

# The Effect of Soil Texture on Richness and Diversity of Bacterial Communities

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Bacterial diversity in soil is high relative to more homogeneous environments (e.g., freshwater or marine habitats). Isolation imparted by fragmented aquatic microhabitats in unsaturated soil likely plays a large role in creating this diversity. We evaluate the role of soil texture, which determines the extent and connectivity of microhabitats, in constraining bacterial diversity. Soil samples with a range of textures were collected from sixteen sites across Connecticut and Massachusetts. Soil particle size distributions were measured to determine soil texture (% sand, % silt and % clay). Soil chemical characteristics (e.g., pH, % N) that might influence diversity were quantified for each site. Terminal restriction fragment length polymorphism (T-RFLP) analysis was performed to characterize the diversity (richness, Shannon's  $H'$ , and evenness) of soil bacterial communities. Bacterial species richness increased significantly ( $p = 0.04$ ) with the coarseness of the soil, quantified as % sand. No trend in  $H'$  or  $E$  were observed; all communities exhibited high diversity and evenness. The increase in species richness in coarser soils is likely due to the increased number of isolated water films in soils with larger pores, suggesting that pore-scale hydrologic regime constrains bacterial richness in soil.

Keywords: bacterial diversity, soil texture, terminal restriction fragment length polymorphism (T-RFLP)

## Introduction

Soil microbial communities develop in response to constraints and selection pressures in their environment (physical, chemical, and biological). The chemical and biological constraints have been studied extensively (e.g., Bending and Lincoln, 2000; Bressan et al., 2008; Crecchio et al., 2007; Hackl et al., 2004). In contrast, ways in which the physical environment of soil exerts control over community structure and diversity are more poorly understood.

Nonhomogeneous distributions of bacteria are created by numerous environmental factors and have been observed at a range of spatial scales, from sub-centimeter (Becker et al., 2006; Dechesne et al., 2003; Nunan et al., 2003; Grundmann and Debouzie, 2000) to meter (Bent et al., 2003; Nunan et al., 2002; Bundt et al., 2001) to kilometer or more (Fierer and Jackson, 2006; Cho and Tiedje, 2000). Micro-scale distribution is influenced by, among other factors, pore structure (Nunan et al., 2003), chemical conditions (Becker et al., 2006), and proximity to other bacterial taxa (Grundmann and Debouzie, 2000).

Pore-scale physical considerations are crucial for understanding spatial dynamics of bacterial diversity because this is the

largest scale at which individual bacterial cells or colonies experience the soil environment, the smallest scale being the microhabitat. There is no accepted or defined size for a bacterial microhabitat (Metting, 1993, p. 6), although one could reasonably assume it to be on the scale several cell lengths (tens of microns). Because bacterial cells depend on hydration for cell maintenance and diffusive transport of nutrients, spatial and temporal patterns of water retention are important determinants of the habitability of soil microhabitats. Since water-retention behavior in soil is well understood (see Raats and van Genuchten [2006] for an overview), this aspect of the physical soil environment is amenable to study at the pore scale.

In unsaturated soils, the parameter that determines water-phase connectivity is the pore size distribution, which is in turn controlled by bulk density and particle size distribution (soil texture). Pore size and shape determine the relative magnitudes of capillary, viscous and gravitational forces, thereby controlling the water content and configuration within a pore. The location of a microhabitat in the three-dimensional pore network is of utmost importance in determining the hydrologic regime it experiences. In moist soils (e.g., those at field capacity), water films in larger pores are held by surface tension (capillary forces) in crevices on the surfaces and at the edges of pores where soil grains meet. Smaller pores in which capillary forces are stronger may be saturated at field capacity.

Studies of bacterial communities in soils and sediments (Carson et al., 2010; Zhou et al., 2002) and in microcosms

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(Treves et al., 2003) indicate that hydraulically-induced spatial isolation in drier soils leads to higher diversity (richness and evenness) relative to wetter, more hydraulically connected soil or sediment environments. Increased fragmentation of the water phase, within three-dimensional habitable pore space in soil, results in a larger number of hydrated microhabitats available for colonization. This in turn increases the potential for multiple species to coexist in close proximity without directly competing for resources.

Increased spatial isolation in soil systems has the potential to increase bacterial diversity. For two soils at the same water content and same porosity, a finer-textured soil would have more water-filled pores, whereas a coarser soil would have more isolated water films. This could lead to higher diversity in the coarser soil, because more isolated microhabitats exist in which to harbor species that might otherwise compete with their neighbors.

The objective of this study was to evaluate the hypothesis that because soil texture determines pore-scale hydrologic connectivity, texture should have a measurable effect on bacterial diversity. We used terminal restriction fragment length polymorphism (T-RFLP), a technique in which each peak in the electropherogram represents at least one DNA sequence or terminal restriction fragment (T-RF) of a given size, and peak height indicates relative abundance. The number and heights of peaks can be used to calculate measures of diversity (e.g., richness, evenness) (Braker et al., 2001; Fierer and Jackson, 2006; Liu et al., 1997; Sessitch et al., 2001). Because many distinct T-RF sequences may share a common length, the richness estimate obtained from this technique is an extreme lower bound on the true value. Nevertheless, it can be used in a relative way, to determine trends in richness and diversity among samples.

