The diversity and biogeography of soil bacterial communities

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For centuries, biologists have studied patterns of plant and animal diversity at continental scales. Until recently, similar studies were impossible for microorganisms, arguably the most diverse and abundant group of organisms on Earth. Here, we present a continental-scale description of soil bacterial communities and the environmental factors influencing their biodiversity. We collected 98 soil samples from across North and South America and used a ribosomal DNA-fingerprinting method to compare bacterial community composition and diversity quantitatively across sites. Bacterial diversity was unrelated to site temperature, latitude, and other variables that typically predict plant and animal diversity, and community composition was largely independent of geographic distance. The diversity and richness of soil bacterial communities differed by ecosystem type, and these differences could largely be explained by soil pH ($r^2 = 0.70$ and $r^2 = 0.58$, respectively; $P < 0.0001$ in both cases). Bacterial diversity was highest in neutral soils and lower in acidic soils, with soils from the Peruvian Amazon the most acidic and least diverse in our study. Our results suggest that microbial biogeography is controlled primarily by edaphic variables and differs fundamentally from the biogeography of "macro" organisms.

biodiversity | microbial ecology | soil bacteria | terminal-restriction fragment length polymorphism

Although microorganisms are perhaps the most diverse (1, 2) and abundant (3) type of organism on Earth, the distribution of microbial diversity at continental scales is poorly understood. Ecologists describing microbial biogeography typically invoke Beijerinck (4) from a century ago: "Everything is everywhere, the environment selects." However, few studies have attempted to verify this statement or specify which environmental factors exert the strongest influences on microbial communities in nature (5, 6). With the advent of ribosomal DNA-analysis methods that permit the characterization of bacterial communities without culturing (7, 8), it is now possible to examine the full extent of microbial diversity and describe the biogeographical patterns exhibited by microorganisms at large spatial scales.

Scientific understanding of microbial biogeography is particularly weak for soil bacteria, even though the diversity and composition of soil bacterial communities is thought to have a direct influence on a wide range of ecosystem processes (9, 10). Much of the recent work in soil microbial ecology has focused on cataloging the diversity of soil bacteria and documenting how soil bacterial communities are affected by specific environmental changes or disturbances. As a result, we know that soil bacterial diversity is immense (11, 12) and that the composition and diversity of soil bacterial communities can be influenced by a wide range of biotic and abiotic factors (13). However, almost all of this work has been site-specific, limiting our understanding of the factors that structure soil bacterial communities across biomes and regions.

We hypothesize that soil bacterial communities do exhibit biogeographical patterns at the continental scale of inquiry and that these patterns are predictable. Whereas previous studies have examined the biogeographical distributions of soil fungal communities (14) and individual strains of soil bacteria (15, 16), to our knowledge, no previous study has examined how entire soil bacterial communities are structured across large spatial scales. We hypothesize that the biogeographical patterns exhibited by soil bacteria will be fundamentally similar to the patterns observed with plant and animal taxa and that those variables which are frequently cited as being good predictors of animal and plant diversity, particularly those variables related to energy, water, or the water–energy balance (17–19), will also be good predictors of bacterial diversity. To test these hypotheses, we used a ribosomal DNA-fingerprinting method to compare the composition and diversity of bacterial communities in 98 soils collected from across North and South America.

Results and Discussion

Soil bacterial diversity, as estimated by phylotype richness and diversity (Shannon index) (20), varied across ecosystem types (Fig. 1). Of all soil and site variables examined, soil pH was, by far, the best predictor of both soil bacterial diversity ($r^2 = 0.70$, $P < 0.0001$; Table 1 and Fig. 1*A*) and richness ($r^2 = 0.58$, $P < 0.0001$; Fig. 1*B*) with the lowest levels of diversity and richness observed in acid soils (Fig. 1). Because soils with pH levels >8.5 are rare, it is not clear whether the relationship between bacterial diversity is truly unimodal, as indicated in Fig. 1, or whether diversity simply plateaus in soils with near-neutral pHs. Likewise, because our fingerprinting method underestimates total bacterial diversity (see *Methods*), we cannot predict how the absolute diversity of bacteria changes across the pH gradient. When we compare paired sampling locations with similar vegetation and climate but very different soil pHs, we find evidence for the strong correlation between bacterial diversity and soil pH at the local scale. For example, two deciduous forest soils collected in the Duke Forest, North Carolina (see Table 3, which is published as supporting information on the PNAS web site), showed that the soil with the higher pH (DF2, pH = 6.8) had an estimated bacterial richness 60% higher than the more acidic soil (DF3, pH = 5.1). Similarly for two tropical forest soils collected <1 km apart in the Peruvian Amazon, the soil with the higher pH (PE8, pH = 5.5) had an estimated bacterial richness 26% higher than the more acidic soil (PE7, pH = 4.1).

