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## Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles

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**Abstract** Heavy metal contamination in an area immediately surrounding a zinc smelter has resulted in destruction of over 485 hectares of forest. The elevated levels of heavy metals in these soils have had significant impacts on the population size and overall activity of the soil microbial communities. Remediation of these soils has resulted in increases in indicators of biological activity and viable population size, which suggest recovery of the microbial populations. Questions remain as to how the metal contamination and subsequent remediation at this site have impacted the population structure of the soil microbial communities. In the current study, microbial communities from this site were analyzed by the phospholipid fatty acid (PLFA) procedure. Principal component analysis of the PLFA profiles indicated that there were differences in the profiles for soils with different levels of metal contamination, and that soils with higher levels of metal contamination showed decreases in indicator PLFAs for mycorrhizal fungi, Gram-positive bacteria, fungi, and actinomycetes. PLFA profiles for remediated sites indicated that remediated soils showed increases in indicator PLFAs for fungi, actinomycetes, and Gram-positive bacteria, compared to unremediated metal contaminated soils. These data suggest a change in the population structure of the soil microbial communities

resulting from metal contamination and a recovery of several microbial populations resulting from remediation.

**Keywords** Heavy metals · Soil · Remediation · Microbial communities · PLFA profiles

### Introduction

The impacts of elevated heavy metal levels on the size and activity of natural soil microbial communities have been well documented. Field studies of metal contaminated soils have demonstrated that elevated metal loadings can result in decreased microbial community size (Jordan and LeChevalier 1975; Brookes and McGrath 1984; Chander and Brookes 1991; Konopka et al. 1999) and decreases in activities such as organic matter mineralization (Chander and Brookes 1991) and leaf litter decomposition (Strojan 1978). Remediation strategies for metal contaminated soils seek to reduce the biological impact of the metals by removing the metals or reducing their bioavailability. Soils at a field site which was contaminated with heavy metals due to the operation of a zinc smelter were remediated by surface application of sewage sludge. This remediation program resulted in increases in microbial community size and total heterotrophic activity, suggesting a recovery of the microbial community (Kelly and Tate 1998). However, measures of total microbial community size or activity focus on the community as a whole, and questions remain as to how metal contamination and remediation affect specific populations within soil microbial communities. It has been demonstrated that changes in specific microbial populations can occur even when total microbial community size remains unchanged (Pennanen 2001). Therefore, it has been suggested that measures of microbial community structure may be more sensitive to disturbance than assays which focus on general microbial processes or overall community size (Kennedy and Smith 1995). Thus, microbial community structure may be

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useful as a highly sensitive bioindicator of disturbance and of the progress of remediation.

Several researchers, using isolation-based techniques, have demonstrated that heavy metal contamination can cause shifts in microbial populations (Barkay et al. 1985; Doelman et al. 1994; Gingell et al. 1976; Roane and Kellogg 1996). However, isolation-based techniques are limited as they provide information on only a small component of the microbial community since only a small percentage of soil microbes are culturable (Ward et al. 1990). Phospholipid fatty acid (PLFA) analysis is a procedure which is useful for evaluating microbial community structure. Changes in PLFA profiles are indicative of changes in the overall structure of microbial communities (Frostegård et al. 1996) and "signature" PLFAs can provide information on specific groups of microorganisms present in a community (Frostegård et al. 1993a). PLFA analysis offers an advantage over isolation-based techniques because it avoids the selectivity inherent in the isolation of microorganisms (Cavigelli et al. 1995). Previous work has shown that metal contamination can result in shifts in PLFA profiles for soil microbial communities (Pennanen et al. 1996; Griffiths et al. 1997).

The goal of this research project was to use PLFA analysis to assess the impacts of heavy metal contamination and subsequent remediation on soil microbial communities from a field site which had been contaminated due to the operation of a zinc smelter. The hypothesis was that soils with higher levels of heavy metals would show shifts in PLFA profiles and changes in specific microbial populations as compared to soils with lower metal levels, and that remediated soils would show recovery of these microbial populations.