The structure and diversity of soil bacterial communities is of interest in environmental forensic applications because “snapshots” of community structure (presence/absence and relative abundance of taxa) may be used as a signature for a given soil. Soil community metagenomic techniques such as T-RFLP (Heath and Saunders, 2006; Horswell et al., 2002; Macdonald et al., 2011; Meyers and Foran, 2008), denaturing gradient gel electrophoresis (DGGE) (Lerner et al., 2006), and amplicon length heterogeneity-polymerase chain reaction (ALH-PCR) (Moreno et al., 2006) have been evaluated for their usefulness in forensic investigations. Lerner et al. (2006) concluded that DGGE was potentially useful but applied a long list of caveats to its wide adoption by forensic practitioners. Macdonald et al. (2011) evaluated T-RFLP on soil bacterial and fungal communities as a method of discriminating among sites of similar geology. They were able to discriminate statistically among 93% of their sites, although principal component analysis revealed high similarity among sites.

Researchers have highlighted the need for standardized protocols for soil sampling and storage, DNA extraction, and subsequent analysis of PCR products (e.g., T-RFLP, DGGE). In addition to agreed-upon protocols, an accessible database of profiles for comparison is critical for soil microbial metagenomics tech-

niques to have widespread usefulness. In light of these considerations, soil community structure and diversity shows promise as part of a set of criteria used to identify or match forensic soil samples, since temporal variability due to moisture, temperature, or other environmental factors complicates their use as stand-alone indicators (Meyers and Foran, 2008).

## Materials and Methods

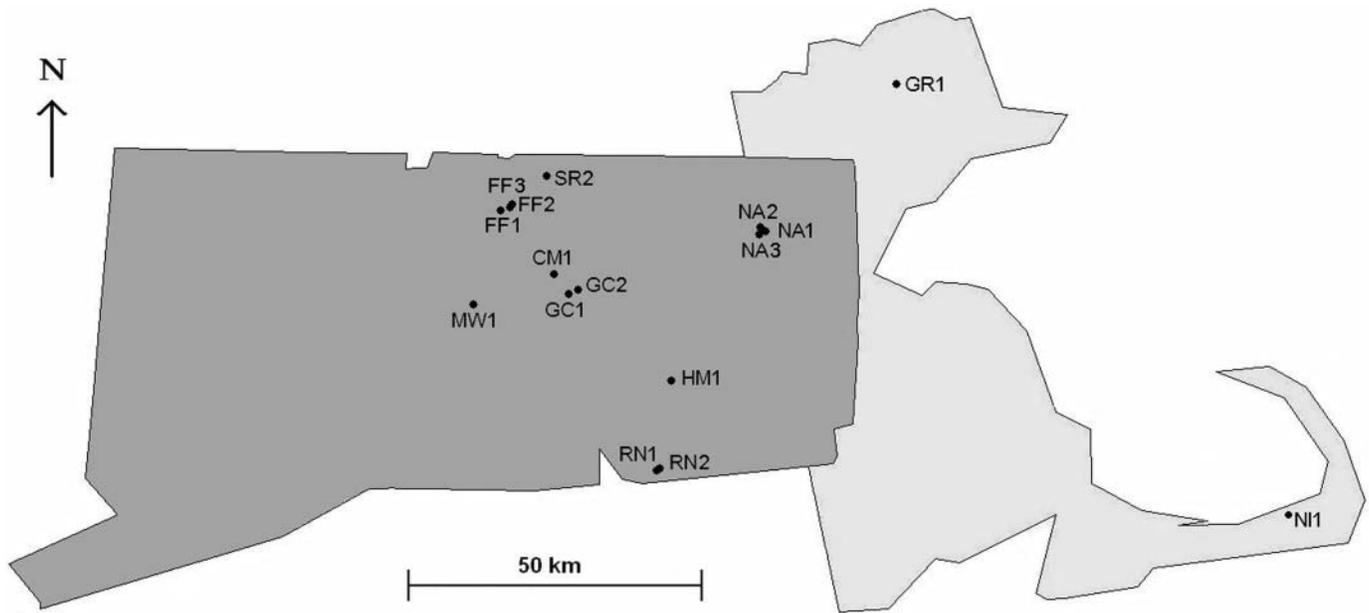
### *Site Selection*

The overarching strategy guiding site selection was to minimize differences in soil properties other than the property of interest (texture). Soil survey maps from NRCS (Natural Resources Conservation Service [NRCS], 2011) were used to find forested sites on publicly owned land (state parks and forests). Fourteen sites in Connecticut and two in Massachusetts were selected (Figure 1). Some sites were located in the same park or forest but in different parts of the terrain (e.g., three sites in Natchaug State Forest: NA1 in alluvial soil, NA2 in glacial till, and NA3 in glacial outwash).

