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Shifts in Microbial Community Composition Following Surface Application of Dredged River Sediments

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Abstract Sediment input to the Illinois River has drastically decreased river depth and reduced habitats for aquatic organisms. Dredging is being used to remove sediment from the Illinois River, and the dredged sediment is being applied to the surface of a brownfield site in Chicago with the goal of revegetating the site. In order to determine the effects of this drastic habitat change on sediment microbial communities, we examined sediment physical, chemical, and microbial characteristics at the time of sediment application to the soil surface as well as 1 and 2 years after application. Microbial community biomass was determined by measurement of lipid phosphate. Microbial community composition was assessed using phospholipid fatty acid (PLFA) analysis, terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes, and clone library sequencing of 16S rRNA genes. Results indicated that the moisture content, organic carbon, and total nitrogen content of the sediment all decreased over time. Total microbial biomass did not change over the course of the study, but there were significant changes in the composition of the microbial communities. PLFA analysis revealed relative increases in fungi, actinomycetes, and Gram positive bacteria. T-RFLP analysis indicated a significant shift in bacterial community composition within 1 year of application, and clone library analysis revealed relative increases in Proteobacteria, Gemmatimonadetes, and Bacteriodetes and relative decreases in Acidobacteria, Spirochaetes, and Planctomycetes. These results provide insight into microbial community shifts following land application of dredged sediment.

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Introduction

Human modifications of terrestrial ecosystems can have significant negative impacts on aquatic habitats. Conversion of terrestrial ecosystems from forest or grasslands to row agriculture, residential development, or commercial development can lead to significant increases in erosion, which removes nutrient rich topsoil and transfers it to surface waters [7, 16, 40], and erosion can lead to sediment buildup in surface waters, which can decrease water depth and reduce habitats for fish and other aquatic organisms [7, 41]. Illinois provides a good example of these phenomena, as the Illinois River watershed has been modified over the past 100 years from primarily forests, prairies, and wetlands to a mixture of agriculture and urban and suburban development [17]. These land use changes have resulted in increased erosion and sediment input to the Illinois River, which has drastically decreased river depth and reduced habitats for aquatic organisms [9].

Dredging can be used to remove sediment and restore freshwater habitats [7, 35], but for the Illinois River system, this would involve the removal of millions of cubic meters of sediment [3], and the relocation of this dredged sediment is a significant challenge. One possible solution is to return dredged sediment to the soil surface. Illinois River sediments have some desirable soil characteristics, including high nutrient content and good water holding capacity [9], suggesting that sediment application may be beneficial for

some terrestrial soils. One concern with the application of sediments to land is the possible presence of contaminants in the sediments, including heavy metals and organic pollutants [33]. A recent survey of Illinois River sediments found high levels of lead and other contaminants in sediments located close to Chicago [27]. However, sediments taken from lower sections of the river had contaminant concentrations below levels of regulatory concern [27].

Previous studies have documented the beneficial use of a variety of dredged sediments. Canet et al. [7] found that application of sediment dredged from a lake in Eastern Spain to an agricultural soil improved the water retention and cation exchange capacity of the soil and increased the yield of lettuce. Similarly, Lembke et al. [24] reported that application of lake sediments to an agricultural soil in Illinois resulted in a significant increase in corn yield as compared to unamended farmland. In addition, application of Illinois River sediment to an eroded soil resulted in increased plant growth [23]. Therefore, several recent studies have demonstrated the beneficial impacts of dredged sediments on plant growth.

Microorganisms are critical components of terrestrial soils due, for example, to the critical roles they play in organic matter decomposition and nutrient cycling. The transfer of sediments from an aquatic ecosystem to the soil surface represents a drastic change in habitat that will result in significant changes in physical and chemical conditions within the sediment, most notably a significant reduction in moisture content. Several previous studies have demonstrated that significant changes in moisture content can alter soil microbial community composition [5, 10, 19, 36] and that microbial communities found in terrestrial soils are distinct in composition from microbial communities found in aquatic sediments [37]. However, no previous studies that we are aware of have examined shifts in microbial community composition following land application of dredged sediment.

In this study, we investigated the changes in microbial community composition associated with surface application of dredged sediment at a site in Chicago, IL, USA. This site, which was formerly the location of the US Steel Southworks mill, occupies 232 ha bordering Lake Michigan on the south side of Chicago. Since the closure of the mill in 1992, all structures (with the exception of the ore walls) have been removed from the site, leaving behind highly compacted, barren soil. In 2004, a 12-ha section of the site was covered with Illinois River sediment with the goal of revegetating the site. We hypothesized that physical and chemical changes occurring in the surface-applied sediment over time would result in shifts in microbial community composition, with an increase in the relative contributions of common terrestrial microorganisms. In this study, we tested these hypotheses using physical and chemical analyses of the sediments as well as phospholipid fatty acid analysis (PLFA), terminal restriction fragment length polymorphism analysis (T-RFLP), and clone library sequencing to profile the microbial communities. Results from these different methods of microbial community analysis provided a view into microbial community shifts following the surface application of dredged sediment.