## Materials and methods

### Site and sampling

Soil samples, selected based on degree of contamination (distance from contamination source) and time since initiation of remediation, were collected from a field site in Palmerton, Pa., which is downwind from a zinc smelter (Table 1). Early data on the level of biological activity in relationship to the smelter have been reported (Jordan and LeChevalier 1975). The remediation management procedure used at the site was a one time application of a 2:1 (wet weight) mixture of municipal sewage sludge and power plant fly ash. The sludge/fly ash mixture was surface applied at a rate of approximately 73.5 Mg hectare<sup>-1</sup>, which corresponds to a layer of approximately 2.5–4 cm. For sites F and G (Table 1), limestone was also added to the mixture in an amount equal to 3.7 Mg hectare<sup>-1</sup>.

Soils were sampled on two occasions: the first sampling occurred during the fall of 1995 and encompassed soils from unremediated sites, and the second sampling occurred in the summer of 1996 and encompassed soils from both remediated and unremediated sites (Table 1). From each sampling site, a composite sample consisting of three subsamples was collected (with a minimum distance between subsamples of 3 m) from the top 15 cm of soil. For remediated sites, any remaining sludge layer on the surface of the soil was removed before the soil sample was collected. Soil samples were stored on ice during transport to the laboratory. In the laboratory soil samples were sieved (<2 mm) and stored at -20°C until analysis. Descriptions of the sampling sites and characterizations of each of the soils collected, including total and soluble heavy metal levels, have been previously reported (Kelly and Tate 1998). For the purposes of discussion, levels of total soil zinc, soluble zinc, and soil organic matter have been included in Table 1.

### Phospholipid fatty acid assay

Four PLFA profiles were produced for the soil samples from each of the sampling sites. Fatty acids were extracted from the soil using a modification of the method of White et al. (1979) as previously described (Kelly et al. 1999). Fatty acids were prepared according to the MIDI protocol (MIDI 1995) and analyzed using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE). Fatty acids with carbon chain lengths between 9 and 20 were identified. The abbreviated names for the fatty acids refer to the total number of carbon atoms in the chain, the number of double

**Table 1** Description of soil sampling sites

Site	Soil series	Distance from source	Remediation	Time since remediation <sup>a</sup>	Total Zn (ppm) <sup>b</sup>	Soluble Zn (ppm) <sup>b</sup>	Organic matter (%) <sup>b</sup>
Fall							
A	Dekalb	6.5 km east	None	NA <sup>c</sup>	551	104	2.2
B	Klinesville	4.8 km east	None	NA	2,616	1,038	17.9
C	Dekalb	1.6 km west	None	NA	4,032	951	29.8
D	Holly	1.6 km east	None	NA	13,656	1,136	11.7
Spring							
A	Dekalb	6.5 km east	None	NA	199	101	2.9
D	Holly	1.6 km east	None	NA	5,335	735	4.8
E	Klinesville	0.8–1.2 km east	None	NA	8,719	1,085	6.4
F	Dekalb	0.6–0.8 km east	Sludge/fly ash	10 years	11,944	865	16.4
G	Klinesville	0.5–0.6 km east	Sludge/fly ash	5 years	12,881	158	20.8
H	Dekalb	1.6–2.4 km east	Sludge/fly ash	3 years	807	6	15.0
I	Dekalb	3.2–4.0 km east	Sludge/fly ash	1 year	533	3	3.9

<sup>a</sup> Time elapsed between remediation and collection of soil samples

<sup>b</sup> Previously published (Kelly and Tate 1998)