Sampling sites were chosen based on a variety of considerations. The primary factor was land use: forested sites were chosen as a way to homogenize the effects of disturbance by recent human activity. Second-growth forests were chosen as representative of most of the forested area in New England. Another factor that influences diversity is pH (Fierer and Jackson, 2006). Most soils in Connecticut and Massachusetts are acidic, so the few sites with soils formed in alkaline materials were excluded from consideration, as were soils characterized as “slightly acid” or “neutral” in NRCS soil series descriptions (NRCS, 2011).

Drainage class was also a major consideration for site selection, as a convenient way to control for both average water content and organic carbon content. The degree of aeration in a soil is a major driver of carbon accumulation. Under aerated conditions, any available organic material will be readily utilized by microbes, whereas prolonged saturation and anaerobic conditions will allow organic carbon to accumulate in the soil. Drainage class describes the typical water content of a soil, and encompasses aspects of both soil texture and landscape position. For example, most soils that are “excessively drained” are coarse-textured, whereas a “moderately well-drained” soil could be coarse or fine-textured, and the water content would depend on its position on the landscape. To obtain as wide a range of texture as possible while minimizing differences in organic content, the moderately-well-drained drainage class was chosen. In the main study area (Connecticut), 24 soil series are members of this drainage class. Soil samples were taken from areas where these series have been identified based on soil survey maps.

Because bacterial abundance varies with depth in the vadose zone (Holden and Fierer, 2005), all samples were taken from the same depth range below the surface (13 to 19 inches [33 to 48 cm]). This range was chosen as a consensus depth from the B horizon of all soil series sampled (based on NRCS soil series



**Figure 1.** Map of Connecticut (dark grey) and eastern Massachusetts (light grey) showing locations of sampling sites.

descriptions). Sampling in the B horizon has the added advantage of eliminating the effects of the rhizosphere associated with understory plants (if any) in the forest soils.

In summary, site selection criteria were implemented to allow for a range of soil textures while variability in chemical characteristics that might have confounding effects on diversity measures. This limits the inference space of the study to unsaturated acidic bulk forest soils with low organic content. A similar trend may be observable in other soils, although bacterial community structure is dependent on a large number of factors. Therefore, our results may not be directly transferable to other soil types.

#### *Sampling Protocol*

Samples were collected between August 2 and October 9, 2007. Much of the sampling was conducted during a long dry period in the study area (approximately 22 August 22 to October 7, 2007). Sample NA3 was collected on October 9, the day after the first heavy rain of the fall season.

Soil samples were obtained from hand-dug trenches that were 1 m long by 0.5 m deep. Each sample was taken along the walls of a trench at a depth between 30–50 cm below ground surface. Using a sterilized augur, three small (~5 g) subsamples for DNA analysis were collected at 0.5 m intervals along the trench and deposited into sterile Whirl-pak bags (Nasco, Fort Atkinson, WI, USA). Using a soil corer (SoilMoisture Equipment Corp., Santa Barbara, CA, USA), four intact 3-cm-long cores were collected to determine water content. In some cases, intact cores could not be collected due to the rocky or loose nature of the soil; in these cases, cores were repacked using dug-out material to approximately the same bulk density as the in-situ soil. An estimate of soil water content was also obtained using

a Hydrosense device (Decagon Devices, Pullman, WA, USA). An additional ~3 kg of soil was collected along the trench for estimation of particle size distribution and nutrient content. All samples were transported to the laboratory on ice and stored at  $-20^{\circ}\text{C}$  prior to analysis.

#### *Physical Characterization*

The particle size distribution, in the form of a percent finer curve, was obtained for each site using ASTM protocol D422-63 (ASTM, 1985d), modified as follows: Soil was oven-dried and sieved to remove material finer than  $75\ \mu\text{m}$  (silt and clay fractions), then washed with tap water through the  $75\ \mu\text{m}$  sieve again to remove silt and clay particles adhering to the sand fraction. The residual was oven-dried and the amount of fine material lost during washing was determined by mass. The washed sand was passed through a sieve stack to obtain the particle size distribution of the sand fraction. The hydrometer method (Bouyoucos, 1936) was used to obtain the size distribution of the silt and clay fractions obtained in the first step.

For most soils, volumetric water contents ( $\theta$ ) were determined gravimetrically from the soil cores. Water contents for the two clayey soils, which came out of the ground as hard clumps, were determined using the clod method (soil volume determined by displacement).