Materials and Methods

Site Description

Sediment was collected from Lower Peoria Lake, a large (surface area 1,093 ha) shallow (average depth 0.76 m) section of the Illinois River approximately 265 km southwest of Chicago. Over 100,000 tons of sediment were collected in summer 2004 with a clamshell bucket and transported in barges to the US Steel South Works site, located in southeastern Chicago, IL, USA. On the south side of the property sediment was spread with a bulldozer over an area of approximately 4.7 ha and allowed to dry for up to 2 weeks prior to being bulldozed into piles up to 2 m high. The dry sediment was later spread with a bulldozer to depth of 0.5 to 1 m and seeded with annual rye grass. On the north side of the property sediment was placed on an area of approximately 8 ha and left to dry in place without mechanical mixing. It dried to a depth of 0.4 to 0.8 m and was hand seeded with annual rye grass. The north side and south side treatment plots were approximately 1 km apart.

Sample Collection

Soil samples were collected from three locations within the US Steel site during the summer in three consecutive years: July 12, 2004, July 26, 2005, and August 23, 2006. Site 1 was located in the northern treatment plot and sites 2 and 3 were located within the southern treatment plot. Sites 2 and 3 were 100 m apart. In 2004 single samples were collected from each site for a total of three samples, in 2005 four replicate samples were collected from each site for a total of 12 samples, and in 2006, five replicate samples were collected from each site for a total of fifteen samples. Replicate samples for a site were collected from spots at least 1 m apart. Each sample consisted of the top 10 cm of soil collected with a hand trowel. All plant material including roots as well as other debris were removed by hand, and each sample was placed in a Ziploc bag, homogenized by manual mixing, and stored on ice for transport to the laboratory. Within 2 h of collection 0.5 g subsamples from each soil sample were transferred to 2 ml microcentrifuge tubes and stored at −80°C for subsequent molecular analyses and 5 g subsamples for microbial biomass and phospholipid fatty acid analyses were transferred to screw

cap test tubes and stored at −20°C. Remaining soil was stored at 4°C for physical and chemical analyses which were performed within 2 weeks of sample collection.

Soil Physical and Chemical Analyses

In the laboratory, all bulk soil samples were sieved through a 2 mm sieve. Soil moisture content was determined gravimetrically by drying at 100°C [15], soil organic matter content was determined by loss on ignition at 500°C [2], and pH was determined in a 1:2 soil/water slurry [39]. Total Kjeldahl nitrogen was measured by acid digestion (EPA Method 3050) [11] and distillation by the Kjeldahl method (EPA Method 351.2) [12]. Soil physical and chemical data were analyzed by analysis of variance (ANOVA) using Systat version 11 (Systat Software, Inc., Point Richmond, CA, USA), and pair-wise comparisons were done by Tukey's post hoc test. ANOVA was used to assess differences between sites 1, 2, and 3 for samples collected in 2005 and 2006. There were no significant differences in soil physical or chemical properties between sites 1, 2, and 3 in 2005 or 2006 (data not shown). Therefore, samples from all sites were treated as replicates for subsequent ANOVA analyses of samples from 2004, 2005, and 2006.

Microbial Community Analyses

Microbial Biomass

Microbial biomass was determined by measurement of total phospholipids using the lipid phosphate assay [14]. This method is widely used in microbial ecology and has been shown to correlate well with measurements of microbial biomass based on substrate-induced respiration and ATP content [14]. Lipid phosphate data were analyzed by ANOVA using Systat version 11 (Systat Software, Inc.) with sites 1, 2, and 3 treated as replicates.