<sup>c</sup> NA not applicable

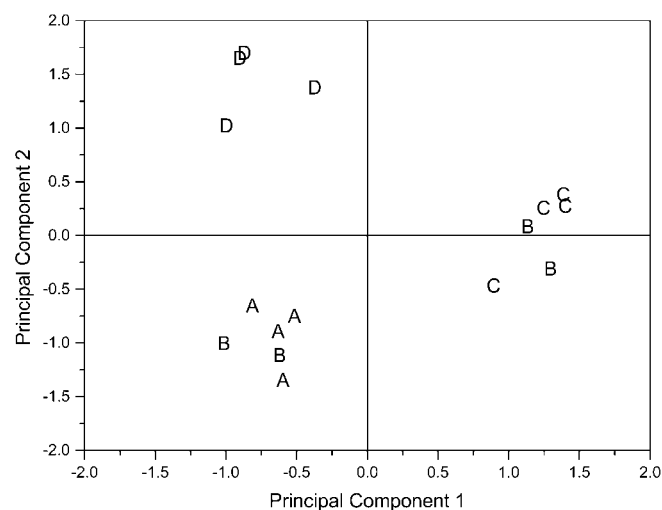
bonds, followed by the position of the double bond from the methyl end of the chain and conformation (e.g. 16:1 $\omega$ 7 t).

Principal component analysis (PCA) was used to analyze the PLFA profiles using the SAS statistical package (SAS Institute, Cary, N.C.). Three separate and independent PCA analyses were run. PLFA profiles for unremediated soils from the fall sampling were compared, PLFA profiles for unremediated soils from the spring sampling were compared, and PLFA profiles for both unremediated and remediated soils from the spring sampling were compared. For each PCA analysis, individual PLFA scores were expressed as a percentage of the total PLFAs in the sample.

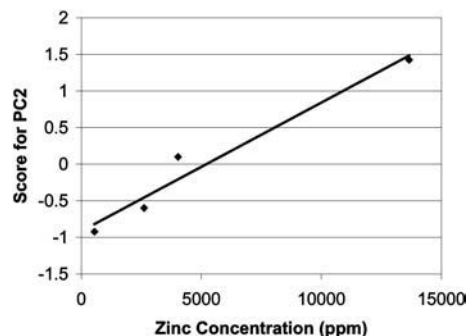
## Results

### Fall: unremediated sites

Principal component analysis (PCA) of the PLFA profiles for the soils from the fall sampling indicated that there were differences in PLFA profiles among the samples and that the samples tended to group based on sample location (Fig. 1). The PCA graph (Fig. 1) suggested that separation of samples on PC2 (which accounted for 23.1% of the variance in the data set) was related to the level of zinc in the soils. For example, the site most distant from the smelter (A) had the lowest total and soluble zinc metal concentrations (Table 1), and in the PCA analysis it showed a trend toward lower scores on PC2 (Fig. 1). Linear regression analysis of the PCA data demonstrated that scores on PC2 correlated very well ( $R^2=0.9606$ ) with the levels of zinc in these soils (Fig. 2). Fatty acids with high correlation coefficients (cc) for PC2 included 16:1 $\omega$ 5c (cc=-0.945) and 15:0iso (cc=-0.861). Comparison of the means for these fatty acids indicated that there were statistically significant decreases in 16:1 $\omega$ 5c and



**Fig. 1** Principal component analysis of phospholipid fatty acid profiles for unremediated sites from the fall sampling. A, B, C and D represent soil sampling sites. For site descriptions see Table 1. Scores for each fatty acid were the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. PC1 accounted for 31.5% of the variance in the data, and PC2 accounted for 23.1% of the variance in the data



**Fig. 2** Correlation between zinc concentrations and mean scores on PC2 for unremediated sites from the fall sampling,  $R^2=0.9606$

**Table 2** Comparison of mean values for indicator phospholipid fatty acids from unremediated sites from the fall sampling

Site <sup>a</sup>	Indicator phospholipid fatty acids	
	16:1 $\omega$ 5c	15:0iso
A	2.97 <sup>b</sup> a <sup>c</sup>	8.70 a
B	2.87 ab	9.04 a
C	2.66 b	8.60 a
D	1.63 c	5.75 b

<sup>a</sup> For site descriptions see Table 1

<sup>b</sup> Scores for each fatty acid are the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. Each score is the mean of four replicate extractions

