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Soil microbial diversity, community structure and denitrification in a temperate riparian zone

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Abstract. Nitrate (NO_3^-) removal in riparian zones bordering agricultural areas occurs via plant uptake, microbial immobilisation and bacterial denitrification. Denitrification is a desirable mechanism for removal because the bacterial conversion of NO_3^- to N gases permanently removes NO_3^- from the watershed. A field and laboratory study was conducted in riparian soils adjacent to Carroll Creek, Ontario, Canada, to assess the spatial distribution of denitrification relative to microbial community structure and microbial functional diversity. Soil samples were collected in March, June, and August 1997 at varying soil depths and distances from the stream. Denitrification measurements made using the acetylene block technique on intact soil cores were highly variable and did not show any trends with riparian zone location. Microbial community composition and functional diversity were determined using sole carbon source utilization (SCSU) on Biolog® GN microplates. Substrate richness, evenness and diversity (Shannon index) were greatest within the riparian zone and may also have been influenced by a rhizosphere effect. A threshold relationship between denitrification and measures of microbial community structure implied minimum levels of richness, evenness and diversity were required for denitrification.

Key words: denitrification, functional diversity, microorganisms, sole carbon source utilization, temperate riparian zone

Introduction

Riparian buffer zones are recommended as a means of decreasing nitrate (NO_3^-) concentrations in groundwater draining from agricultural land (Gilliam 1994; Martin et al. 1998; Muscutt et al. 1993; Vought et al. 1994). Nitrate as a nonpoint-source pollutant can be linked to agriculture through excess fertilizer use and the improper disposal of livestock wastes. The accumulation of NO_3^- in groundwater is a concern in eutrophication (Jacobs and Gilliam 1985) and as a hazard to human and animal health (Prasad and Power 1995; Sotomayor and Rice 1996). Riparian zones, located between agricultural fields and aquatic ecosystems, are in the physical position to modify, incorporate, and dilute NO_3^- in ground water before it enters waterways (Osborne and Kovacic 1993). Numerous studies have documented the NO_3^- attenuating capacity of riparian zones (Haycock and Burt 1993; Jordan et al. 1993; Osborne and Kovacic 1993; Simmons et al. 1992). However, the primary mechanism responsible

for NO_3^- attenuation is unclear. Three processes are capable of removing NO_3^- from groundwater: plant uptake, microbial immobilization and biological denitrification. Of these three mechanisms, biological denitrification is the most desirable means of NO_3^- removal. The end product of this bacterial process is molecular N_2 (1), a gas that is released to the earth's atmosphere.

$$\underset{\text{nitrate}\rightarrow\text{nitrite}\rightarrow\text{nitric oxide}\rightarrow\text{nitrous oxide}\rightarrow\text{nitrogen}}{NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2} \quad (1)$$

If immobilized by plants or microbes, NO_3^- is returned to the landscape upon death and decomposition (Groffman et al. 1991; Hanson et al. 1994). Denitrification, however, has the potential to produce long term improvements in water quality, as it represents a true removal of NO_3^- from the watershed (Groffman et al. 1991). Although the benefits of denitrification as a NO_3^- removal mechanism are known, the perspective from which the process has been studied needs to be expanded. This includes the need for further investigation into biological denitrification in subsurface soils (Martin et al. 1998).

Current protocols for determining the desired characteristics of riparian buffer zones are made on the basis of physical size, vegetation and hydrological parameters. However it is difficult to predict the denitrifying ability of riparian soils based on these characteristics. As recognized by Kennedy and Smith (1995) assumptions of soil microbiology derived from the above ground ecology of plants and animals are not valid. These considerations may not encompass spatial and temporal variables that regulate the microbial community, and consequently, denitrification. A possible solution is the development of a classification system for riparian soils based on function as well as structure. This may be accomplished by exploring relationships between riparian soil microbial processes (i.e. denitrification) and soil microbial community structure.

The paucity of studies linking the structure and function of microbial communities may be the result of limitations in classical methods of microbiology. Methods dependent on culturing exclude many microbes due to the selective nature of media (Garland and Mills 1991). Other techniques, molecular approaches and phospholipid fatty acid analysis, are limited by their technical demands (Buyer and Drinkwater 1997). Garland and Mills (1991) developed an approach to the characterization of soil microbial communities based on sole carbon source utilization (SCSU) in Biolog® GN microplates (Biolog Inc. Hayward, CA, USA). Although several limitations of SCSU have been identified (Garland 1998; Glimm et al. 1997; Konopka et al. 1998; Haack et al. 1995; Winding 1994), the method is rapid, inexpensive, and relatively easy to perform, making it useful for preliminary studies of microbial community composition. Furthermore, metabolic fingerprints of bacterial communities obtained with Biolog plates are reproducible (Haack et al. 1995) and are ideal for detecting site-specific differences between closely related habitats (Pankhurst et al. 1996; Winding 1994).

The objective of this study was to relate biological denitrification in a temperate riparian zone to soil microbial community composition and microbial functional diversity. Effective characterization of these relationships could have implications for sustainable riparian zone management. In addition, microbial community structure and biological denitrification were examined at varying distances from the stream and at increasing depths in the soil profile, to improve our understanding of the spatial distribution of microbes and their activities.

Methods

Site characterization

The study was conducted adjacent to Carroll Creek (43°39′ N 80°29′ W), a headwater tributary of the Grand River approximately 24 km northwest of Guelph, Ontario, Canada. The study site was divided into four sections based on land use; (i) agricultural field, (ii) meadow, (iii) woody riparian zone, and (iv) grassy riparian zone (Figure 1). Woody and grassy riparian zones were approximately 70 m wide. Vegetation in the woody riparian zone was characterized by mixed tree and shrub species. The dominant tree species in the zone were willow (*Salix* spp.) and basswood (*Tilia americana*). A transitional area (approximately 10 m wide) separated both riparian zones from the meadow. Meadow, as defined in this study, is a low-lying, previously cultivated agricultural field dominated by grasses, weeds and wildflowers. Located 160 m from the stream was an agricultural field, planted in soybeans. The slope from the soybean field to the stream edge was calculated as 3%. Soils for the watershed are a blend of well-drained silty loam and sandy loam and is part of the Brant–Harriston complex (Hoffman et al. 1963). Analysis of core subsamples at the site indicated soils between 0–300 cm deep were fine–medium grained sand with trace amounts of clay. Soils between 300–600 cm deep were described as clayey sand and clayey silt and were characterized by the presence of stones, cobbles, and pebbles (Davidson 1997).