Phospholipid Fatty Acid Analysis

Microbial community composition was determined by the PLFA method [22]. Briefly, lipids were extracted from 5 g (wet weight) of soil with a chloroform–methanol–phosphate buffer mixture (1:2:0.8) and the lipids were separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column [43]. The phospholipids were saponified, the resultant fatty acids were methylated [30] and the fatty acid methyl esters (FAMEs) were analyzed using the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). Several samples whose fatty acid concentrations were too low for detection with the MIDI system were analyzed by Gas chromatography mass spectrometry (GC-MS). In order to allow identification of FAMEs based on GC-MS retention times, several samples were analyzed by both MIDI and GC-MS to generate a conversion table. For each sample, individual PLFA values were expressed as a percentage of the total PLFAs in the sample. The total PLFA data set was analyzed by nonmetric multidimensional scaling (MDS) using Primer V.5 software package (Primer-E Ltd, Plymouth, UK). For a full description of the MDS procedure, see Clarke and Warwick [8]. Briefly, the PLFA dataset was imported into the Primer V.5 software package (Primer-E Ltd), and a similarity matrix was calculated using the Bray–Curtis similarity coefficient [6]. The MDS procedure was then used to ordinate the similarity data after 100 random restarts. The stress value of the ordination indicates how well the plot represents the similarity data. The analysis of similarity (ANOSIM) routine in Primer V.5 was then used to examine the statistical significance of differences between groups of samples. ANOSIM reports R-statistics and p-values. An R-statistic close to 1 indicates that samples in the same group are more similar to each other than samples in different groups. An R-statistic close to 0 indicates that samples in the same group are not more similar to each other than samples in different groups. The p value reflects the statistical significance of the R-statistic [8]. In addition to MDS analysis, signature PLFAs as defined by Zak et al. [44] (Gram positive bacteria: 15:0 iso, 16:0 iso, 16:0 10 Me; Gram negative bacteria: 16:1ω7c, 17:0 cyclo, 18:1ω7c, 19:0 cyclo; general bacteria: 14:0, 15:0 anteiso, 15:0, 16:0 anteiso, 16:1ω5c, 17:0 anteiso, 17:0, 17:0 iso; actinomycetes: 10Me 18:0; fungi: 18:2ω6,9c and 18:1ω9c) were analyzed by ANOVA and Tukey's post hoc test, treating sites 1, 2, and 3 as replicates, using Systat version 11 (Systat Software, Inc.). Since PLFA data were based on percentages, data were transformed prior to ANOVA analysis by taking the arcsine of the square root of each percentage value [45].

Terminal Restriction Fragment Length Polymorphism Analysis

DNA was extracted from each soil sample using the MoBio UltraClean Soil DNA Kit (MoBio Laboratories, Solana Beach, CA, USA) and confirmed by agarose gel electrophoresis. Bacterial 16S rRNA genes were amplified via polymerase chain reaction (PCR) using bacterial domain primers 8F and 926R [25]. Primer 926R was obtained from Operon (Alameda, CA, USA) and primer 8F (labeled at the 5′ end with Well Red Dye D4) was obtained from Sigma-Proligo (The Woodlands, TX, USA). PCR conditions and cycling parameters were described in Janus et al. [18]. Duplicate PCR reactions were run for each sample and pooled. PCR products were purified with the UltraClean PCR Cleanup Kit (MoBio Laboratories) and DNA concen-

Table 1 Soil properties

Sample	Soil moisture content (%)*	pH*	Organic matter content (%)*	Total Kjeldahl nitrogen (ppm)**	Microbial biomass (nmoles P g dry soil^{-1})**
2004	68.5 (0.6) a	6.1 (0.12) a	7.7 (0.1) a	656 (53) a	26.8 (3.1) a
2005	6.1 (0.6) b	7.2 (0.02) b	5.6 (0.3) b	695 (161) a	33.4 (6.2) a
2006	20.0 (0.9) c	6.0 (0.43) a	7.2 (0.1) c	230 (68) b	29.9 (2.8) a

Each value represents mean (2004 n=3; 2005 n=12; 2006 n=15), with standard error values in parentheses
*Data points with different letters are significantly different (p<0.001), **data points with different letters are significantly different (p<0.05)

trations were determined by agarose gel electrophoresis. Thirty nanograms of each sample were digested with MspI (New England BioLabs, Beverly, MA, USA) according to the manufacturer's instructions. After digestion samples were ethanol precipitated and resuspended in nuclease free water. Terminal restriction fragments were resolved using CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) and fragment sizes were determined by comparison to DNA Size Standard Kit 600 (Beckman Coulter) using CEQ 8000 Genetic Analysis Software (Beckman Coulter). Binary T-RFLP data (presence vs. absence) were analyzed by MDS and ANOSIM using Primer V.5 Software as described above for PLFA.

Clone Library Analysis

Four representative soil samples were chosen for clone library analysis: one from 2004, two from 2005, and one from 2006. Bacterial 16S rRNA genes were amplified from soil DNA isolated with the MoBio UltraClean Soil DNA Kit (MoBio Laboratories) by PCR using unlabeled bacterial domain primers 11F [20] and 926R [25], which were obtained from Operon. PCR amplicons were cloned with the TOPO-TA cloning kit (Invitrogen, Carlsbad, CA, USA) using either PCR-2.1 or PCR-4.0 vectors and

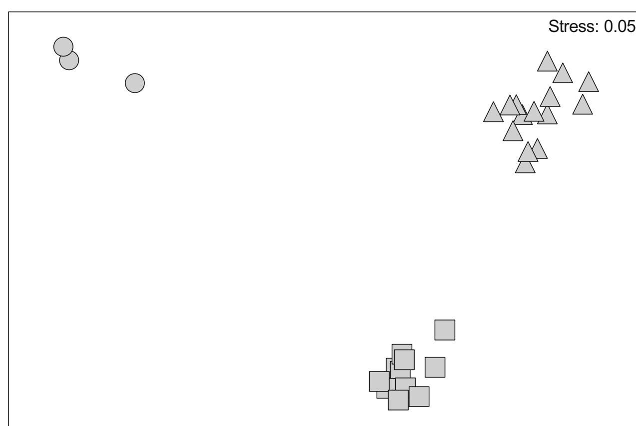

Figure 1 Nonmetric multidimensional scaling (MDS) analysis of microbial communities based on PLFA data: 2004 (*circle*; n=3), 2005 (*square*; n=12), 2006 (*triangle*; n=15)