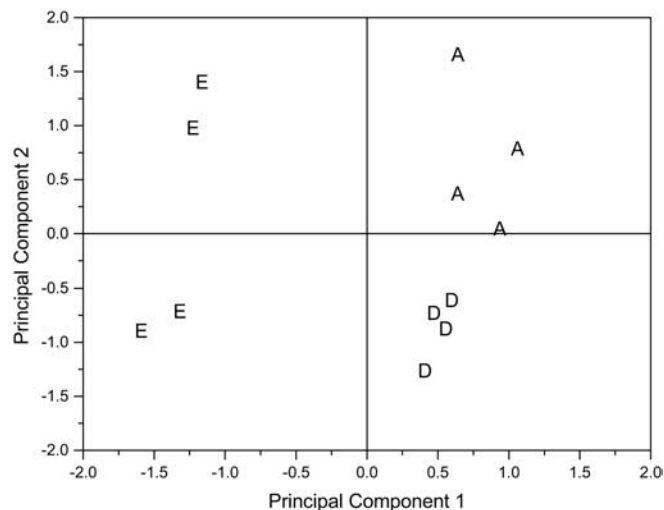
<sup>c</sup> Values in same column followed by a different letter are significantly different ( $\alpha=0.05$ ), using Duncan's New Multiple Range Test

15:0iso for the site with the highest metal levels (D; Table 2).

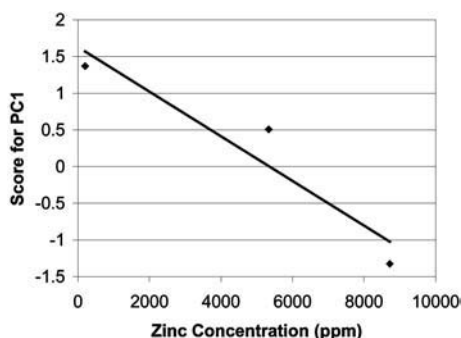
PCA analysis of the fall sampling soils also indicated that 31.5% of the variance in the data was accounted for by PC1 (Fig. 1). PC1 showed a strong separation of site C and some separation of site B from sites A and D, which clustered close together with respect to PC1. Since site A was the least contaminated site and site D was the most heavily contaminated site, separation along PC1 was not based on metal contamination. The differences in microbial communities demonstrated by PC1 may relate to some other differences in soil physico-chemical properties. One possibility could be soil organic matter content. Sites B and C had significantly higher soil organic matter than sites A and D (Table 1).

### Spring: unremediated sites

A separate PCA analysis of the PLFA profiles for the unremediated soils from the spring sampling confirmed the impact of metal contamination on PLFA profiles (Fig. 3). PLFA profiles for sites A, D, and E were separated along PC1 (which accounted for 20.4% of the variance in the data set), with the least contaminated site (A) having the highest scores and the most contaminated site (E) having the lowest scores. Linear regression

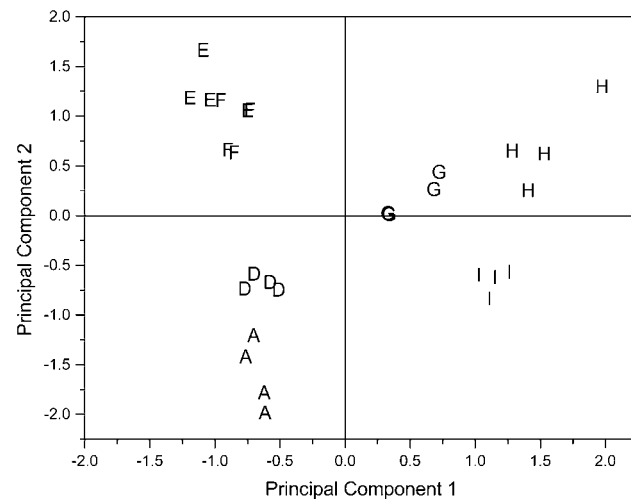


**Fig. 3** Principal component analysis of phospholipid fatty acid profiles for unremediated sites from the spring sampling. A, D and E represent soil sampling sites. For site descriptions see Table 1. Scores for each fatty acid were the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. PC1 accounted for 20.4% of the variance in the data, and PC2 accounted for 9.2% of the variance in the data