Soil collection

Soil cores were collected three times during the 1997 study period in March, June, and August. Samples collected on March 10 and 11, 1997 were obtained using a split spoon core sampler attached to a drill rig (Davidson Environmental Drilling, Waterloo, ON, Canada). A total of 48 soil cores 2 cm in diameter and 7 cm in length were obtained from depths ranging from 100 to 600 cm below soil surface, at four sampling locations (Figure 1). Fourteen additional cores were collected on June 9, 1997 using a hand operated stainless steel core sampler (sanitized with 75% ethanol and air

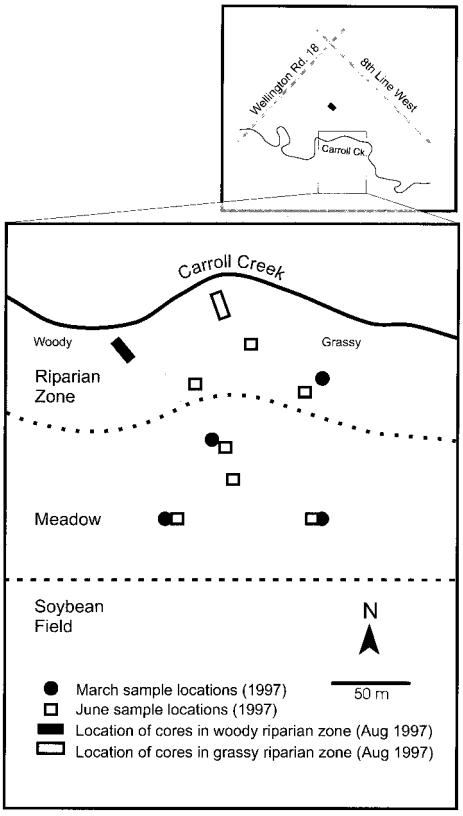

Figure 1. Study site at Carroll Creek, Ontario, Canada.

dried between samples). Samples were taken from two shallow depths (0–9 cm) and (21–30 cm) at seven random sampling locations at the site (Figure 1). Soil collected in June was not of a consistency able to retain core formation; however, the volume of each sample was known to be 113 cm^3. A third set of soil cores collected on August 5 and 6, 1997 was removed using a prototype of the drive point/piston sampler (DPPS) described in Starr and Ingleton (1992). Replicate soil cores were randomly collected along transects in the grassy and woody riparian zones (Figure 1), at three distances from the stream (4 m, 10 m, 15 m). The DPPS allowed for extraction at discrete depths of 10, 100, 250 and 350 cm below the soil surface. Each of the 42 soil cores measured 5 cm in height and 4.5 cm in diameter.

For all three sampling dates, cores were sealed in sterile 250 ml glass mason jars and placed on ice for transport to the laboratory. Soil cores were stored at 4 °C in the dark until analyses could be performed. March samples were subjected to the longest storage times with denitrification analyses begun within 30 days of collection and SCSU analyses begun within 79 days of storage. Although this is an extended

time for environmental samples to be stored, it is within the guidelines suggested by Anderson (1987) who recommended soil testing be performed within 3 months of collection. Tate (1995) has indicated that storage of soil at 4 °C in the dark results in minimal changes in experimental data, even after several weeks. Breitenbeck and Bremner (1987) found storage of soils at 4 °C is a satisfactory method of preserving denitrification activity for 1 month. Since March denitrification assays were performed within 30 days from time of collection, denitrification data were not considered to have been jeopardized. All analyses performed for June and August soil samples were completed within 3 weeks of sample collection.

Percent soil water content was determined gravimetrically by placing soil samples in a drying oven at 105 °C for 24 hours. Soil samples collected in March and August were extracted for NO_3^- by adding 20 g of soil to 60 ml of 2 N KCl and shaking vigorously at 300 rpm for 1 h. Extracts were analyzed for NO_3^- using the cadmium reduction method on a Technicon Autoanalyzer II system (Technicon Industrial Systems, Tarrytown, NY, USA). Percent organic carbon was determined in soil samples collected in March and August using the Leco Induction Furnace (Leco Corporation, St. Joseph, MI, USA) dry combustion method (Tabatabi and Bremner 1970).

Denitrification activity

Soil cores were analyzed for both natural and potential denitrification rates. For March and August cores, natural denitrification was measured using a modified version of the static core method and acetylene (C_2H_2) inhibition assay outlined in Tiedje et al. (1989). Briefly, soil cores were contained in 250 ml mason jars, each with an embedded serum stopper (Suba Seal, Barnsley, UK) in the lid. Jars were alternately evacuated with a vacuum pump and flushed with 100% helium to create an anaerobic environment. Purified, acetone free, C_2H_2 was added to each core to a final concentration equivalent to 10 kPa. This concentration of C_2H_2 has been shown to be effective in blocking the final denitrification reaction that oxidizes nitrous oxide (N_2O) to N_2 gas (Tiedje 1982). Nitrous oxide accumulates in the headspace and can be measured using gas chromatography. Sampling consisted of the removal of 1 ml of headspace using a glass gastight syringe after 1, 3, 4, and 6 h of incubation time. Samples were analyzed by a gas chromatograph (Gow-Mac Instrument Co. Series 150 Gas Chromatograph, Shannon, Ireland) equipped with a Porapak Q column and thermal conductivity detector operated at 150 mA. Oven and detector temperatures were 50 °C; the carrier gas was He at a flow rate of 40 ml/min. Because June soil samples collapsed upon removal from the corer, the static core method could not be used to determine natural denitrification rates. Alternatively, a modified version of Duff and Triska's (1990) slurry method was used. A sample (10 g) of field moist soil was placed in a sterile 125 ml Erlenmeyer flask and slurried with 5 ml of sterile Optima water (Fisher Scientific, Fair Lawn, NJ, USA). As described previously, an anaerobic environment was created and C_2H_2 was added to each sample jar. Jars

were incubated at room temperature and shaken continuously (150 rpm) on an orbital shaker for the 6 h incubation period.