transformed into chemically competent *Escherichia coli.* Transformed *E. coli* were grown overnight on LB agar plates containing 50 ug/ml kanamycin. Randomly selected colonies were transferred to LB broth containing 50 ug/ml kanamycin, grown overnight at 37°C, and PCR-screened for the presence of inserts using M13F and M13R primers. Plasmids containing the insert were isolated using the Mini Plasmid Prep Kit (MoBio Laboratories), and plasmid concentrations were determined by ultraviolet visible spectrophotometry or by Quant-iT DNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Forty clones were sequenced for each soil sample. Each clone was sequenced bidirectionally via Dye Terminator Cycle Sequencing performed with Quick Start Kit (Beckman-Coulter, Fullerton, CA, USA) and M13F and M13R primers. Sequencing reactions were run on CEQ 8000 DNA sequencer (Beckman-Coulter). The SeqMan component of the Lasergene software package (DNASTAR, Inc., Madison, WI, USA) was used to assemble a consensus sequence for each clone. Clone sequences were deposited to Genbank. Sequences from 2004 were deposited under accession numbers EU234541 to EU234580. Sequences from 2005 and 2006 were deposited under accession numbers EU262303 to EU262415. Clone sequences were identified by comparison to the Genbank nucleotide database using BLAST [1] via the National Center for Biotechnology Information (NCBI) web page (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

There were significant changes in soil physical and chemical properties with time. Moisture content, which was very high at the time of placement, decreased drastically within 1 year (Table 1). The soil pH increased slightly in 2005 to near neutral but, in 2006, returned to the slightly acidic pH observed in 2004 (Table 1). Organic matter content decreased significantly between 2004 and 2005 (Table 1), but between 2005 and 2006, the organic matter composition increased, although it did not reach the original level observed in the 2004 samples (Table 1). Soil

Table 2 ANOSIM values for PLFA data

Comparison	R statistic	p value (p<)
2004, 2005	1.000	0.003
2004, 2006	1.000	0.001
2005, 2006	0.999	0.001

nitrogen remained steady for the first year after sediment application, but there was a significant decrease in total soil nitrogen by 2006 (Table 1).

Total microbial biomass did not change significantly in the 2 years since the material was placed on site (Table 1). However, MDS analysis of PLFA data demonstrated a clear difference in microbial community composition between samples from 2004, 2005, and 2006 (Fig. 1). The very low stress value of 0.05 indicated that this ordination was an excellent representation of the similarity data, and ANOSIM analysis indicated that the differences between samples collected in 2004, 2005, and 2006 were highly significant (Table 2).

Signature PLFAs indicated that there were some significant changes in the relative numbers of some microbial populations over the course of the study (Fig. 2). There were significant relative increases in two fungal-specific PLFAs, 18:2ω6,9c and 18:1ω9c, with time since application (Fig. 2, $p<0.001$). A significant increase over time was also seen in the relative concentration of the actinomycete-specific PLFA 10Me18:0 (Fig. 2, $p<0.001$). When all the bacterial-specific PLFAs were summed as a group, there was a significant decrease in the relative amount of bacteria-specific PLFAs over time (Fig. 3A, $p<0.001$). Signature PLFA analysis also revealed a shift within the soil bacterial communities over the course of the study, with a significant relative increase over time in total Gram positive bacteria-specific PLFAs as a fraction of the total bacterial PLFA pool ($p<0.001$) and a significant relative decrease over time in total Gram negative bacteria-specific PLFAs as a fraction of the total bacterial PLFA pool ($p<0.001$; Fig. 3B).

MDS analysis of T-RFLP data revealed a separation of 2004 samples from 2005 and 2006 samples but only a slight separation of 2005 and 2006 samples (Fig. 4). The low stress value of 0.13 indicated that this ordination was a very good representation of the similarity data. ANOSIM confirmed that there was a statistically significant difference in bacterial community composition between 2004 and 2005 samples and between 2004 and 2006 samples, but there was not a significant difference between 2005 and 2006 samples (Table 3). Binary T-RFLP data (presence vs. absence of peaks) was used in the MDS analysis (Fig. 4 and Table 3) to avoid any potential bias introduced by PCR amplification. However, when relative peak height values were used for the MDS and ANOSIM analyses in place of the binary data, the results did not vary in a significant way from the results for the binary data (results for peak height data are not shown).

