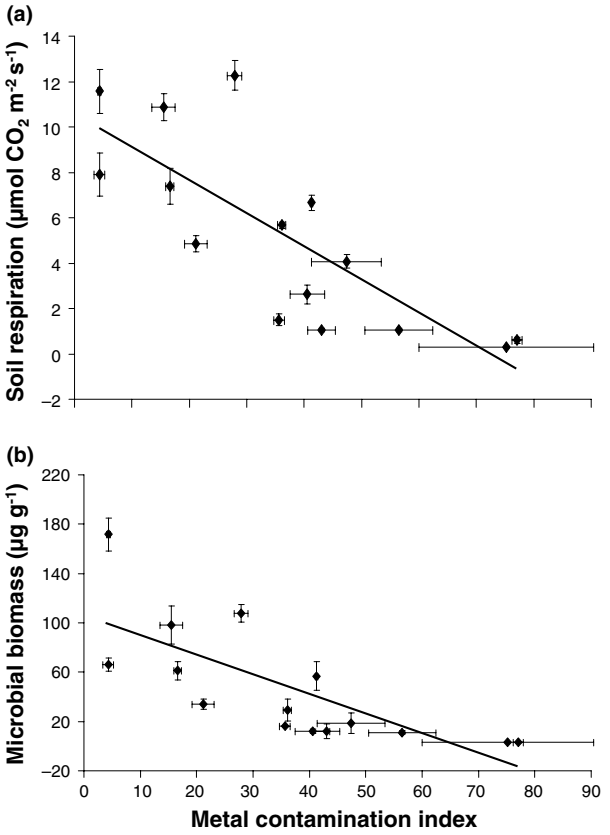

Figure 2 (a) Soil respiration (y) as a function of the contamination index (x) on 14–18 September. Vertical and horizontal bars represent 1 SE ($n = 6$). The regression: $y = -0.145x + 10.5$ ($R = -0.783$, $P = 0.002$, $n = 15$). (b) Microbial biomass (y) as a function of the contamination index (x) on 14–18 September. Vertical and horizontal bars represent 1 SE ($n = 3$). The regression: $y = -1.60x + 105$ ($R = -0.741$, $P = 0.002$, $n = 15$).

significant through the last measurement period on 2–3 November ($R = -0.580$, $F = 7$, $P = 0.023$). Breakpoints (flat followed by sloped response variable distributions) with respect to the CI were not detected.

Microbial biomass, as estimated by the chloroform fumigation/extraction method (once) and by total microbial PLFAs (twice), was negatively correlated to the CI (Table 3). The relationship was negative and linear over the CI range. Breakpoints in microbial biomass with respect to the CI were not detected by either method (Fig. 2b). Both methods indicated a negative relationship between microbial biomass and CI.

At the first sampling period, 14–18 September, microbial PLFAs ranged from 362 to 50 nmol g^{-1} (mean = 156 nmol g^{-1}, SE = 22) while at the second soil sampling period, 11–16 October, microbial PLFAs ranged from 226 to 60 nmol g^{-1} (mean = 141 nmol g^{-1}, SE = 14). Over the *c.* 1 month interval between measurements, the mean soil temperature dropped by 7 °C from 14.6 °C to 7.5 °C (Table 1). The strength of the relationship between PLFA biomass and the CI increased over the time interval from $R = 0.663$, $P = 0.007$, to $R = 0.810$, $P \geq 0.001$), as the slope declined from −2.5 to −1.9.

Principal components analysis of microbial phospholipids followed by linear and break point regression were used to explore the relationship between microbial community structure and the CI. PLFA PC 1 accounted for 31% and 35% of the variation in the data set at the first and second measurement periods, respectively. No significant relationships were found between principal component axes 2, 3, or 4 and the CI at either soil sampling, indicating that PLFA PC 1 was the only axis to describe a contaminant effect. A breakpoint in PLFA PC 1 with respect to the CI was detected at 29.8 CI units in the first measurement period and at 27.6 CI units in the second (Table 3). To the left of the break points (data $x <$ BP) the slope and P-value of the regression lines for PLFA PC 1 with respect to the CI were flat and insignificant, while to the right of the break points (data $x >$ BP) slopes and P-values were steep and significant (Table 3, Fig. 3a).