Potential denitrification rates are representative of denitrification under optimum conditions and are a reflection of the initial activity of denitrifying enzymes in the soil samples. Replicate slurries were made for each soil sample by amending 10 g of field moist soil with 20 ml of solution containing nitrate (KNO_3^- (0.2 g/l)), carbon (dextrose (2 g/l)) and chloramphenicol (1 g/l), a protein synthesis inhibitor that prevents *de novo* enzyme synthesis. Flasks were sealed with serum stoppers (Suba Seal, Barnsley, UK) and alternately evacuated and flushed with helium gas three times. The appropriate volume of C_2H_2 was added to each flask to achieve a final headspace partial pressure of 10 kPa. Slurries were incubated on an orbital shaker at room temperature. Gas samples (1 ml) were taken at regular intervals over a one hour period and analyzed for an accumulation of N_2O by injection into a gas chromatograph equipped with thermal conductivity detector.

Natural and potential denitrification rates were corrected for the amount of N_2O dissolved in the soil water using the Bunsen equation (Tiedje 1982) and expressed in μmol/g dry soil/h.

Sole carbon source utilization

Biolog GN microplates comprise a 96-well microtitre plate with 95 different carbon sources and a negative control well without a carbon source. Each well also contains nutrients, salts, peptone, and the redox dye tetrazolium violet in dried film form (Biolog Inc. 1991). Wells are colourless at the time of inoculation, but those that undergo respiration oxidize the carbon substrate and reduce the tetrazolium dye to an insoluble violet formazan (Biolog Inc. 1991). This results in a metabolic fingerprint of the microbial community that can be characterized by the rate and pattern of colour development, as well as the combined richness and evenness of colour response among wells (diversity) (Staddon et al. 1998; Zak et al. 1994).

Isolation of microorganisms for inoculation into Biolog GN microplates was performed by modifying the method of England et al. (1995). A 5 g soil sample was suspended in 15 ml of sterile 0.1% (w/v) sodium pyrophosphate (pH adjusted to 7.0) with 3 g of glass beads and shaken on an orbital shaker for 1 hour at 170 rpm at room temperature. The resulting soil suspension was centrifuged (680 $\times$ *g* for 10 min at 4 °C) and 10 ml of supernatant was removed, placed in a sterile centrifuge tube and the soil extracted again with 10 ml of sterile 0.1% (w/v) sodium pyrophosphate. This procedure was repeated twice, giving a total of 30 ml of pooled supernatant. The pooled supernatant was centrifuged at 1000 $\times$ *g* for 15 min 4 °C to remove remaining plant and soil debris and then transferred to a sterile centrifuge tube. This process was repeated to ensure the final cell suspension was relatively free of colloids other than bacteria. The resulting supernatant was centrifuged at 8000 $\times$ *g* for 10 minutes at 4 °C to pellet the extracted microorganisms. This bulked pellet of bacteria was washed and

centrifuged twice at 8000 × *g* for 10 minutes at 4 °C in sterile Optima water (Fisher Scientific, Fair Lawn, NJ, USA) and resuspended in 30 ml of sterile 0.85% (w/v) sodium chloride. Inoculum densities were standardized by diluting each sample to a percentage transmittance of 75% at 595 nm using a Spectronic 20 spectrophotometer with 0.85% NaCl as a blank. Each replicate suspension was inoculated immediately onto a Biolog GN microplate (100 μl per well) and incubated at 30 °C in darkness for 7 days.

Colour development in the plates was monitored at 24 h intervals by measurement of absorbance at 595 nm using a Bio-Rad M3550-UV Microplate Reader (Bio-Rad Laboratories Inc., Hercules, CA, USA) and Microplate Manager Software (v.4.0, Bio-Rad Laboratories Inc., Hercules, CA, USA). Data collected after 72 h were used in statistical analyses.

Statistical analyses

SAS (SAS Institute Inc. Cary, NC, USA) was used to conduct all statistical analyses. Absorbance values for microplate wells with C sources were adjusted against the control well and transformed by dividing the raw difference value for each well by the average well colour development (AWCD) for the plate (Garland and Mills 1991). Microbial functional diversity – defined by Zak et al. (1994) as the number, type, activity and rates at which a suite of substrates are utilized by the bacterial community – was calculated using the Shannon index for diversity (Table 1). Although this does not allow the identification of exact numbers or taxonomic identities of bacterial species in the community, patterns of functional diversity with and among communities provide insight to soil microbial communities where little understanding currently exists (Staddon et al. 1998; Zak et al. 1994). Intensity of colour development in each well, reflective of species density, was determined for each sample. Defined as Shannon evenness, this value represented the distribution of the individuals within each species designation, and was calculated for each soil sample using the formula and values provided in Table 1. Substrate richness – the total number of substrates utilized on each Biolog plate – was used to estimate the number of functional groups (species richness) in the soil samples. Substrate utilization in this study was defined as significant catabolic activity (optical density values greater than 0.1) to eliminate the influence of weak false positive responses (Garland 1998; Heuer and Smalla 1997).

Differences in denitrification activity, diversity, richness and evenness were compared among March and June data using regression analyses to model equations for the data. Equations were tested for homogeneity of slopes using PROC Mixed (SAS 1990). August data were analyzed using a three-way mixed model analysis of variance (ANOVA). Comparisons of population means were made using least square mean tests. Since soil cores obtained on each of the three sampling dates were collected from different locations at the study site, and were subject to different sampling protocols and storage times, the data were not compared between sample dates.

Table 1. Formulae for calculations.

Index	Purpose	Formula	Definitions
Shannon[a]	To measure species richness and evenness	$H' = -\sum p_i \ln p_i$	p_i = proportional colour development of the *i*th well over total colour development of all wells of a plate N = sum of positive optical densities on a Biolog plate
		$H' = C/N\ (N \ln N - \sum n_i \ln n_i)$	n_i = zero or positive optical density of a test well on a Biolog plate $C = 2.3$
Shannon evenness[a]	To calculate species evenness based on the Shannon Index	$E = H'/\ln S$	H' = Shannon index of diversity S = number of wells on a Biolog plates with colour development (OD > 0.1)

[a] Staddon et al. (1998).

Pearson correlation coefficients were calculated on individual data sets for March, August and June 1997 as well as pooled data sets (comprising all three collection dates), to delineate relationships between microbial community composition and denitrification activity.