**Fig. 4** Correlation between zinc concentrations and mean scores on PC1 for unremediated sites from the spring sampling,  $R^2=0.8992$

analysis of the PCA data demonstrated that scores on PC1 had a strong negative correlation ( $R^2 = 0.8992$ ) with the levels of zinc in these soils (Fig. 4). Fatty acids with high positive correlation coefficients for PC1 included TBSA18:0 10Me ( $cc=0.848$ ), 17:0 10Me ( $cc=0.797$ ), 18:2 $\omega$ 6,9c ( $cc=0.872$ ), 16:1 $\omega$ 5c ( $cc = 0.860$ ), 18:1 $\omega$ 9c



**Fig. 5** Principal component analysis of phospholipid fatty acid profiles for unremediated sites and remediated sites from the spring sampling. A, D, E, F, G, H and I represent soil sampling sites. For site descriptions see Table 1. Scores for each fatty acid were the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. PC1 accounted for 19.8% of the variance in the data, and PC2 accounted for 10.2% of the variance in the data

( $cc=0.739$ ), 15:0iso ( $cc=0.653$ ), 17:0cyclo ( $cc=0.768$ ), and 18:1 $\omega$ 7c ( $cc=0.836$ ). Fatty acids with high negative correlation coefficients included 16:1 $\omega$ 11c ( $cc=-0.721$ ). Comparison of the mean scores for these fatty acids indicated that there were statistically significant decreases in 16:1  $\omega$ 5c, 18:2  $\omega$ 6,9c, 17:0 10Me, TBSA 18:0 10Me, 15:0iso, 18:1 $\omega$ 9c, and 18:1 $\omega$ 7c for the most heavily contaminated site (E), and a statistically significant increase in 16:1 $\omega$ 11c (Table 3).

#### Spring: remediated and unremediated sites

A separate PCA was run combining the PLFA profiles for the remediated and unremediated soils from the spring sampling (Fig. 5). This PCA indicated that there were differences in PLFA profiles and that these differences were related to sample location and remediation. In this analysis (Fig. 5), unremediated sites (A, D, and E) differed along PC2 (which accounted for 10.2% of the variance in the data set). Fatty acids with high negative

**Table 3** Comparison of mean values for indicator phospholipid fatty acids from unremediated sites from the spring sampling

Site <sup>a</sup>	Indicator phospholipid fatty acids (TBSA)								
	16:1 $\omega$ 5c	18:2 $\omega$ 6,9c	17:0 10Me	18:0 10Me	15:0iso	18:1 $\omega$ 9c	18:1 $\omega$ 7c	16:1 $\omega$ 11c	17:0cyclo
A	3.07 <sup>b</sup> a <sup>c</sup>	1.48 a	1.31 a	2.33 a	11.88 a	5.12 a	5.75 a	0.34 b	2.91 b
D	2.87 a	1.68 a	0.89 a	1.71 a	9.23 b	4.54 ab	6.14 a	0.11 b	3.64 a
E	1.94 b	0.35 b	0.19 b	0.00 b	8.78 b	4.03 b	4.57 b	1.13 a	2.01 c

<sup>a</sup> For site descriptions see Table 1

<sup>b</sup> Scores for each fatty acid are the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. Each score is the mean of four replicate extractions

<sup>c</sup> Values in same column followed by a different letter are significantly different ( $\alpha=0.05$ ), using Duncan's New Multiple Range Test

**Table 4** Comparison of mean values for indicator phospholipid fatty acids from remediated and unremediated sites from the spring sampling