Clone library results also revealed shifts in microbial community composition over the course of the study. Specifically, clone library analysis indicated that in 2004, the soil bacterial community was dominated by Gram-negative bacteria; in 2005, it was dominated by Gram-positive bacteria; and in 2006, Gram-negative bacteria were again more numerous (Fig. 5). Examination of the division or phylum level affiliations of the sequences in the clone libraries showed some informative trends (Fig. 6). Proteobacteria

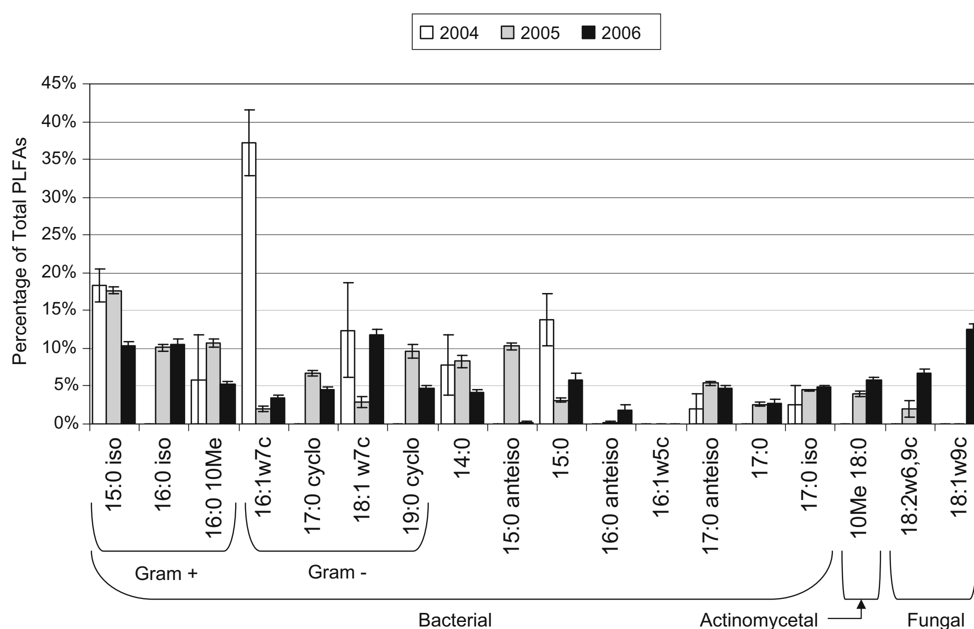

Figure 2 Phospholipid profiles for soils from 2004, 2005, and 2006. Each data point is mean ± standard error (2004 n=3; 2005 n=12; 2006 n=15). Groupings of signature phospholipids based on Zak et al. [44]

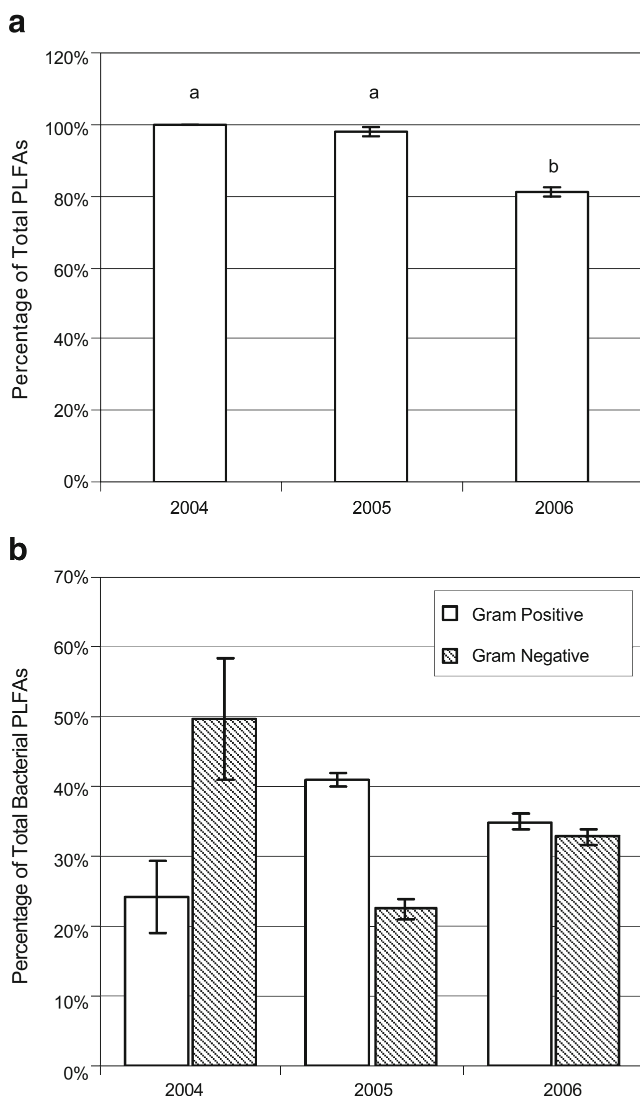

Figure 3 Total bacterial PLFAs as a percentage of total PLFAs (**A**) and relative amounts of PLFAs specific for Gram-positive and Gram-negative bacteria expressed as a percentage of total bacterial PLFAs (**B**). Each data point is mean ± standard error (2004 n=3; 2005 n=12; 2006 n=15). Data points with different letters are significantly different (p<0.001)