Factor loadings for specific fatty acids were as follows: fatty acids with negative factor loadings indicating enrichment in low CI samples were monoenoic fatty acids (18:1ω9*c*, 16:1ω9*c*, 16:1ω7*c* 16:1ω7*t*, 16:1ω5*c*), cyclic fatty acids (*cy*17:0, *cy*19:0), fatty acids methylated at the tenth carbon from the aliphatic end (10*me*16:0, 10*me*17:0, and 10*me*18:0), and the branched PLFA, *i*15:0. Several of the monoenoic fatty acids and the cyclic fatty acids are biomarkers for the Gram-negative bacteria. The methylated

Table 3 Break points (BP), correlation coefficients, and slopes of replicated biological parameters with CI

Date	Measuremt	Break point	All data				Data (x < BP)				Data (X > BP)			
			R	P-value	Slope	N	R	P-value	Slope	N	R	P-value	Slope	N
14–18 September	Microbial carbon (μg g^{-1})	–	0.741	0.002	−1.6	15	–	–	–		–	–	–	
14–18 September	Total PLFAs (ng g^{-1})	–	0.663	0.007	−2.5	15	–	–	–		–	–	–	
11–16 October	Total PLFAs (ng g^{-1})	–	0.810	0.001	−1.9	15	–	–	–		–	–	–	
14–18 September	PLFA PC 1	29.8	0.839	0.00	0.036	15	0.003	> 0.99	−9.80E-05	6	0.860	0.003	0.054	9
11–16 October	PLFA PC 1	27.62	0.556	0.03	0.025	15	0.343	0.40	−3.5E-2	5	0.609	0.062	0.038	10
14–18 September	Microbial species richness	47.99	0.592	0.02	−0.18	15	0.136	0.19	−.047	12	0.755	0.456	−0.40	3
11–16 October	Microbial species richness	55.27	0.725	0.00	−0.18	15	0.371	0.24	−.089	12	0.989	0.098	−0.60	3

fatty acids are biomarkers for the actinomycete group of Gram-positive bacteria. Fatty acids with positive factor loadings indicating enrichment in higher CI samples were saturated fatty acids (14:0, 15:0, 16:0, 17:0), several branched fatty acids (a15:0, i16:0, i17:0), and the fatty acid 18:2ω6,9*c*. The branched fatty acids and 18:2ω6,9*c* have been identified as biomarkers for Gram-positive bacteria and fungi, respectively.

Microbial community richness, as estimated by PLFA peak number, was also correlated with CI (Fig. 3b). Significant negative linear relationships between richness and the CI were detected with all data included in the analysis ($R = 0.592$, $P = 0.02$, and $R = 0.725$, $P < 0.001$, for 14–18 September and 11–16 October, respectively). Breakpoints were detected at 47.9 richness units and 55.3 richness units for the two sampling periods respectively. Slopes and P-values of regression lines for data $x <$ BP were flat and insignificant. However, because of the small number of points ($n = 3$) falling to the right of the breakpoint, the regression lines for data $x >$ BP were insignificant too. This indicates that effects of the contaminants on microbial community richness were only marginally detectable and then at only the highest CI values.

Above-ground plant biomass was negatively linearly correlated with the CI: $y = -2.73x + 223$ ($R = -0.66$, $P = 0.013$, $n = 15$). Root biomass was also negatively linearly correlated with the CI: $y = -0.560x + 47.6$ ($R = -0.52$, $P = 0.046$, $n = 15$). Litter biomass was not linearly correlated with CI. A trend between litter accumulation and the CI was indicated by quadratic regression ($R = 0.61$, $P = 0.059$) in which litter tended to accumulate more at sites with intermediate CI values. No breakpoints in plant biomass parameters with respect to the CI were detected.