Results

Effect of location on denitrification activity

Table 2 shows the mean natural and potential denitrification rates for soil samples collected in March, June and August 1997. For all three sampling dates, no significant changes were observed in natural denitrification rates for different soil depths, distances from the creek or among the different types of above ground vegetation (data not shown). Similarly, sampling location did not have a significant effect on potential denitrification rates in March or June samples (data not shown). Potential denitrification rates for August cores were significantly affected by an interaction between above-ground vegetation and soil depth (Figure 2). In all cases, potential denitrification rates were higher than natural denitrification rates (Table 2). Coefficients of variation were high, but were lower for potential denitrification rates than natural denitrification rates (Table 2).

Effect of location on microbial community composition

The highest Shannon evenness values among March data generally occurred at a distance of 48 m from the creek, the only March sampling location situated within the riparian zone (Figure 3). Modelled response of Shannon evenness to increasing

Table 2. Mean natural and potential denitrification rates and corresponding coefficients of variation for soil samples collected at Carroll Creek, Ontario, Canada. Rates are averaged over all soil depths, distances and vegetation types for each sampling date. For all 3 sampling dates, mean potential denitrification rate was significantly higher than mean natural denitrification rate (Student *t*-test, $P < 0.05$).

	Natural denitrification activity		Potential denitrification activity	
Sampling date	Mean rate (μmol/g/h)	Coefficient of variation	Mean rate (μmol/g/h)	Coefficient of variation
March 1997	1.947 (1.90)[a]	97.7%	15.749 (12.044)	76.5%
June 1997	0.998 (1.35)	135.0%	11.606 (9.877)	85.0%
August 1997	0.258 (0.50)	192.0%	15.428 (18.06)	117.0%

[a] Values in parentheses are standard deviation (March $n = 48$, June $n = 14$, August $n = 48$).

soil depth was represented by a positive quadratic function in all cases except for the sampling location furthest from the stream, which resembled an inverse parabola (Figure 3). Functional diversity and substrate richness did not differ significantly with sampling locations for March samples.

Microbial community composition for June soil samples was significantly affected by sampling depth and distance from the creek, but was not affected by the type of above-ground vegetation. Mean substrate utilization richness on pooled

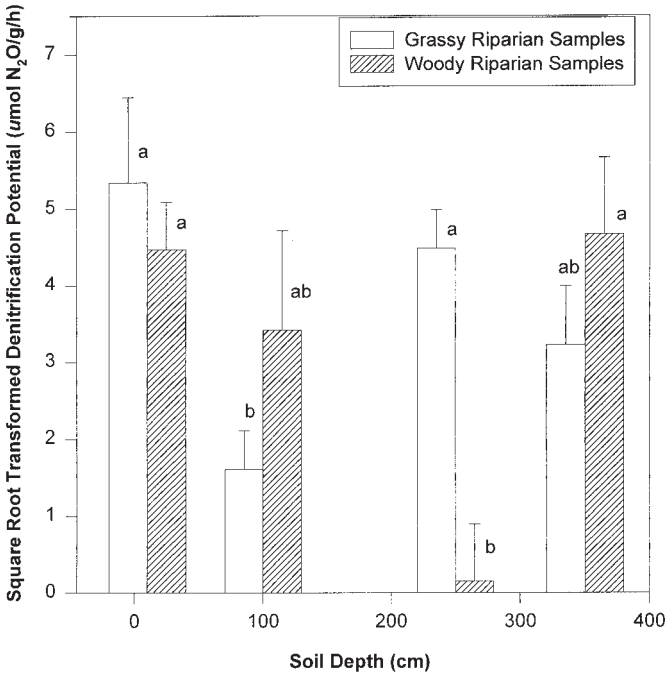

Figure 2. Relationship between potential denitrification rates (square root transformed) and soil depth in grassy and woody riparian zones at Carroll Creek, Ontario, Canada. Soil cores collected August 5–6, 1997. Treatments with the same letters are not significantly different ($P < 0.05$).

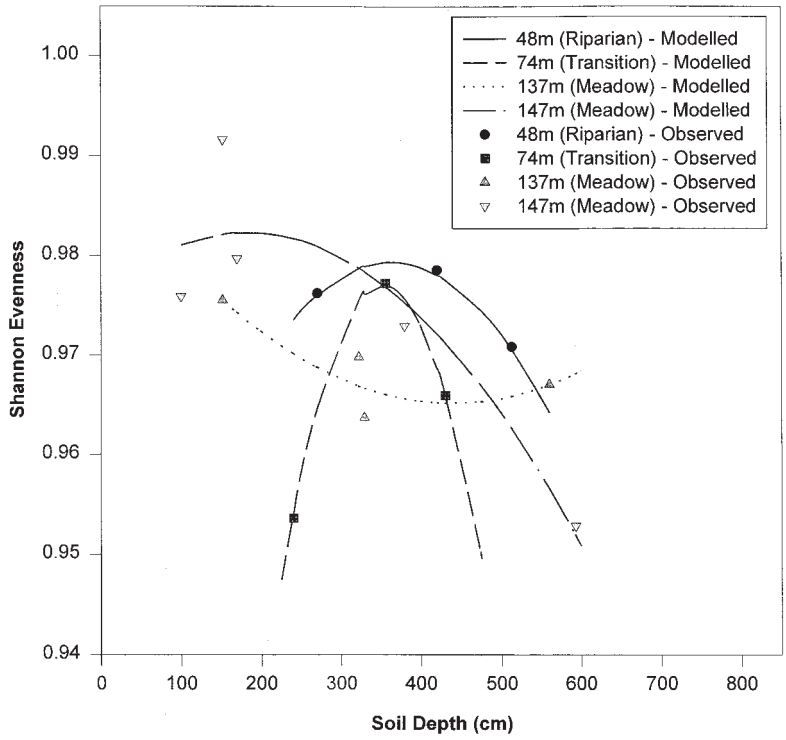

Figure 3. Modelled and observed response of Shannon Evenness of the soil microbial population with increasing soil depth and distance from the stream. Soil samples collected March 10–11, 1997 from Carroll Creek, Ontario, Canada. Slopes of lines are significantly different ($P < 0.05$).

riparian and meadow soil samples was significantly higher at the 0–9 cm soil depth (82.8 ± 3.8; $n = 12$) than at the 21–30 cm soil depth (76.6 ± 4.8; $n = 11$). Similar trends were noted for the Shannon evenness of the samples (Figure 4a) and the Shannon indices of the samples (Figure 4b). At the 0–9 cm soil depth, Shannon evenness and Shannon index values were highest adjacent the stream, within the riparian zone (Figures 4a, 4b). The converse was true for the 21–30 cm soil depth, which indicated a rise in Shannon evenness and Shannon indices of the microbial community with increasing distance from the creek (Figures 4a, 4b).