#### *Chemical Characterization*

Composite samples from each trench were analyzed for chemical properties. Percent C and N were measured with a PerkinElmer CHN 2400 Analyzer at the Center for Environmental Sciences and Engineering, University of Connecticut. Percent organic matter was measured by loss on ignition, pH quantified by equilibration of equal volumes of soil and water,

and Morgan extraction (extraction in ammonium acetate, pH 4.8, followed by ICP analysis) (Northeast Region Coordinating Committee on Soil Testing, NEC-67, 1995) was conducted for Ca, Mg, P, K, B, Cu, Fe, Mn, Zn, and Al by the University of Connecticut Soil Testing Lab.

### Biological Characterization

T-RFLP was used to characterize the bacterial community of each subsample. DNA was extracted from 0.25 g of soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). One extraction was done for each of three subsamples taken along each trench. Extracted DNA was quantified using a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA). PCR amplification reactions were performed using the *Taq* PCR Core Kit (Qiagen, Valencia, CA, USA) with bacterial primers 63F, labeled at the 5' end with WellRED D-4 (Sigma-Aldrich, St. Louis, MO, USA), and 1387R (Marchesi et al., 1998). Each 100  $\mu$ L reaction contained 2.5 U *Taq* polymerase, primers at a concentration of 0.2  $\mu$ M, dNTP at 200  $\mu$ M each, manufacturer's PCR buffer at 1X concentration, and 10–55 ng of template DNA (10  $\mu$ L extracted DNA solution). Amplification was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), with reaction conditions as follows: denaturing at 94° C for 1 min, followed by 30 cycles of 95° C for 1 min, 55° C for 1 min, 72° C for 1.5 min, and final incubation at 72° C for 5 min. PCR products were cleaned with the QIAquick PCR Purification Kit (Qiagen).

Restriction digests were performed on approximately 200 ng of DNA with 10 U of *HaeIII* (New England Biolabs, Ipswich, MA, USA) in 50  $\mu$ L reactions, incubated at 37° C for 1 hour and denatured at 80° C for 20 minutes per manufacturer instructions. Digests were cleaned with QIAquick Nucleotide Removal Kit (Qiagen). The resulting DNA fragments were dried at 37° C, then resuspended in 39.75  $\mu$ L of deionized formamide and 0.25  $\mu$ L of 600-bp Beckman size standard (Beckman-Coulter, Fullerton, CA, USA). T-RF lengths in base pairs (bp) were analyzed with a Beckman-Coulter CEQ8000 capillary electrophoresis machine.

After T-RF lengths (bp) and abundance (in fluorescence units) were estimated using the Beckman-Coulter CEQ post-processing algorithm, the lengths were binned and the total fluorescence of profiles standardized according to the method of Dunbar et al. (2001). T-RF sizes from all subsamples were binned at 0.6 bp resolution using hierarchical clustering, and peaks with heights below 150 fluorescence units were discarded as noise (based on visual analysis of the raw profiles). The total fluorescence (sum of peak heights) in all subsamples was standardized so that the subsample with the highest fluorescence had a total fluorescence within 1% of the sample with the lowest fluorescence.

### Diversity Measures

Using T-RFLP data, we calculated the richness, evenness, and diversity of the bacterial communities. Richness ( $S$ ) is the num-

ber of unique taxa present in the sample (here represented by the number of peaks in the electropherogram), while evenness describes the equitability with which T-RFs in the sample are distributed among taxa (Magurran, 1988). The Shannon index ( $H'$ ) is a measure of diversity that combines information about richness and evenness and ranges between 0 and  $\log_2(S)$ ; its value increases with the number of individuals sampled (Hill et al., 2003). The Shannon index is computed as:

$$H' = - \sum_{i=1}^S p_i \log_2 p_i \quad (1)$$

where  $p_i$  are the relative heights of the peaks ( $p_i = \text{height of peak}(i)/\text{sum over all } i$ ). The evenness ( $E$ ) of the community structure was calculated as  $E = H'/H_{max}$ , where  $H_{max} = \log_2(S)$  represents the diversity of a community in which all taxa are equally abundant (Magurran, 1988).

### Statistical Analysis

The dataset resulting from the physical, chemical and biological characterization (Table 1) contained 5 physical parameters (% sand, silt and clay; bulk density; and volumetric water content), nine chemical parameters (pH, % organic matter, %C, %N, Fe, Mn, Al, Cu, Zn), and four biological or ecological parameters ( $S$ ,  $H'$ ,  $E$ , and average quantity of DNA extracted per gram of dry soil for all subsamples at each site).

Preliminary evaluation of the dataset was conducted using a variety of techniques, including Q-Q plots for normality testing, variance inflation factor (VIF) analysis for collinearity testing (Krzanowski, 2000), as well as simple plots of each independent variable with the dependent variable to determine whether linear modeling was appropriate. We also utilized principal component analysis (PCA) (Jolliffe, 1986) to examine the overall chemical profiles of the soils.