Regressions were used to evaluate the relationship between microbial and plant biomass, respiration, and microbial community structure. Microbial biomass (total PLFAs) was tightly correlated with soil respiration (for the 14–18 September 2000 measurement period $R = 0.921$, $F = 72$, $P < 0.001$). The correlation of respiration for the 14–18 September 2000 measurement to the retrospectively measured plant variables was: $R = 0.547$, $F = 5.6$, $P = 0.036$ and $R = 0.503$, $F = 4.41$, $P = 0.056$ for above ground and root biomass, respectively. Microbial biomass was less well correlated with PLFA PC 1 than with respiration or the CI (for the 14–18 September measurement period $R = 0.566$, $F = 6.1$, $P = 0.027$). The regression analysis indicates that the relationship between PLFA PC 1 and the CI was not due solely to biomass or activity differences.

DISCUSSION

We explored the relationship between ecosystem-level functioning and community structure by use of a 93-year-old mine-waste contamination gradient in alluvial soil. The sensitivity of broad functional parameters (*in situ* soil respiration, microbial and plant biomass) and patterns of microbial diversity in terms of abundance and richness were used to evaluate whether community level change compensates for depression of ecosystem function in a system subjected to long-term contamination. Previous studies have examined the effects of metals on soil respiration, microbial biomass, and microbial diversity (see Pennanen 2001, for review). However, this study is the first, to our knowledge, to examine these three parameters concomitantly along an alluvial mine-waste contamination gradient to gain insight into the long-term influence of contaminants on the relationship between communities and processes. Our data do not support the hypothesis that functional redundancy at the community structural level prevents depression of

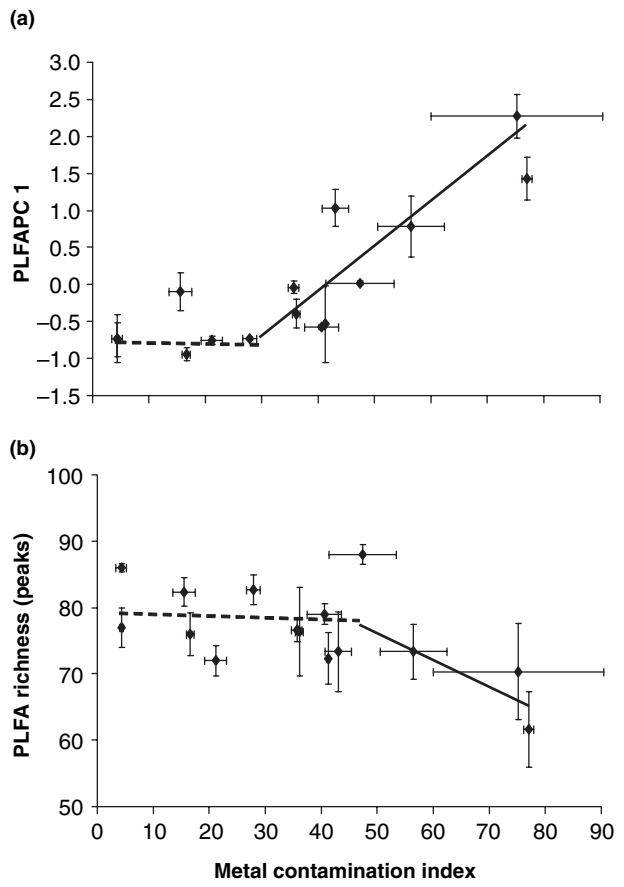

Figure 3 (a) Change in microbial community structure (y) as a function of the contamination index (x) at sampling time 1. Vertical and horizontal bars represent 1 SE ($n = 3$). PLFA PC 1 accounted for 31% of the variation in the data set. A break point in the dependent variable distribution was detected at $x = 29.8$. The regression for $x < 29.8$: $y = -0.000098x - 0.665$ ($R < 0.001$, $P = 0.995$, $n = 6$). The regression for $x > 29.8$: $y = 0.536x - 2.25$ ($R = 0.860$, $P = 0.003$, $n = 9$). The regression for all plots combined (regression line not shown): $y = 0.036x - 1.3$ ($R = 0.839$, $P < 0.001$, $n = 15$). (b) Microbial community diversity as estimated by total PLFA peaks (y) as a function of the contamination index (x). Vertical and horizontal bars represent 1 SE ($n = 3$). A break point in the dependant variable distribution was detected at $x = 47.9$. The regression for $x < 47.9$: $y = -0.047x + 79.8$ ($R = -0.136$, $P = 0.674$, $n = 12$). The regression for $x > 47.9$: $y = -0.402x + 96.4$ ($R = -0.755$, $P = 0.456$, $n = 3$). The regression for all plots combined (regression line not shown): $y = -0.176x + 82.9$ ($R = -0.592$, $P = 0.020$, $n = 15$).

ecosystem function in the long-term in contaminated soil systems (Fig. 1b).