Substrate utilization richness among August soil samples was highest in the 10–15 cm depth than at lower depths (Table 3). Richness was also affected by an interaction between sampling depth, distance from the creek and above ground vegetation (Table 3). A significant above ground vegetation $\times$ distance from creek interaction affected both the Shannon index (Figure 5a) and Shannon evenness of the microbial community in the August data (Figure 5b).

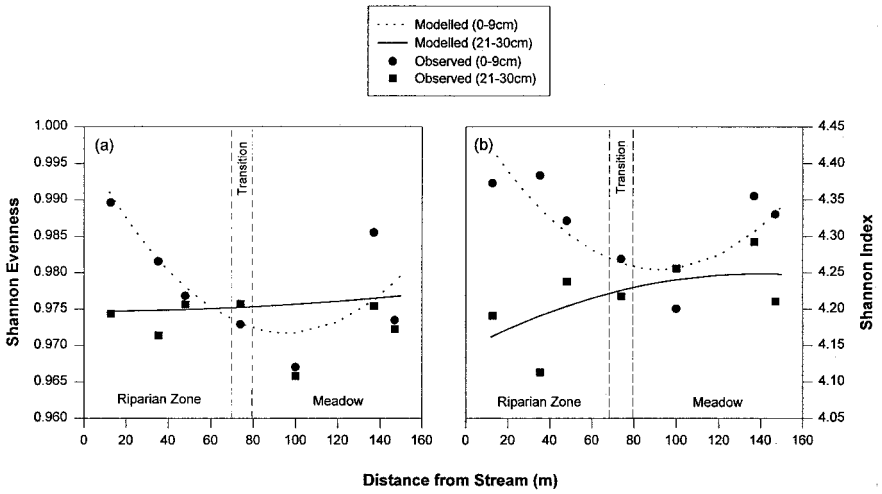

Figure 4. Modelled and observed response of (a) Shannon evenness and (b) Shannon index of soil microorganisms with increasing depth and distance from the stream. Microbes extracted from soil samples collected at Carroll Creek, Ontario, Canada, June 8–9, 1997.

Relationship between denitrification activity and microbial community structure

Correlation analyses on individual data sets did not reveal any significant relationships between microbial community structure (diversity, richness, evenness and denitrification activity). When the data were pooled over all three sampling dates, positive relationships between natural denitrification activity and substrate richness, evenness and functional diversity were observed (Table 4). When illustrated graphically, the relationship was not linear, but showed a threshold effect (Figures 6a–6c). Although correlation coefficients between measures of the soil microbial community and potential denitrification rates were non-significant (Table 4), a similar relationship was observed (Figures 6d–6e).

Table 3. Variation in the richness of soil microorganisms in the riparian zone adjacent Carroll Creek, Ontario, August 1997. Species richness is based on the number of utilized substrates on BIOLOG plates, after 3 days of incubation at 30 °C. Values with the same letters indicate no significant differences at $P = 0.05$.

	Grassy Riparian Zone Distance from stream (m)			Woody Riparian Zone Distance from stream (m)		
Soil depth (cm)	4	10	15	4	10	15
10–15	78^{a}	MD	82^{a}	69^{ad}	MD	MD
100–105	68.5(4.5)[1],^{ac}	62(4)^{ad}	62.5(5.5)^{ad}	32(5)^{be}	81.5(0.5)^{a}	69.5(2.5)^{a}
250–255	68(3)^{ac}	63.5(3.5)^{ad}	51.5(14)^{ade}	53(11)^{ad}	74(4)^{a}	59.5(3.5)^{ad}
350–355	40(26)^{bd}	40.5(15)^{bcd}	69(2)^{ac}	54(10)^{ad}	68.5(2.5)^{ac}	40.5(3.5)^{bcd}

[1] Values in parentheses are standard error ($n = 42$). MD = missing data.

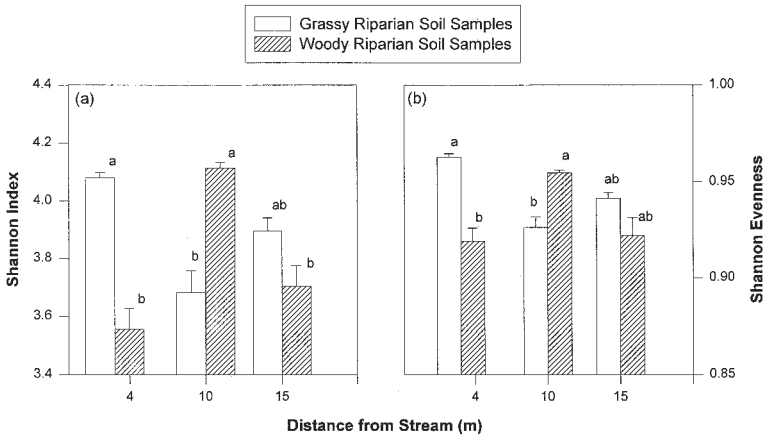

Figure 5. Variation in (a) Shannon index and (b) Shannon evenness of soil microbial populations in woody and grassy riparian zones with increasing distance from stream. Microbes extracted from soil samples collected August 5–6, 1997. Bars with the same letters are not significantly different ($P < 0.05$).

Discussion

Denitrification data from this study were subject to high spatial variability typically observed when soil microbial processes are measured. Parkin (1987) concluded that the patchy distribution of denitrification activity in soil was the result of denitrifying 'hot spots', induced by discrete anaerobic microsites and/or small patches of organic matter. While most soil samples tend to exhibit low rates of denitrification, samples containing hot spots will produce high rates of denitrification, resulting in skewed data and high spatial variability (Jacinthe et al. 1998; Parkin 1987). High spatial variability was observed at this study site, as illustrated by the large coefficients of variation. Other researchers have presented high coefficients of variation on soil denitrification data (Ambus and Christensen 1993; Parkin 1987). This high spatial variation may partially account for a lack of significant trends observed in the distribution of natural and potential denitrification rates at the site. High coefficients of variation may have masked any differences in denitrifying activity with soil depth, distance from the creek or within different types of above ground vegetation.