The goal of the statistical analysis was to determine whether other measured variables besides the primary variable of interest (texture, quantified as percent sand) had a significant effect on diversity ( $S$ ,  $H'$ ,  $E$ ). Separate hierarchical multiple regressions were carried out using SPSS (SPSS, Inc.) to evaluate the effects of nutrient parameters on the diversity indices, over and above the effects of texture. Each independent variable was tested individually, as well as in blocks with similar variables (e.g., redox-active metals Fe and Mn; toxic metals Zn, Al, Cu). The significance of the change in  $R^2$  was assessed using the F statistic. The associated  $p$  value indicated whether the increase in  $R^2$  due to the addition of the parameter to the baseline linear model was significant. Finally, the relationships between percent sand and  $S$ ,  $H'$ , and  $E$  were determined through linear regression.

## Results

### Soil Physical and Chemical Characteristics

Soil textures ranged from coarse sand to silty clay loam (representative particle size distributions shown in Figure 2). For the

**Table 1.** Soil properties and biodiversity measures for all samples. Samples are listed from coarsest to finest texture.  $\theta$  indicates volumetric water content, OM is organic matter content, H' is Shannon's diversity index, E is evenness.

Site	% Texture	% Sand	% Clay	pH	$\theta$ (%)	OM (%)	$\mu\text{g DNA g}^{-1}$ dry soil	Average T-RFs, $n$	Max. # T-RFs	H'	E
NI1	Coarse sand	97	0	5.0	9	2.0	1.0	81.7	94	5.61	0.88
NA3	Coarse sand	90	0	5.5	4	1.2	1.3	42.0	42	3.10	0.57
NA1	Loamy sand	85	1	4.9	17	2.0	2.2	111.0	130	6.15	0.91
SR2	Loamy sand	80	0	5.0	9	1.9	0.7	29.5	34	1.61	0.33
RN2	Sandy loam	70	4	5.1	19	2.4	2.0	87.0	118	4.82	0.75
GC2	Sandy loam	65	7	5.0	13	1.3	0.7	96.3	100	5.98	0.91
RN1	Sandy loam	63	5	4.8	11	3.8	1.3	89.7	107	5.52	0.85
CM1	Sandy loam	66	3	4.7	7	2.3	1.4	90.5	100	5.64	0.87
GR1	VF sandy loam	65	2	4.8	7	2.5	0.6	35.0	35	2.26	0.44
NA2	Sandy loam	63	1	5.2	15	3.6	2.5	92.7	98	5.65	0.87
HM1	Sandy loam	53	5	4.8	11	3.3	1.6	85.0	85	5.82	0.91
MW1	Silt loam	47	6	4.6	9	1.6	1.3	85.5	108	5.63	0.88
FF3	Silt loam	43	5	4.9	16	1.7	0.8	20.5	25	1.67	0.38
GC1	Loam	45	10	4.7	8	2.8	0.7	61.0	90	4.25	0.72
FF2	Silt	6	11	5.3	9	1.6	1.2	83.0	98	5.78	0.91
FF1	Silty clay loam	4	35	5.7	32	2.0	1.3	62.0	62	5.39	0.91

most common soil textures (sandy loams and silt loams), several sites with similar textures were included for comparison. The coarse sand and loamy sand categories were represented by two sites each. Nutrient parameter values varied from site to site (Table 1). Box-and-whisker diagrams for individual parameters showed only one outlying value for pH; all other nutrients showed no outliers.

#### T-RFLP Results

Four of the sampling sites (NA3, GR1, SR2, FF3) did not produce a T-RELFP profile at all due to excessive impurities (possibly salts) not removed in the DNA extraction process, resulting in twelve remaining sites for regression analysis. At least two subsamples from each remaining site successfully produced a T-RFLP profile, with the exception of two sites (FF1, HM1) in which only one subsample apiece yielded results. After binning

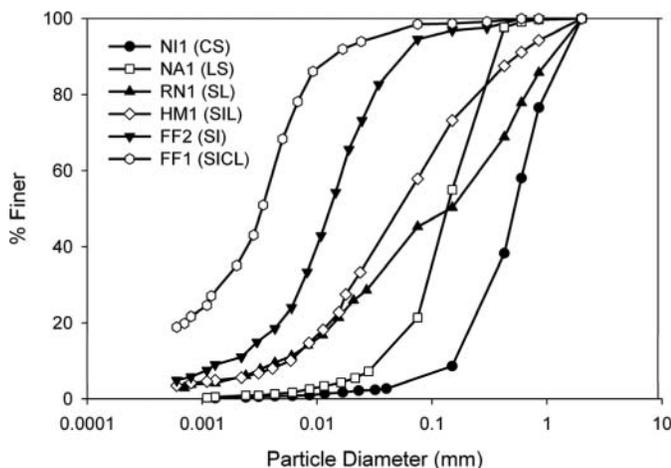
and standardizing total fluorescence for all electropherograms, the number of T-RF peaks for all viable subsamples at each site were averaged to obtain the mean richness of the bacterial communities at that site (an unbiased estimator). The maximum number of T-RF peaks per site was also considered as a measure of richness. The number of T-RF peaks in the subsamples before total fluorescence standardization ranged from 59 to 125; after standardization the range was 59 to 109.