were significant components of the communities from all years, and the relative percentage of Proteobacteria within the clone libraries increased over time. Gemmatimonadetes did not appear in 2004, but they accounted for 4% of the community in 2005 and 13% in 2006. Planctomycetes represented a small fraction of the communities in 2004 and 2005 and did not appear in 2006. Similarly, Spirochaetes were present in 2004 but were not seen in the 2005 and 2006 samples. The clone library analysis also showed that Bacteriodetes and Acidobacteria were significant components of the communities in all years. However, there were changes in their relative contributions to the clone libraries, namely, Bacteriodetes increased over time while Acidobacteria decreased over time.

Discussion

The drastic decrease in moisture content that was observed (Table 1) was expected due to the movement of the sediment from the river bottom to the soil surface. This drastic decrease in moisture content should have resulted in an increase in oxygen availability that would be expected to have encouraged the activity of aerobic heterotrophic microorganisms which, in turn, likely contributed to the significant decrease in organic matter content between 2004 and 2005 (Table 1). The mixing of the sediment that occurred during collection and deposition would also be expected to have promoted the decomposition of organic matter by disrupting the structure of the material and increasing organic matter availability. However, between 2005 and 2006, the organic matter content of the soil rebounded, although it did not reach the level observed in 2004 (Table 1). This increase in soil organic matter in 2006 was likely due to the significant increase in plant growth on the soil. In 2004, there was no visible plant growth at the site. In 2005, the site was mainly covered in rye grass, and by 2006, there was a dense and diverse mixture of rye grass and volunteer plants growing at the site. This plant growth certainly would be expected to have contributed to the soil organic matter pool. A previous study at a different site also reported an increase in soil organic matter in land-applied sediments due to plant growth [26]. The increase in plant growth at our site may have also led to the observed decrease in total soil nitrogen levels in 2006 (Table 1), as a larger plant community would be expected to have increased nitrogen incorporation into plant biomass.

Despite these significant changes in soil physical and chemical parameters, the total microbial biomass did not change significantly in the 2 years since the material was placed on site (Table 1). This result is somewhat surprising,

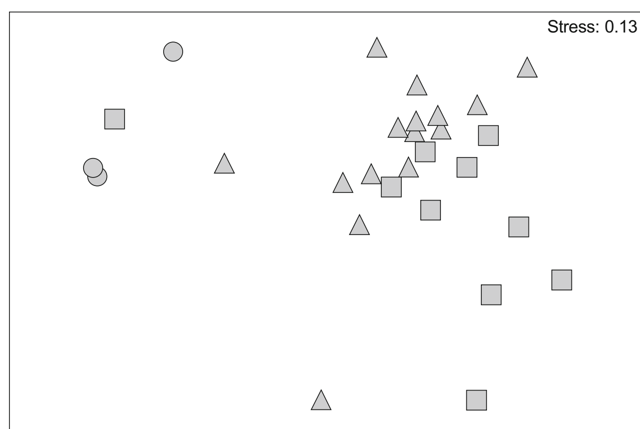

Figure 4 Nonmetric multidimensional scaling (MDS) analysis of bacterial communities based on T-RFLP Data: 2004 (circle;n=3), 2005 (*square*; n=12), 2006 (*triangle*; n=15)

Table 3 ANOSIM values for T-RFLP data

Comparison	*R* statistic	*p* value (*p*<)
2004, 2005	0.857	0.001
2004, 2006	0.699	0.007
2005, 2006	0.212	0.016

especially given the large changes in soil moisture, organic matter, and total soil nitrogen that occurred (Table 1). However, biomass is not the most sensitive indicator of changes in microbial communities. Changes in specific microbial populations within a community have often been shown to occur even when total microbial community size remains unchanged [21, 34]. Therefore, molecular methods were used in this study to examine the composition of the soil microbial communities in more detail.

MDS analysis of PLFA data indicated that there were significant shifts in microbial community composition at the site over time (Fig. 1 and Table 2), and analysis of signature PLFAs provided more specific information on the changes occurring within the soil microbial communities. The observed relative increases in two fungal specific PLFAs (Fig. 2) are consistent with the shift from primarily anaerobic sediment to a more aerobic terrestrial soil, as fungi typically represent a substantial portion of the microbial biomass in terrestrial soils [32]. The increase in fungi may also reflect the increasing contribution of plants to the system, as fungi form symbiotic associations with roots of a range of plant species [38], and fungi play an important role in the degradation of lignin.