Coefficients of variation were lower for potential denitrification data. In this case, the spatial variation was likely reduced because potential denitrification measurements were made on core subsamples which had been mixed and from which large pieces of decaying organic material had been removed (Murray et al. 1995). In addition, all major factors limiting denitrification were optimized, thus the variability associated with potential denitrification is the variability due only to the dispersion of potentially active denitrifying enzymes in the soil (Parkin 1987). Reduced spatial variability allowed the significant interactive effect of above ground vegetation and soil depth to be revealed for August 1997 data. Despite the presence of this interactive

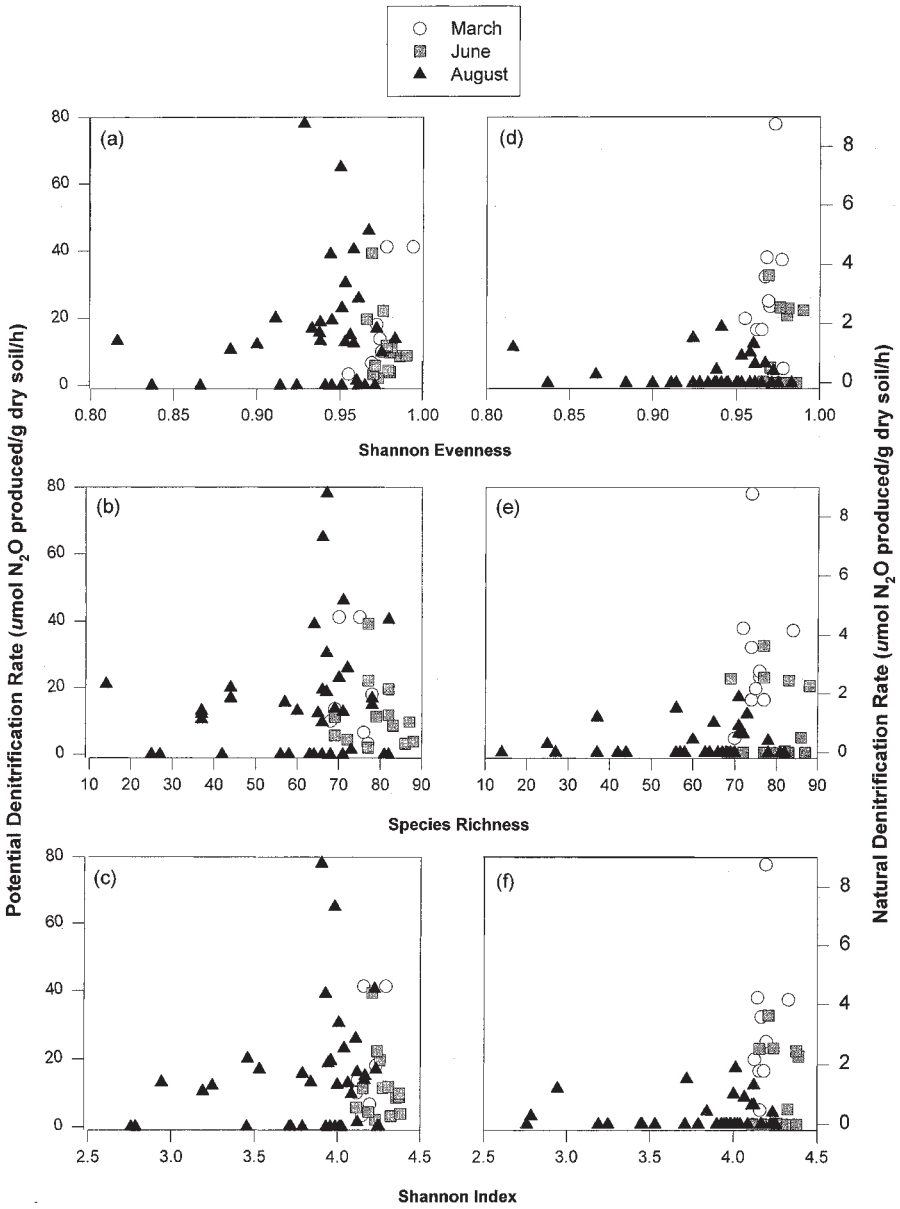

Figure 6. Relationship between potential denitification rates and the (a) Shannon evenness, (b) species richness and (c) Shannon index of extracted soil microorganisms collected in March, June and August 1997 from the riparian zone and meadow at Carroll Creek, Ontario, Canada. Correlations between natural denitrification rates and microbial structure variables. ((d) Shannon evenness, (e) species richness and (f) Shannon index) are based on measurements taken from the same soil samples.

Table 4. Pearson correlation coefficients for microbial structure and function parameters determined from soil samples collected at Carroll Creek, Ontario, Canada (March, June and August 1997). Shannon index, Shannon evenness and species richness based on SCSU of Biolog plates incubated at 28 °C for 3 days. Coefficients marked by * are statistically significant at $P < 0.05$, coefficients marked by ** are significant at $P < 0.01$.

	Shannon index	Shannon evenness	Species richness	Potential denitrification rate
Shannon evenness	0.94 *n* = 91 **			
Species richness	0.96 *n* = 91 **	0.83 *n* = 91 **		
Potential denitrification rate	0.13 *n* = 59	0.17 *n* = 59	0.08 *n* = 60	
Natural denitrification rate	0.25 *n* = 63 *	0.26 *n* = 63 *	0.28 *n* = 64 *	0.06 *n* = 66

effect, no obvious trend with respect to the influence of above ground vegetation on denitrification was displayed. The effect of vegetation on NO_3^- removal remains a major question in riparian zone research. Results of this study neither support nor dispute the findings of Groffman et al. (1991) or Schnabel et al. (1997) who observed higher denitrification rates in grassy riparian plots than in forested or woody vegetative riparian areas.

In general, potential denitrification rates for August samples were not affected by increased soil depth. This observation is useful from a management perspective as it indicates that this site has the potential for NO_3^- removal below the root zone.