#### Results of Statistical Analysis

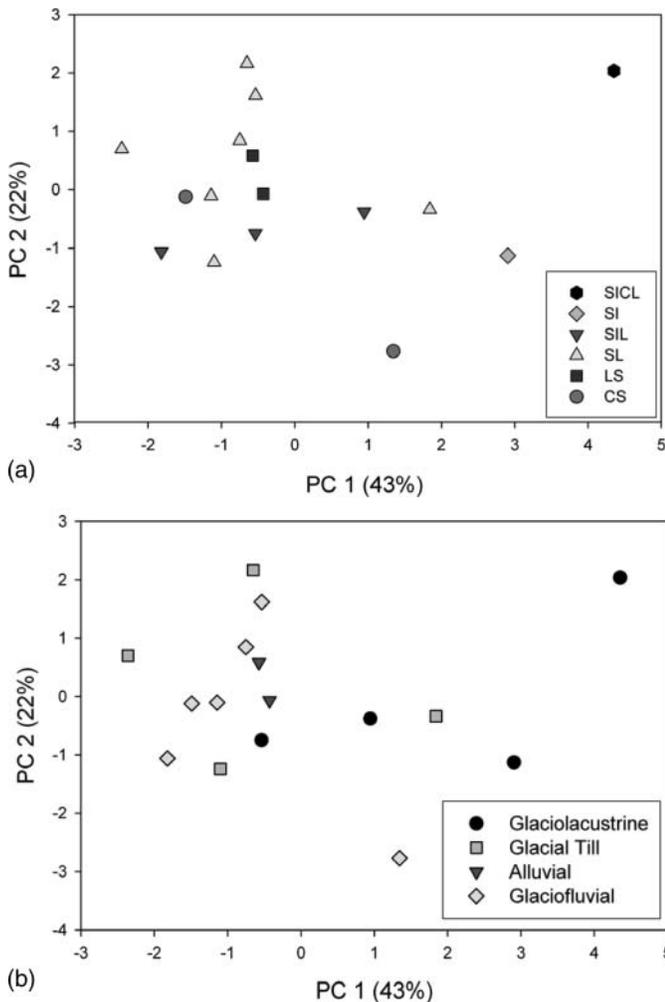
Prior to analysis, collinear variables were removed from the dataset. VIF analysis showed that %C and %N were highly collinear with % organic matter, so the organic matter parameter was chosen to represent the group.

PCA performed on the remaining nutrient parameters showed little evidence of clustering according to texture, with the exception of the two loamy sands (Figure 3a). The first principal component (PC), representing 43% of the total variance, primarily reflected pH, Mn, and  $-Al$  content (absolute value of loadings approximately equal), while the second PC (22% of total variance) reflected water content. The third PC (16% of total variance, not shown) mostly reflected Fe content. The sandy loam soils had low pH and Mn values and high amounts of Al, relative to the other samples in the dataset (Figure 3a). The two finest soils appear at the right side of the plot due to their relatively low Al content and high pH and Mn content. While these parameters were indicated by PCA as being important in characterizing the variability of soil properties, ultimately none of them was found by hierarchical multiple regression to be significant in determining species richness.

In the same PCA plot overlaid with parent material from which each soil was derived (Figure 3b), the only cohesive cluster was the alluvial soils, which are the same two loamy sands which clustered together (Figure 3a). Their particle size distributions are almost identical, and they were both collected in sandy



**Figure 2.** Graph of particle size distributions for a representative selection of sampling sites. Codes in parentheses are textural classifications: CS, coarse sand; LS, loamy sand; SL, sandy loam; SIL, silt loam; SI, silt; and SICL, silty clay loam.



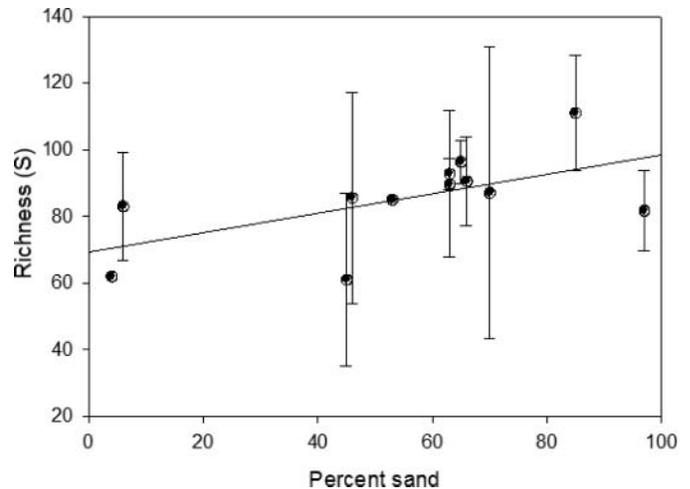
**Figure 3.** Principal component analysis on soil nutrient characteristics (not including %C and %N) overlaid with a) soil texture and b) soil parent material. In Figure 3a), SICL indicates silty clay loam, SI is silt, SIL is silt loam, SL is sandy loam, LS is loamy sand, and CS is coarse sand. Both plots show the same points; only the categories overlaid have been changed.

alluvial deposits along the banks of small rivers. Their nutrient profiles were also similar (Figure 3a); however, only one successfully produced T-RFLP results, indicating problematic soil constituents present to a higher degree in one soil (SR2).