A significant increase was also seen in the relative concentrations of an actinomycete-specific PLFA (Fig. 2),

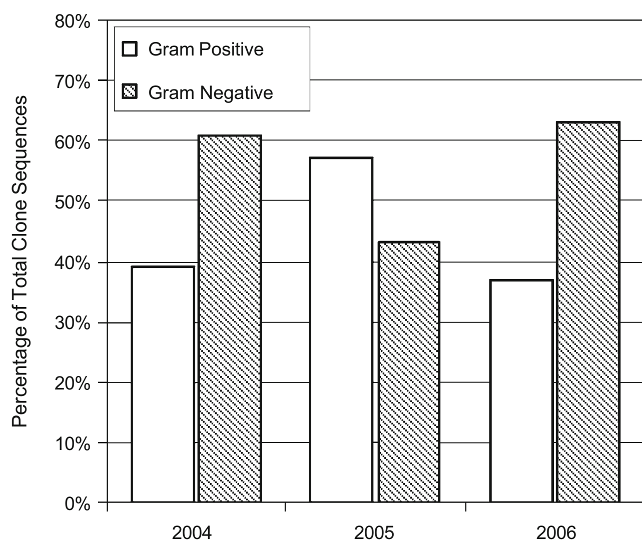

Figure 5 Percentage of Gram-positive and Gram-negative sequences obtained from clone library analysis

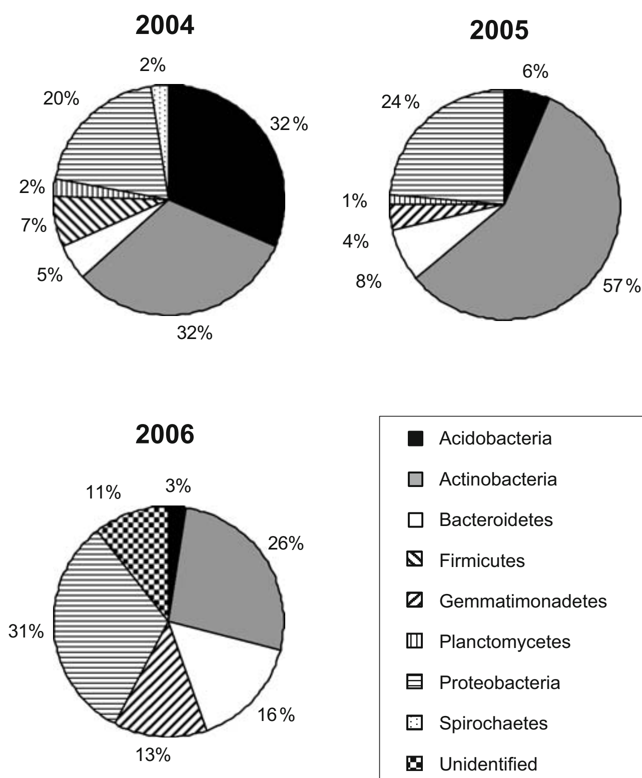

Figure 6 Composition of clone libraries from 2004, 2005, and 2006

which is also consistent with the shift from primarily anaerobic to more aerobic conditions, as the majority of soil actinomycetes are aerobic, and they can be present in high numbers in terrestrial soils [42]. The increase in actinomycetes may also reflect the increasing influence of plants, as some actinomycetes form symbiotic associations with the roots of some nonleguminous plants [31], and actinomycetes contribute to the breakdown of plant material by producing lignin and cellulose-degrading enzymes [29].

Signature PLFA analysis also revealed a shift within the soil bacterial community over time with a significant relative increase in Gram-positive bacteria and a significant relative decrease in Gram-negative bacteria (Fig. 3B). Previous work has shown that Gram-positive bacteria dominate in bulk soils whereas Gram-negative bacteria dominate in the high nutrient environment of the rhizosphere [28]. Therefore, the dominance of Gram positives in 2005 may reflect the transition to a more terrestrial ecosystem prior to the presence of a large plant community, and the increase in relative numbers of Gram negative bacteria between 2005 and 2006 may reflect the increasing influence of plants.

The resolution of PLFA analysis is limited to these broad categories of microorganisms, so the composition of the bacterial portion of the microbial communities was examined in more detail using DNA-based techniques. T-RFLP analysis revealed a significant difference in bacterial

community composition between 2004 and 2005 samples and between 2004 and 2006 samples but no significant difference between 2005 and 2006 samples (Fig. 4 and Table 3), suggesting that the main shift in bacterial community composition occurred in the first year after surface application of the sediment. This corresponds to the drastic decrease in moisture content (Table 1), which would be expected to have had a significant impact on bacterial community composition due to the increased availability of oxygen. Another interesting aspect of the MDS analysis is that both the ordination (Fig. 4) and the ANOSIM (Table 3) indicated that the 2004 samples were more similar to the 2006 samples than they were to the 2005 samples. This is somewhat surprising since the 2006 communities have had more time to diverge from the original communities than the 2005 communities. However, this pattern fits with the patterns observed for soil moisture, pH, and organic matter content, namely, that the levels for 2006 are closer to the levels for 2004 than they are to the levels for 2005 (see Figs. 1A, B and 2A). Clone library data showed a similar trend in Gram-negative and Gram-positive bacteria (Fig. 5) to that observed with PLFA analysis (Fig. 3B), namely, PLFA and clone library analysis both indicated that in 2004, the soil bacterial community was dominated by Gram-negative bacteria, and in 2005, it was dominated by-Gram positive bacteria. As stated above, this increase in Gram-positive bacteria may reflect the shift towards a more terrestrial soil community prior to the presence of a large plant community. However, PLFA and clone library analysis varied for 2006 samples: PLFA showed similar relative amounts of Gram-negative and Gram-positive bacteria in 2006, whereas clone library analysis showed higher levels of Gram-negative bacteria. This discrepancy may be due to the differences in the two methods: PLFA analyzes cell membrane components extracted from the microbial community as a whole, whereas clone library analysis represents a limited, random sampling of the bacterial community. Thus, clone library analysis is subject to errors associated with random sampling.