Potential denitrification rates for all three sampling dates were significantly higher than their corresponding natural denitrification rates. This suggests that NO_3^- and/or carbon was limiting in collected soil cores. These substrates, along with oxygen (O_2), are the primary regulators which dictate whether denitrification will occur (Knowles 1982). Addition of these substrates to soil cores typically increases denitrification rates to some extent (Ambus and Christensen 1993; Davidsson and Leonardson 1996; Groffman et al. 1991; Schnabel et al. 1996). Despite the dependence of denitrification on these substrates, organic carbon content of the soil samples and soil extracted nitrate were not found to be predictive of denitrification in this study. This observation is similar to that of Zak and Grigal (1991) who observed denitrification rates were not correlated with extractable NO_3^-, microbial biomass C or microbial biomass N in both upland and wetland ecosystems. Similarly, Schnabel et al. (1997) found no correlation between denitrification rates, organic carbon content or extracted soil NO_3^-.

Patterns of SCSU were effective in providing a metabolic fingerprint of the soil microbial community at the site for each of the three sampling dates. Table 5 (adapted from Derry et al. 1999) illustrates the similarity of microbial functional diversity values for this study site with other ecosystems.

Table 5. Table Shannon diversity indices ($H' = -p_i \ln p_i$) between different ecosystems (adapted from Derry et al. 1999) compared with the research presented here. Values in brackets are standard deviations.

Reference	Ecosystem	Community	Shannon index (Incubated at Temp = 30 °C, time = 72 h)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Scarified, mineral forest soil	2.65 (0.41)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Standing timber, mineral forest soil	2.97 (0.21)
Derry et al. (1999)	High arctic tundra (69°22′N, 81°47′W)	Unenriched mineral tundra soil	3.62 (0.73)
This study	Riparian zone (43°39′N, 80°29′W)	Woody riparian soil, August (mineral soil: 350–355 cm deep)	3.64 (0.42)
This study	Riparian zone (43°39′N, 80°29′W)	Woody riparian soil, August (100–105 cm deep)	3.74 (0.62)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Clear-cut, mineral forest soil	3.75 (0.31)
This study	Riparian zone (43°39′N, 80°29′W)	Grassy riparian zone soil, August (mineral soil: 350–355 cm deep)	3.75 (0.55)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Scarified, organic forest soil	3.92 (0.10)
This study	Riparian zone (43°39′N, 80°29′W)	Grassy riparian zone soil, August (100–105 cm deep)	3.94 (0.15)
Derry et al. (1999)	High arctic tundra (69°22′N, 81°47′W)	Human enriched organic tundra soil	4.04 (0.17)
Derry et al. (1999)	High arctic tundra (69°22′N, 81°47′W)	Animal enriched organic tundra soil	4.12 (0.06)
This study	Riparian zone (43°39′N, 80°29′W)	Woody riparian zone soil, August (rhizosphere: 10–15 cm)	4.16 (na)
This study	Riparian zone (43°39′N, 80°29′W)	Grassy/woody riparian zone soil June (21–30 cm deep)	4.18 (0.06)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Clear-cut, organic forest soil	4.21(0.13)
This study	Riparian zone (43°39′N, 80°29′W)	Grassy riparian zone soil, August (rhizosphere soil: 10–15 cm)	4.23 (0.01)
This study	Meadow (43°39′N, 80°29′W)	Meadow soil, June (21–30 cm)	4.25 (0.044)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Organic forest soil following prescribed burn	4.26 (0.13)
This study	Meadow (43°39′N, 80°29′W)	Meadow soil, June (rhizosphere: 0–9 cm)	4.28 (0.08)
Staddon et al. (1998)	Pine forest (46°00′N, 77°33′W)	Standing timber, organic forest soil	4.32 (0.11)
This study	Riparian zone (43°39′N, 80°29′W)	Grassy/woody riparian zone soil June (rhizosphere: 0–9 cm)	4.36 (0.05)

The extent of substrate utilization (evenness) of microbes extracted from soil samples collected in March 1997 was generally highest in the riparian zone. Similarly, June 1997 riparian zone samples generally exhibited higher Shannon indices and higher Shannon evenness values. A possible explanation for this observation is that the microbial community is enriched by the frequent periods of wetting and drying that occur in riparian soils compared to meadow soils. This may also explain the observation that the March evenness data followed a quadratic function with depth. Deeper samples are continuously wet and anaerobic, shallow samples are mostly dry, but samples collected from the 200–400 cm range are more likely to be influenced by fluctuations in the groundwater table, undergoing periods of wetting and drying. Since water is a vector for the movement of materials through soil, these groundwater fluxes might have provided these soil depths with increased nutrients and minerals. For example, Keeney (1980) noted that the diffusion of NO_3^- and carbon was promoted in saturated soils. However it is difficult to determine precisely what factors are affecting the composition of the microbial community in this study. The soil environment is heterogeneous (as indicated by the denitrification data from these samples) and subject to high spatial variability. This is also evident by the observed data from the March soil samples, which often deviate from the modelled data. These data are hampered by a small sample size. More extensive sampling of the field site would have improved the chances of transcending the natural variation of these soil samples; displaying real differences in their spatial distribution.

Microbial functional diversity and substrate utilization richness for March populations did not display any significant trends in their distribution. This observation concurs with that of Derry et al. (1999) who noted that differences between Shannon diversity indices of soil samples occurred only when both richness and evenness components of the measure were significantly different.

Measures of microbial community structure for June and August 1997 samples were generally higher at shallow depths. This may indicate the microbial community in these soils are under the influence of a rhizosphere effect. August richness was greater at the 10–15 cm soil depth and June Shannon index and evenness tended to be higher at 0–9 cm than 21–30 cm. The rhizosphere, with its increased concentrations of carbohydrates, amino acids and organic acids from root exudates, is known to stimulate microbial activity (Beare et al. 1995; Knowles 1982; Sorensen 1997). Even in the woody riparian zone, where deeper roots may place the rhizosphere below the 10–15 cm soil horizon, a decomposing litter layer could increase the nutrient content of the shallow soil samples at the site.

Shannon index, richness and evenness data for August were affected by an interaction between above ground vegetation and distance from the creek. As was true for the denitrification data for this sampling date, the interaction of these variables did not affect the microbial community in a definitive manner. Differences observed may be another example of the influence of high spatial variability affecting microbes. Organic matter availability, temperature, pH, redox potential, percent soil

water and soil structure are known physicochemical factors that affect the composition and distribution of soil biotic communities (Beare et al. 1995; Stotzky 1997). The patchy distribution of these factors in soil is reflected in the heterogeneity of richness, evenness and diversity in this riparian zone in August 1997.