The lack of clustering of sites based on soil chemical characteristics with respect to both soil texture and parent material suggests that the chemical profiles of the soils are not strongly influenced by these factors. In particular, there is no accompanying trend in chemical profile with soil texture that could complicate the interpretation of our results (Figure 3a).

To examine the possible effects of bias in DNA extraction from different soil textures, we assessed the DNA quantity extracted per gram of dry soil via linear regression, and found no significant relationship with any soil fraction (sand, silt, or clay,  $p \geq 0.51$ ).

Regression analysis revealed the sand fraction (% sand) to be the sole soil parameter that exhibited a statistically signif-



**Figure 4.** Trend in bacterial richness with percent sand. Richness is estimated as the number of T-RF peaks in T-RFLP profile, averaged for sites with multiple profiles. Slope is significant ( $p = 0.04$ ). Error bars represent 1 standard deviation of richness for each site.

icant linear relationship with average number of T-RF peaks per site ( $p = 0.04$ , Figure 4). Furthermore, hierarchical multiple regression showed that no additional physical or chemical parameter, whether alone or in combination with similar parameters, caused a significant increase in  $R^2$  over the baseline model (T-RF number as a linear function of % sand). A regression of the maximum number of T-RFs (highest T-RF number of all subsamples for the site) yielded similar regression coefficients and level of significance (Table 2).

The slopes of the regression lines between  $H'$  and % sand, and between  $E$  and % sand, were not significantly different from zero (Table 2). Diversity values (4.25 to 6.15) were higher than, and evenness values (0.72 to 0.91) comparable to, those in other T-RFLP soil bacterial community studies (Asuming-Brempong et al., 2008; Dunbar et al., 2000; Girvan et al., 2003; Hackl et al., 2004).

## Discussion

### Interpretation of Findings

Bacterial richness increases with the coarseness of soil, quantified as the sand content (percentage by mass of particles > 50  $\mu\text{m}$ ). Regression significance was obtained despite the small

**Table 2.** Regression parameters for viable sites ( $n = 12$ ).  $S_{\text{avg}}$  indicates average richness in all subsamples per site, while  $S_{\text{max}}$  indicates the maximum richness. Regression parameters for  $S_{\text{avg}}$  and  $S_{\text{max}}$  were similar; slopes for  $H'$  and  $E$  were not significantly different from zero.

Parameter	Slope	Intercept	$R^2$	$p$
$S_{\text{avg}}$	$2.91 \times 10^{-1}$	69.39	0.35	0.04
$S_{\text{max}}$	$3.63 \times 10^{-1}$	79.13	0.35	0.04
$H'$	$2.81 \times 10^{-3}$	5.37	0.02	0.64
$E$	$-2.74 \times 10^{-4}$	0.88	0.01	0.71

sample size ( $n = 12$ ). Furthermore, the relationship between % sand and richness was the only statistically significant relationship in our dataset; other factors that could have a confounding effect on the relationship were not significant. For example, soil type (which encompasses texture and chemical properties) has the potential to influence the amount of DNA extracted (Burgmann et al., 2001; Schneider et al., 2009), which could in turn bias the observed T-RFLP profile. We tested for this influence, but found no significant effect of % sand, silt or clay on the quantity of DNA extracted.

The observed trend is an important finding in that it indicates that the physical environment of soil plays an important role in shaping the structure of bacterial communities, along with chemical and biological factors such as amount and type of organic matter (Crecchio et al., 2007; Nusslein and Tiedje, 1999; Zhou et al., 2002), pH (Fierer and Jackson, 2006), redox potential (Braker et al., 2001), inhibitory substances (Kandeler et al., 2000; Moffett et al., 2003), predation (Jousset et al., 2008; Rosenberg et al., 2009). Our study design sought to limit the influence of such confounding factors and so does not address the magnitude of their effects on richness relative to the texture effect.

The finding of increased richness in coarser soils is in agreement with previous research (Carson et al., 2010; Treves et al., 2003) indicating that maintaining lower (more negative) matric potentials in soil results in higher bacterial richness. The more highly fragmented water phase present in coarser soils exerts control over bacterial community structure by providing more isolated microhabitats, thereby fostering higher richness. Although attached bacterial colonies depending on small and transient sources of hydration in coarser soils would be at increased risk of desiccation relative to those in finer-textured soils, the development and maintenance of diversity would be enhanced by the hydrologic disconnectedness. Furthermore, there is ample evidence that soil bacteria have developed mechanisms to resist desiccation, including the ability to enter dormant or resting states (Amako et al., 2008; van Keulen et al., 2007) and production of extra-polymeric substances/biofilms (Foster, 1981; Hartel and Alexander, 1986; Roberson and Firestone, 1992). Therefore, larger pore spaces, though more prone to desiccation, do not preclude bacterial colonization, and may in fact enhance the survival of rare species by providing isolated habitats in which competition for resources is reduced.