Site <sup>a</sup>	Indicator phospholipid fatty acids (TBSA)					
	16:1 $\omega$ 5c	18:2 $\omega$ 6,9c	17:0 10Me	18:0 10Me	15:0iso	16:0iso
A	3.07 <sup>b</sup> a <sup>c</sup>	1.48 ab	1.31 a	2.33 a	11.88 a	3.62 a
D	2.87 a	1.68 ab	0.89 ab	1.71 ab	9.23 b	2.42 bc
E	1.94 b	0.35 c	0.19 c	0.00 d	8.78 b	2.65 b
F	1.96 b	0.17 c	0.39 bc	0.93 bcd	7.57 c	2.04 c
G	1.85 b	1.20 b	0.42 bc	1.05 bc	7.68 c	2.49 bc
H	1.61 b	1.79 a	0.00 c	0.61 cd	9.18 b	2.53 b
I	1.59 b	1.74 ab	0.00 c	0.76 bcd	9.66 b	3.53 a

<sup>a</sup> For site descriptions see Table 1

<sup>b</sup> Scores for each fatty acid are the amount of that fatty acid present in a sample expressed as a percentage of the total fatty acids in the sample. Each value is the mean of four replicate extractions

<sup>c</sup> Values in same column followed by a different letter are significantly different ( $\alpha = 0.05$ ), using Duncan's New Multiple Range Test

correlation coefficients for PC2 included TBSA 18:0 10Me ( $cc = -0.708$ ) and 17:0 10Me ( $cc = -0.566$ ), 18:2 $\omega$ 6,9c ( $cc = -0.560$ ), 16:1 $\omega$ 5c ( $cc = -0.587$ ), 15:0iso ( $cc = -0.637$ ), and 16:0iso ( $cc = -0.621$ ). Comparison of the mean values for these fatty acids confirmed that there was a statistically significant decrease in all six of these indicator fatty acids for the most contaminated site (E; Table 4).

The remediated sites (F, G, H, and I) showed values on PC2 which were intermediate between the most contaminated site (E) and the least contaminated site (A; Fig. 5). The most recently remediated site (I) had a score on PC2 most similar to the least contaminated site (A), and the earliest remediated site (F) had a score on PC2 most similar to the most contaminated site (E). Comparison of the mean values for the fatty acids discussed above indicated that there were significant increases in 18:2 $\omega$ 6,9c, TBSA 18:0 10Me, and 16:0iso with remediation (Table 4).

PCA analysis of the remediated and unremediated soils from the spring sampling also indicated that 19.8% of the variance in the data was accounted for by PC1 (Fig. 5). PC1 showed a strong separation of the more recently remediated sites G, H, and I from the unremediated sites A, D, and E, which clustered close together with respect to PC1. Since site A was the least contaminated site and site D and E were more heavily contaminated, separation along PC1 was not based on metal contamination. Fatty acids with high negative correlation coefficients for PC1 included 16:1 $\omega$ 5c ( $cc = -0.589$ ) and 16:0 10Me ( $cc = -0.902$ ). Fatty acids with a high positive correlation coefficient for PC1 included 18:2 $\omega$ 6c ( $cc = 0.600$ ).

## Discussion

Analysis of the PCA data for the fall samples (Fig. 1) showed a correlation between soil metal levels and scores on PC2 (Fig. 2). Fatty acids with high negative correlation coefficients for PC2 included 16:1 $\omega$ 5c, which is an indicator of arbuscular mycorrhizal fungi (Haack et al. 1994) and 15:0iso, which is an indicator of Gram-positive bacteria (Frostegård et al. 1993b; Konopka et al. 1999).

The levels of 16:1 $\omega$ 5c and 15:0iso were significantly lower in site D soils, which had the highest metal loadings, than in site A, B and C soils (Table 2). These data indicate that soils from the most contaminated site tended to have lower levels of the indicator fatty acids for arbuscular mycorrhizal fungi and Gram-positive bacteria than soils from the less highly contaminated sites.