Ecosystem function as measured by soil respiration, microbial biomass, above ground plant biomass, and root biomass declined linearly with respect to contamination. Soil respiration values ranged from 10 μmol CO_2 m^{-2} s^{-1} in the least contaminated sites to less than 1 μmol CO_2 m^{-2} s^{-1} in the most contaminated sites (Fig. 2a). A decline in soil respiration is a frequently observed response to elevated heavy metal concentrations in soil (Bååth 1989; Dahlin *et al.* 1997), but metals also stimulate respiration in the days to weeks following metal additions (Doelman & Haanstra 1984). Presumably, a short-term increase occurs because stress exerts increased metabolic demands on organisms before activity declines because of toxic effects over longer incubations (Odum 1985). Decline in microbial biomass is also a frequently documented effect of heavy metal accumulation in soil (McGrath *et al.* 1995), and has been observed in soils containing as little as 30–60 mg Cu kg^{-1} (Dahlin *et al.* 1997). Microbial biomass may decline because the energetic cost of stress makes the utilization of low-energy substrates unfavourable (Burkhardt *et al.* 1993).

Microbial biomass, above-ground biomass, and root biomass were linearly correlated with soil respiration. This observation is consistent with the interpretation that the producer–decomposer relationship between plants and soil microbes drives soil respiration, and that along the contamination gradient a fairly smooth decline in soil function occurred (Wardle 2002). The response of litter to increasing contamination was not linear. The weak trend ($P = 0.056$), indicated by quadratic regression leads us to speculate that the lack of a linear relationship was because of the tendency of litter to degrade quickly in productive, slightly contaminated sites, while litter was not produced in highly contaminated sites because few plants occurred there.

Community structure, assessed by PLFA analysis, was not strongly affected by low contaminant concentrations (four to 30 times background), but higher concentrations dramatically altered community structure (Fig. 3a). PLFA analysis has been shown to be an accurate and reproducible method for demonstrating change in microbial community structure (Dierksen *et al.* 2002). However, the use of PLFAs to represent specific populations of microorganisms can be problematic because of overlap in the PLFA composition of organisms, because the PLFA composition of most organisms living in complex soil communities is unknown, and because shifts in membrane PLFAs occur as microbes respond to stress (Guckert *et al.* 1986). Here, changes in broad classes of lipids regularly used to identify microbial groups were the source of the variation along PC 1 (White & Ringelberg 1998). The PLFA patterns suggest that microbial community composition shifted from a community consisting of more Gram-negative bacteria and Actinomycetes below the breakpoint to a community containing greater proportions of non-actinomycete Gram-positive bacteria and fungi above the breakpoint. Pennanen *et al.* (1996) observed a similar shift towards Gram-positive bacteria in a study of a metal contamination gradient

produced by smelter outfall in a boreal coniferous forest. However, in that study the fungal marker, 18:2ω6,9*c*, declined in contaminated sites whereas here it increased slightly. This difference may be explained by the disappearance of ectomycorrhizal trees in highly contaminated sites in the forest, while few ectomycorrhizal plant species were present at our study site.