Both  $H'$  and  $E$  showed no change with texture, indicating similarly high levels across all textural classes. By definition,  $H'$  increases with both richness and evenness, so one could expect  $H'$  to increase with richness given the lack of trend in evenness. However, since the  $H'$  values were mostly 80–90% of their maximum value ( $\log(S)$ ), the increases in richness with percent sand did not translate to a significant increase in  $H'$ . The lack of effect of texture on  $H'$  and  $E$  can be explained with reference to a typical bacterial abundance distribution for unsaturated soils. Such distributions typically exhibit high evenness and a long tail representing many rare species (Dunbar et al., 2002; Nusslein and Tiedje, 1999; Zhou et al., 2002). Community analysis using

T-RFLP can be expected to under-count rare taxa due to the finite quantity of soil DNA analyzed and the standardization of fluorescence (total DNA quantity) across all samples resulting in loss of low-abundance (rare) T-RFs. Therefore, the effect of using the T-RFLP method is to cut off part of the long tail of the species abundance distribution. By the same logic, the higher richness observed in coarser soils likely reflects additional rare species (capturing more of the tail). Adding these rare species into the already highly diverse and even abundance distribution had little effect on  $H'$  and  $E$ .

#### *Richness in Relation to Particle Size Fractions*

Our findings of increased richness with percent sand differ from previous studies (Kandeler et al., 2000; Sessitch et al., 2001; Zhang et al., 2007), which observed higher levels of richness and diversity in the finer (silt and clay) fractions of soils, with lower values in the sand fraction. These studies employed a fractionation technique, then examined the bacterial communities attached to the different fractions. The methods employed leave some doubt as to whether the sampled community accurately reflected the microbes attached to each fraction. Zhang et al. (2007) used sonication to disperse the soil fractions, then sequentially removed the larger particle fractions (2000–200  $\mu\text{m}$ , 200–20  $\mu\text{m}$ , then 20–2  $\mu\text{m}$ ). The remaining supernatant was centrifuged to collect the <2  $\mu\text{m}$  fraction. It seems quite likely that the high richness and diversity attributed to the <2  $\mu\text{m}$  fraction includes bacteria dislodged from other fractions during the collection procedure. Sessitch et al. (2001) used a similar sonication and sequential removal protocol, and found extremely low numbers of T-RFs in each sample (between 7 and 26). Such low numbers of sampled taxa cannot be assumed to be representative of the bacterial community in a particular size fraction, and call into question the accepted view that the silt and clay fractions harbor higher diversity.

The interaction between the particle size fractions may also play a role in the different findings. In a well-graded soil (many different particle sizes mixed together), the sand fraction acts as the “skeleton” while silt and clay particles primarily coat the surfaces of the sand grains and act as bridges between them. Consideration of this coating phenomenon, revealed in SEM images of soil (Chenu et al., 2001; Davey, 1978), contradicts the popular conception that the sand fraction (by which we mean sand-sized pore space) is inhospitable for bacteria. Adsorbed small particles on a sand grain would increase the roughness and therefore the water-film-holding capability of a sand grain surface, as well as provide binding sites for adsorption of organic matter and bacterial cells.

#### **Conclusions**

This study examines the role of soil texture in constraining bacterial diversity. Soil texture determines the long-term water retention behavior of the soil, in particular the location and connectivity of hydrated microhabitats. A coarser (sandier)

soil exhibits larger pores that are unsaturated at most matric potentials, and in which water is held in pore corners and as isolated water films. According to the spatial isolation hypothesis (Zhou et al., 2002), isolated water films provide opportunities for increased bacterial diversity. Thus the observed increase in estimated bacterial richness with increasing sand content may be a consequence of the higher number of isolated hydrated microhabitats in coarser soils.

By minimizing variation in and statistically controlling for non-physical parameters that might affect diversity, we isolated the effect of texture on species richness. That our findings contradict previous research in fractionated soils on the effects of particle size on associated bacterial richness suggests that we have tapped into a different phenomenon. Specifically, our results focus on overall richness of the entire soil community, which is affected by differences in pore-scale hydrology brought on by textural variation.

This study is significant because it illustrates the effect of pore-scale hydrologic regime in constraining bacterial diversity in soil and contributes to the usefulness of T-RFLP analysis of soil bacterial communities as a forensic investigation tool. Although it is based on a limited but statistically significant sample size, it offers a glimpse at the role that a physics-based approach can play in understanding problems in soil biology, and represents a promising area for further research.

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