Qualitatively, there was no clear relationship between soil bacterial diversity and plant diversity at the continental scale. Although plant diversity was not determined at each sampling site, ecosystems with the highest levels of bacterial diversity (semiarid ecosystems in the continental U.S.) have relatively low levels of plant diversity (21). Likewise, soils from terra firme sites in the Peruvian Amazon in our analysis had relatively low levels of bacterial diversity (H′ = 2.5–2.7), but

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Abbreviations: MAT, mean average temperature; PET, potential evapotranspiration.

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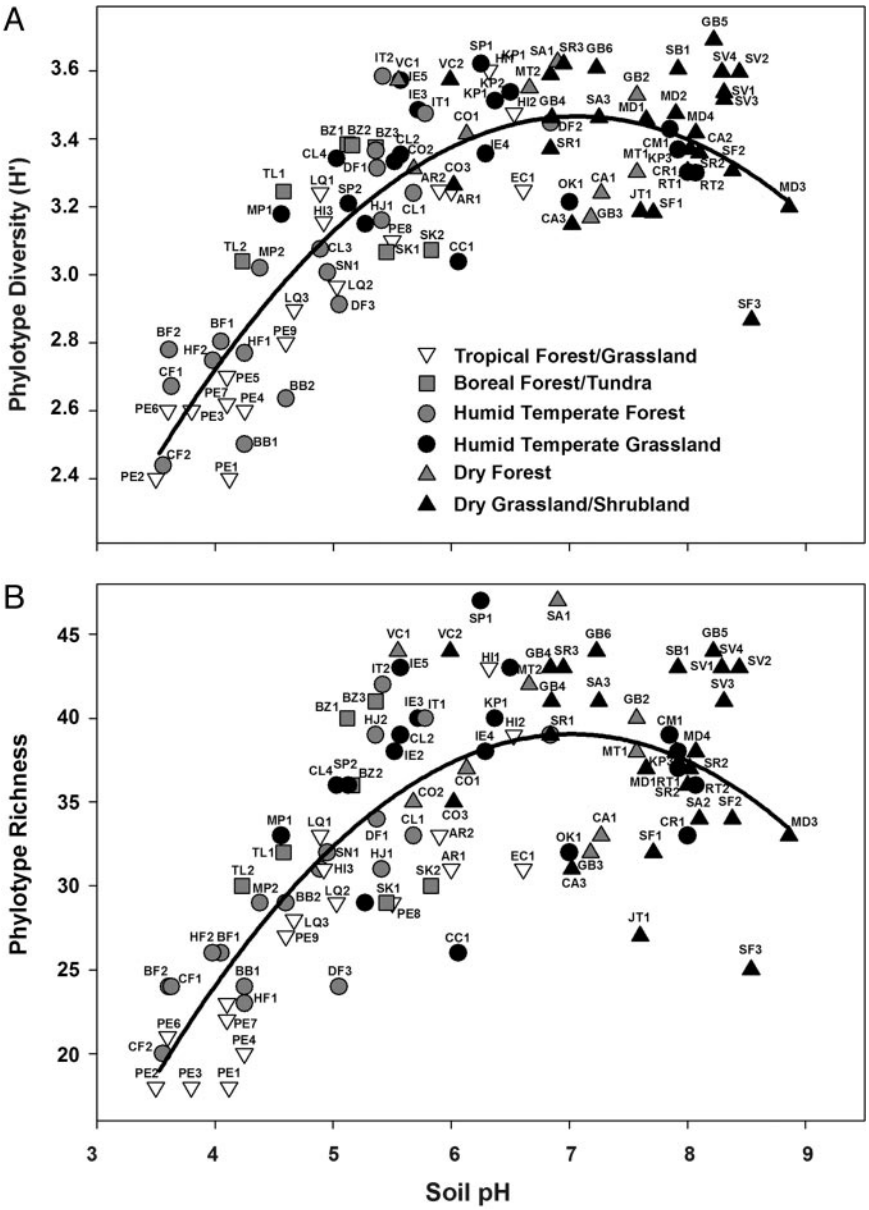

Fig. 1. The relationship between soil pH and bacterial phylotype diversity (*A*) and phylotype richness (*B*), defined as the number of unique phylotypes. Diversity was estimated by using the Shannon index, a summary variable that incorporates the richness and evenness of phylotypes (20). Symbols correspond to general ecosystem categories, and labels denote individual soils (see Table 3). Detailed information on the individual soils is provided in Table 3. Both quadratic regressions ($H' = -0.08pH^2 + 1.12\ pH - 0.5$, $r^2 = 0.70$ for *A* and $Richness = -1.65pH^2 + 23.2pH - 42.3$, $r^2 = 0.58$ for *B*) were statistically significant ($P < 0.0001$). There was no significant correlation between the residuals of the two regressions shown here and any of the other soil and site variables listed in Table 1 ($P >$ 0.25 in all cases).

these sites have some of the highest recorded levels of plant diversity on Earth (22). In fact, we added the tropical sites at Manu National Park, Peru (PE, Table 3) and Missiones, Argentina (AR, Table 3) to test our initial relationship and to contrast microbial diversity at two tropical sites with high plant diversity but contrasting soil pH.