Microbial community richness, estimated by PLFA peak number (Fierer *et al.* 2003), was not responsive to contaminant concentrations up to 50 times background values, but was depressed in the more heavily contaminated sites (Fig. 3b). PLFA peak number, as a surrogate for microbial community richness, has been validated by studies showing it to behave similarly to other measures of diversity. Fierer *et al.* (2003) demonstrated that PLFA peak numbers decline sharply with depth in the soil profile, a finding that has also been shown using terminal restriction fragment length polymorphism analysis of 16s rRNA genes (LaMontagne *et al.* 2003). Metal tolerant microbes have been observed in many contaminated soils (Bååth *et al.* 1998) and metal tolerance mechanisms are well known (Silver *et al.* 1989). Heavy metal tolerance may explain how high levels of microbial diversity are sustained in heavily polluted systems (Zhou *et al.* 2002; Feris *et al.* 2004). Zhou *et al.* 2002 found highly diverse microbial communities in wetland soils containing 20 000 mg Cd kg^{-1} indicating that the number of microbial populations in soil is not necessarily decreased by metal contamination. Instead, low microbial diversity and biomass may be an indirect effect of suppression of plant productivity and subsequent inputs of organic material (Findlay *et al.* 2002; Feris *et al.* 2004), or the degree of spatial isolation (Zhou *et al.* 2002). Here, microbial community richness was affected only in the most contaminated sites, where soils were acidic and plant biomass severely reduced.

Frostegård *et al.* (1993, 1996) showed, using amendments of metal salts to soil, that PLFA patterns change with metal concentration even at low levels of contamination; concentrations of 262 mg Zn kg^{-1} were enough to bring about significant changes in microbial community structure during 6 and 18 month incubation experiments. Soil respiration and microbial biomass were also affected in these experiments, however, the microbial community response was detected earlier and at lower levels of contamination. In the current study, we observed essentially the opposite; microbial community structure was insensitive to contamination concentrations over a range where soil respiration and microbial biomass declined linearly. At high contaminant concentrations both functional and structural variables were dramatically affected (Table 3). Our findings could be explained by an extension of the mechanism which is suggested to account for shorter-term observations that functional redundancy supports ecosystem functions. When an introduced contaminant presents a toxic stress to organisms within a population some organisms will posses or acquire tolerance whereas others will die or suffer suppressed growth. In the short-term, an increased abundance of tolerant populations may compensate functionally for the loss of susceptible populations (Dahlin *et al.* 1997; Palmborg *et al.* 1998), but in the long-term the selective pressure exerted by the contaminants should wane as susceptible populations are excluded. An interpretation consistent with our results would be that long-term, community-level change occurred over a broad range of metal levels such that current microbial community structure shows little response to contaminant concentrations below a threshold level. The current expression of past selective pressure on these microbial communities may be increasing suppression of ecosystem level function and biomass along the gradient that reflects the energetic cost of tolerating metal stress. Additional studies characterizing the energetic cost of tolerance to the dominant organisms found along the gradient would be required to test this explanation. Potentially, re-interpretation of existing data collected in other contaminated systems could be used to test the generality of the phenomena observed here.

Much attention has been given to understanding the consequences of how loss of biodiversity will affect ecosystem functioning (Naeem & Wright 2003), but predictions of responses to anthropogenic environmental change have not addressed how the relationship between communities and processes is effected by toxins in the long-term (Odum 1985; Grime 2001; Loreau 2001; Loreau *et al.* 2002). Heavy-metal laden mine wastes and other persistent pollutants affect ecosystems worldwide (Helgen & Moore 1996). Sound decisions regarding the future management of these ecosystems are impeded by the lack of an ecological understanding of how contaminants, ecosystem function, and communities interact in the long-term.

Further study of the relationship between ecosystem functioning and community structure in contaminated soils may improve our understanding of the effects of anthropogenic global change. The approach employed here illustrates a novel way to use the complex microbial communities found in soil to study the effects of chronic ecosystem contamination. Concerns that findings from short-term, laboratory microcosm or mesocosm scale experiments have little relevance to natural settings could be evaluated using similar experimental designs. Several features of contaminated alluvial floodplains make them valuable systems for studying long-term effects of ecosystem level contamination: (1) Contaminants vary over small spatial scales eliminating potential confounding factors such as climatic or ecotype differences between study sites; (2) contaminant history is generally known or can be reconstructed from mining records; (3) where large floods have deposited

wastes over broad floodplains, ecosystem parameters are set to essentially 'time-zero' as biomass and community complexity are greatly reduced. To predict the effects that pollutants such as heavy metals will have on the future functioning of ecosystems, it will be necessary to include the effects of persistent toxins in the development of models of anthropogenic change.

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