Examination of the division or phylum level affiliations of the sequences in the clone libraries showed some trends consistent with the movement of the sediment from an aquatic habitat to a terrestrial habitat (Fig. 6). For example, Proteobacteria were significant components of the communities from all years, as would be expected since Proteobacteria are commonly found in both aquatic and terrestrial habitats [48]. In contrast, Spirochaetes were present only in the 2004 sample. Spirochaetes are generally anaerobic, and they have been found to be absent from clone libraries of terrestrial soils [4] so their presence in 2004 may reflect the predominantly anoxic conditions in the river sediment and their disappearance from the clone libraries in 2005 and 2006 may reflect the increasingly aerobic conditions that arose as the soil dried. Gemmatimonadetes did not appear in 2004, but their contribution to the clone library increased over time. Gemmatimonadetes are aerobes [47], so their increasing contribution over time may also reflect the increasingly aerobic conditions in the soil. In contrast, Planctomycetes were lost from the clone library over time, and this seems to fit with the transition from an aquatic to a terrestrial system, as Planctomycetes were first isolated from aquatic freshwater habitats, and they have not been isolated from terrestrial soils [46]. However, there is some molecular data suggesting that Planctomycetes actually are present in terrestrial soils [46] so the clone library data may not represent a complete loss of these organisms but only a decrease in their relative abundance.

The clone library analysis also showed that Bacteriodetes and Acidobacteria were significant components of the communities in all years (Fig. 6). This result was expected, as both of these groups are typically found in sediment and soil habitats. However, there were changes in their relative contributions to the clone libraries, namely Bacteriodetes increased slightly over time while Acidobacteria decreased over time. These shifts may reflect the decrease in organic carbon that occurred in the soils, as a study by Fierer et al. [13] demonstrated in field and lab studies that relative numbers of Acidobacteria were negatively correlated with organic carbon availability and relative numbers of Bacteriodetes were positively correlated with organic carbon availability.

There are no other studies we are aware of that have examined shifts in microbial community composition following land application of dredged sediment. However, a number of studies have examined the impact of water level changes on soil microbial community composition. For example, Rees et al. [36] demonstrated that drying of stream sediments due to a natural drought resulted in a significant shift in T-RFLP profiles of sediment microbial communities. Similarly, a recent study by Jaatinen et al. [19] demonstrated that water level drawdown in a boreal soil resulted in a relative decrease in PLFA signatures for Gram-negative bacteria in the upper 10 cm of soil and an increase in fungal PLFAs in the upper 5 cm. These results parallel the results seen in our study in which river sediments went through a significant drying after application to the soil surface and a decrease in Gram-negative bacteria and an increase in fungi were observed via PLFA analysis. Interestingly, several other studies have examined the opposite situation, a drastic increase in soil moisture content due to flooding. Two recent studies of this type demonstrated that flooding resulted in a decrease in the ratio of fungal to bacterial PLFAs [5, 10]. These results are directly opposite to the results observed in our study, which is logical since our study was examining the opposite process, i.e., soil drying as opposed to soil flooding.

Conclusions

The transfer of sediment from an aquatic environment to a terrestrial environment represents a drastic change in habitat. In this study, the application of Illinois River sediment to the US Steel Southworks site resulted in significant changes in the physical, chemical, and biological properties of the sediment over a 2-year period: moisture content, organic carbon, and total nitrogen decreased over time. Total microbial biomass did not change over the course of the study, but there were significant changes in the composition of the microbial communities. PLFA analysis revealed significant relative increases in fungi, actinomycetes, and Gram-positive bacteria. T-RFLP analysis indicated a significant shift in bacterial community composition within 1 year of application, and clone library analysis revealed relative increases in Proteobacteria, Gemmatimonadetes, and Bacteriodetes, and decreases in Acidobacteria, Spirochaetes, and Planctomycetes. Results from these three very different modern methods of microbial community analysis have provided a thorough view of microbial community shifts following a drastic habitat disturbance. These results should be informative for future land applications of aquatic sediment, as they have demonstrated that microbial community composition can effectively transition from aquatic species to terrestrial species without any additional human intervention.

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