Analysis of the PCA data for the spring samples (Fig. 3) showed a negative correlation between soil metal levels and scores on PC1 (Fig. 4). Fatty acids with high positive correlation coefficients for PC1 included the following: TBSA 18:0 10Me and 17:0 10Me, which are both indicators of actinomycetes (Frostegård et al. 1993b); 18:2 $\omega$ 6,9c, which is an indicator of fungi (Guckert et al. 1985); 16:1 $\omega$ 5c, which is an indicator of arbuscular mycorrhizal fungi (Haack et al. 1994); 18:1 $\omega$ 9c and 15:0iso, which are both indicators of Gram-positive bacteria (Frostegård et al. 1993b; Konopka et al. 1999); and 17:0cyclo and 18:1 $\omega$ 7c, which are both indicators of Gram-negative bacteria (Frostegård et al. 1993b; Konopka et al. 1999). All of these indicator fatty acids were significantly lower in site E soils, which had the highest metal loadings (Table 3). Fatty acids with high negative correlation coefficients included 16:1 $\omega$ 11c which is an indicator of Gram-negative bacteria (Zelles 1997). 16:1 $\omega$ 11c was significantly higher in site E soils (Table 3). These data indicated that as proximity to the smelter and metal levels increased, fatty acid indicators of fungi, arbuscular mycorrhizal fungi, actinomycetes, and Gram-positive bacteria decreased. The results were mixed for indicators of Gram-negative bacteria.

The independent PCA analyses for the fall and spring samples both demonstrated that metal contamination had resulted in shifts in the structure of the soil microbial communities as evidenced by the separation of sites on the PCA graphs (Figs. 1, 3, respectively). The interpretation of the two PCA analyses with regard to the signature fatty acids suggested similar trends. At both sampling times a trend toward decreases in arbuscular mycorrhizal fungi and Gram-positive bacteria was observed. The similarity of the results of these two samplings was significant because it indicated that PLFA analysis of these field samples gave reproducible results

after nearly 1 year, which demonstrated that these shifts in microbial populations had persisted over that year.

The decrease in indicator fatty acids of Gram-positive bacteria is not a surprising result since the predominance of Gram-negative bacteria over Gram-positive bacteria has been found previously in metal contaminated soils (Frostegård et al. 1993a). The decrease in the fungal indicator fatty acid with metal contamination which was observed in the Spring sampling is an interesting result. There is evidence in the literature indicating that fungi are more resistant to heavy metals than bacteria (Hiroki 1992; Jordan and LeChevalier 1975). If fungi are more metal resistant than bacteria, their numbers as a percentage of the total microbial population should increase with increased soil metal levels. However, the results of this study suggest that fungi actually decreased with metal contamination. A decrease in the fungal indicator fatty acid 18:2 $\omega$ 6 resulting from metal contamination was seen in another study in which soils from a forest site in the vicinity of a metal smelter in Finland were analyzed (Pennanen et al. 1996). The authors suggested that the decrease in fungal PLFAs which they observed might have been related to an impact of the metals on tree roots. An earlier study conducted at the Finnish smelter site indicated that metal contamination had damaged the fine roots of trees in the vicinity of the smelter (Helmisaari et al. 1995). Pennanen et al. (1996) suggested that damage to fine roots may have resulted in a loss of rhizosphere habitats for mycorrhizal fungi. This theory is supported by findings that indicate that elevated heavy metal levels can decrease (Koomen et al. 1990) or in some cases prevent (Gildon and Tinker 1983) the mycorrhizal infection of plant roots. Pennanen et al. (1996) thus concluded that a decrease in mycorrhizal fungi may have accounted for the observed decrease in 18:2 $\omega$ 6. At the site examined in the current study, trees in the areas closest to the smelter had been destroyed by the smelter emissions. For those trees which were not destroyed, metal contamination may have resulted in root damage. Therefore, both destruction of trees and damage to fine roots may have resulted in a loss of rhizosphere habitats for mycorrhizal fungi at this site. This possibility is supported by the decrease in indicator fatty acids of arbuscular mycorrhizal fungi which was observed in this study in both the fall and spring samplings. If mycorrhizal fungi are one of the dominant fungal groups at this site, then the decrease in mycorrhizal fungi might also account for the overall decrease in the fungal indicator fatty acid 18:2 $\omega$ 6,9c which was observed. The link between fungi and tree roots is further supported by results of several studies in which soils incubated in the laboratory with no plants showed the reverse effect, i.e. increases in fungi with metal amendment, as indicated by increases in 18:2 $\omega$ 6,9c (Kelly et al. 1999) or increases in ergosterol (Khan and Scullion 2000).