Correlation analysis revealed a possible threshold relationship between denitrification activity and all three measures of microbial community composition. The exact nature of this relationship requires further investigation, but a possible interpretation of these findings is that denitrification is negatively affected below critical levels of functional richness, evenness and diversity.

Although few studies linking the microbial community to system activities have been conducted, the concept that there may be minimum biodiversity requirements to maintain system function has been suggested previously (Beare et al. 1995). For example, a study of the metabolic capabilities of forest soil microorganisms demonstrated lowered respiration rates among diluted and filtered microbial populations (Salonius 1981). Salonius (1981) suggested that this observation was not the result of fewer cells but rather a consequence of reduced complexity of enzymes in the soil. Beare et al. (1995) suggested that the importance of microbial biodiversity to biogeochemical cycling arises from the need to maintain the numerous and complex interactions among soil organisms. Positive interactions in soil may confer greater stability and resiliency to ecosystem function, allowing the soil community to lose species with no measurable effect on function. Thus, the number of species (richness), relative distribution of species (evenness) and diversity that support these complex interactions will be important to the regulation of denitrification activity in soils.

This hypothesis seems to be supported by the results of this study, which depict low natural and potential denitrification rates until a certain threshold of diversity, richness and evenness are achieved. However, these results are presented with caution, since the presence of the threshold effect seems reliant on the low measures of microbial community structure observed for August data. This is less of a concern for potential denitrification rates, where the August data indicates the potential for a threshold effect even in isolation from March and June data. Nonetheless, the heterogeneous nature of soil makes it important to consider the possibility that other factors may be influencing the relationship between denitrification activity and microbial community structure. Fluctuations in soil environmental conditions can affect microbial activity without affecting microbial community structure (Griffiths et al. 1997). Similarly, redundancy in function within soil microbial populations may lessen the importance of community structure on microbial function (Kennedy and Smith 1995). Griffiths et al. (1997) suggest that studies attempting to resolve structure–function interactions are best conducted in laboratory microcosms, to ensure that the environmental component remains constant. The current study was likely subject to high environmental variability. Not only were samples collected on three different sample dates, but they were obtained from different locations at the field site.

Although SCSU is regarded as a fast, easily implemented method of describing bacterial communities (Buyer and Drinkwater 1997; Haack et al. 1995; Winding 1994; Zak 1994), there are several limitations to this technique. For example, Garland and Mills (1991) reported that inoculum density had an impact on colour development in Biolog plates and may be responsible for differences observed between samples. In this investigation, cell suspensions were adjusted to the same turbidity, an approach that has been shown to address this problem (Garland 1996; Haack et al. 1995; Staddon et al. 1998). Staddon et al. (1998) proposed that by adjusting extracted samples to the same turbidity, colour development becomes a function of the microbial community and not inoculation density. Confidence in the diversity calculations in this study was further gained by dividing colour development in each well by the average well colour of the plate (sum of optical densities/number of wells). This allows for the comparison of standardized patterns rather than absolute values and has been shown to further correct for slight differences in initial cell densities (Garland and Mills 1991; Heuer and Smalla 1997).

Metabolic redundancy is another problem associated with the use of carbon substrate utilization patterns when assessing microbial community diversity (Konopka et al. 1998). Microbial communities are thought to be functionally redundant, thus many species have the potential to use a specific substrate. As a result, differences in functional diversity may underestimate differences in taxonomic diversity (Konopka et al. 1998; Staddon et al. 1998).

A procedural issue associated with SCSU is appropriate incubation temperature for community analysis. Plates in this study were incubated at 28 °C. This temperature is recommended by the manufacturer for the identification of environmental isolates with Biolog plates (Biolog Inc. 1990) and is commonly used for community level analysis of SCSU patterns (Glimm et al. 1997; Harch et al. 1997; Heuer and Smalla 1997; Laine et al. 1997; Staddon et al. 1998). However, Derry et al. (1999) observed that incubation temperature affected patterns of colour development on Biolog plates for soil microorganisms isolated from high Arctic tundra soil samples. At 28 °C, the metabolic activity of psychrophiles should be virtually zero. While this may be more of a concern for high Arctic tundra soils than temperate riparian soils, the observation illustrates that not all members of the soil microbial community can be active under the specific incubation conditions presented by SCSU. Members of the microbial community are also excluded from characterization by SCSU due to its requirement for culturability. Although inoculation of Biolog plates is much less laborious, and less costly in time and materials than culture-independent approaches, it is important to recognize that Biolog plates represent 95 different enrichment cultures (Konopka et al. 1998). Consequently, it is impossible to determine whether patterns of SCSU are based on dominant members of the microbial community or pseudo-communities which thrive under this selective enrichment (Konopka et al. 1998). Similarly, some bacteria are unable to oxidize any Biolog substrates and are undetectable by this method (Hitzl et al. 1997).

While it is recognized that SCSU has limitations, there is a need to broaden our understanding of microbial community composition and this requires an initial focus on rapid, simple approaches that provide a reasonable chance of deducing biologically relevant patterns. This technique has proven useful in providing initial information on within-site differences in the composition of microbial communities at Carroll Creek, Ontario, Canada. The study further presents evidence for a relationship between denitrification and microbial diversity and community structure. As a novel research area, the importance of a potential correlation between microbial community composition and system stability should be recognized.

It is hoped these observations will provide a starting point for further research, so that we may gain a better understanding of soil quality. In particular, further research is required to elucidate how microbial community composition influences the structure and function of ecosystems. It is recommended that future research adopt a multifaceted approach to this question, incorporating a variety of community level physiological profiling techniques. If subsequent studies, conducted with more precise methods of community structure analysis (e.g. phospholipid fatty acid analysis, temperature gradient gel electrophoresis, 16S ribosomal rRNA analysis) confirm that threshold levels of microbial diversity, richness and evenness are required for denitrification, SCSU may be validated as a useful assay for rapidly determining the denitrification capacity of riparian soils. This information might also be applied to bioremediation efforts against other target pollutants.

Microorganisms are the primary agents responsible for the degradation and detoxification of many environmental contaminants (Lamar and Deitrich 1990; Entry et al. 1994). More information on the distribution and activity of microorganisms will ultimately lead to a better understanding of their degradative abilities and an improved definition of ‘soil quality’.

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