Analysis of the unremediated sites from the spring sampling also indicated that there was a significant decrease in two indicator fatty acids for nocardioform actinomycetes, TBSA 18:0 10Me and 17:0 10Me, at the

most heavily contaminated site. A similar decrease in the actinomycete indicator 18:0 10Me was observed previously in lead contaminated soils (Konopka et al. 1999). Several studies have demonstrated that actinomycetes are less resistant to heavy metals than bacteria and fungi (Zelles 1997; Jordan and LeChevalier 1975). However, in another study actinomycetes were found to be more resistant to cadmium than bacteria (Babich and Stotzky 1977). The results in the literature thus indicate that actinomycetes can respond differently to heavy metals. The decrease in actinomycete indicators observed in this study may have been due to their sensitivity to heavy metal contamination. In addition, some groups of actinomycetes form actinorrhizal association with the roots of a variety of woody plant species. Therefore, the decrease in actinomycete fatty acids which was observed in this study may also relate to an impact of the elevated metal levels on the formation of these associations.

PCA analysis of the PLFA profiles for the remediated and unremediated soils from the spring sampling indicated that there were differences in PLFA profiles and that these differences were related to sample location and remediation. Unremediated sites (A, D, and E) differed along PC2, with samples with higher metal loadings having higher scores on PC2. Fatty acids with high negative correlation coefficients for PC2 included indicators of actinomycetes (TBSA 18:0 10Me and 17:0 10Me), fungi (18:2 $\omega$ 6,9c), arbuscular mycorrhizal fungi (16:1 $\omega$ 5c) and Gram-positive bacteria (15:0iso and 16:0iso). There were significant decreases in all six of these indicator fatty acids for the most contaminated site (E; Table 4). Thus, metal contamination resulted in decreases in indicator fatty acids of actinomycetes, fungi, mycorrhizal fungi, and Gram-positive bacteria.

Examination of the PLFA profiles from the remediated sites indicated that the remediation program had an effect on the composition of the soil microbial communities. PLFA profiles for the recently remediated sites were similar to the less contaminated sites (Fig. 5), and there were significant increases in indicator fatty acids of fungi, actinomycetes, and Gram-positive bacteria with remediation (Table 4). These increases are a reversal of the trends observed for contaminated soils, thus suggesting that the remediation program is resulting in some recovery of the microbial populations. However, there was not a significant increase in the indicator fatty acid of mycorrhizal fungi with remediation (Table 4). Due to the importance of mycorrhizal fungi in nutrient and moisture uptake by plants, this result may be of concern with regards to the revegetation of the site under the current remediation program. A recent study demonstrated that the growth of two grass species in metal contaminated mine tailings was improved with mycorrhizal inoculation (Hetrick et al. 1994). Further research is needed at the current study site to document the potential for eventual recovery of this critical community component.

Although PLFA data presented in this study suggested that the remediation program was having a positive effect on soil microbial communities, the results also suggested

a loss of this effect over time. In PCA analyses of the PLFA data, the more recently remediated sites had profiles most similar to the least contaminated site (A), and the sites which had been remediated earlier had profiles more similar to the most heavily contaminated site (E). This loss of effect may relate to soil pH. Remediation of soils resulted in pH levels higher than the pH levels in the unremediated soils (Kelly and Tate 1998). This increase in pH contributed to a decrease in the solubility and thus the bioavailability of the heavy metals, resulting in a positive effect on the microbial communities in the remediated soils. However, the pH levels for the remediated soils decreased with time since remediation (Kelly and Tate 1998). This decrease in pH over time may have resulted in a gradual re-release of soluble metals, which could account for the loss in the positive microbial effect for the sites which had been remediated earlier. Further research is needed to evaluate the long-term sustainability of the benefits to the soil microbial communities resulting from the remediation program.

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