There was also no apparent latitudinal gradient in diversity (Table 1 and Fig. 2), unlike diversity observations for plants and animals (18). Consequently, the environmental factors frequently cited as good predictors of plant and animal diversity at continental scales, particularly mean annual temperature (MAT) and potential evapotranspiration (PET) (17–19), had little effect on measured soil bacterial diversity (Fig. 2). Sampling resolution can have an important influence on the assessment of diversity patterns (23, 24), and, in this study, soils were collected from plots of $\approx$100 m^2 that are smaller in size than those commonly used to quantify large-scale patterns of plant and animal diversity (17). However, because individual soil bacteria are many orders-of-magnitude smaller than individual plants or animals (25), the number of individuals per plot may be directly comparable. It is also possible that the small size of our plots causes us to overestimate the importance of local parameters, such as soil pH, on bacterial community composition and underestimate the importance of parameters, such as PET and MAT, which are more regional in scale. Nonetheless, our results do suggest that the biogeographical patterns observed in soil bacterial communities are fundamentally different from those observed in well studied plant and animal communities

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Table 1. Univariate models predicting phylotype diversity [Shannon index (20), H′] as a function of various soil and site characteristics

Variable	Model type	AIC value	a	b	c	r^2
Latitude	Quadratic	46.7	2.56	0.03	−0.01	0.16
MAT, °C	Linear	61.5	3.25	−0.01	—	0.01
SMD	Quadratic	20.6	3.29	0.01	0.01	0.33
Organic C, %	Linear	45.2	3.37	−0.03	—	0.15
C:N ratio	Linear	54.1	3.40	−0.01	—	0.05
Silt + clay, %	Linear	61.1	3.25	−0.01	—	0.01
pH	Quadratic	−54.2	−0.49	1.12	−0.08	0.70
CMR	Linear	51.7	3.33	−0.01	—	0.09
PET, mm·yr^{-1}	Linear	61.5	3.27	−0.01	—	0.02

In each case, we fitted a linear ($y = a + bx$) and a quadratic ($y = a + bx + cx^2$) model; results are shown for the model with the lowest Akaike information criteria (AIC) (46) value. Lower AIC values indicate stronger support for the model, balancing model fit and parsimony. CMR, potential carbon mineralization rate (micrograms of C–CO_2 per gram of soil^{-1}·d^{-1}); SMD, soil moisture deficit (mm·yr^{-1}).

that have provided the foundation for biogeographical theory to date.

Not only did we observe that soil pH was the best predictor of bacterial richness and diversity, it was also the strongest predictor of overall community composition (Fig. 3). We observed a general clustering of soil bacterial communities within ecosystems that corresponded to the observed pattern with pH across systems (Fig. 3). For example, the bacterial communities found in soils of arid and semiarid ecosystems, which generally have near-neutral pHs, cluster together, as do bacterial communities from temperate and tropical forest ecosystems, which generally have acidic soils. Of all of the soil and site characteristics examined, soil pH was the best predictor of soil microbial community composition at the continental scale (Table 2), and there was a strong correlation between the primary axis of Fig. 3, which describes 73% of the variation among soil communities, and soil pH ($r^2 = 0.83$ for Axis 1, $P < 0.0001$). There was some correlation between soil pH and a number of other soil properties including soil moisture deficit ($r = 0.68$), soil organic C content ($r = -0.53$), and soil C:N ratio ($r = -0.51$), but the differences in bacterial community composition across ecosystems could largely be explained by differences in soil pH alone ($r_{\mathrm{Mantel}} = 0.75$, $r^2 = 0.56$, $P < 0.001$). These results are not likely to be affected by variation in sampling times, because all soils were collected near the height of the plant growing season at each site and the intrasite variability in bacterial community structure for soils collected at the same location 6 months apart was less than the intersite variability in bacterial community structure (see Fig. 4, which is published as supporting information on the PNAS website).

Whereas vegetation type, carbon availability, nutrient availability, and soil moisture may influence microbial community

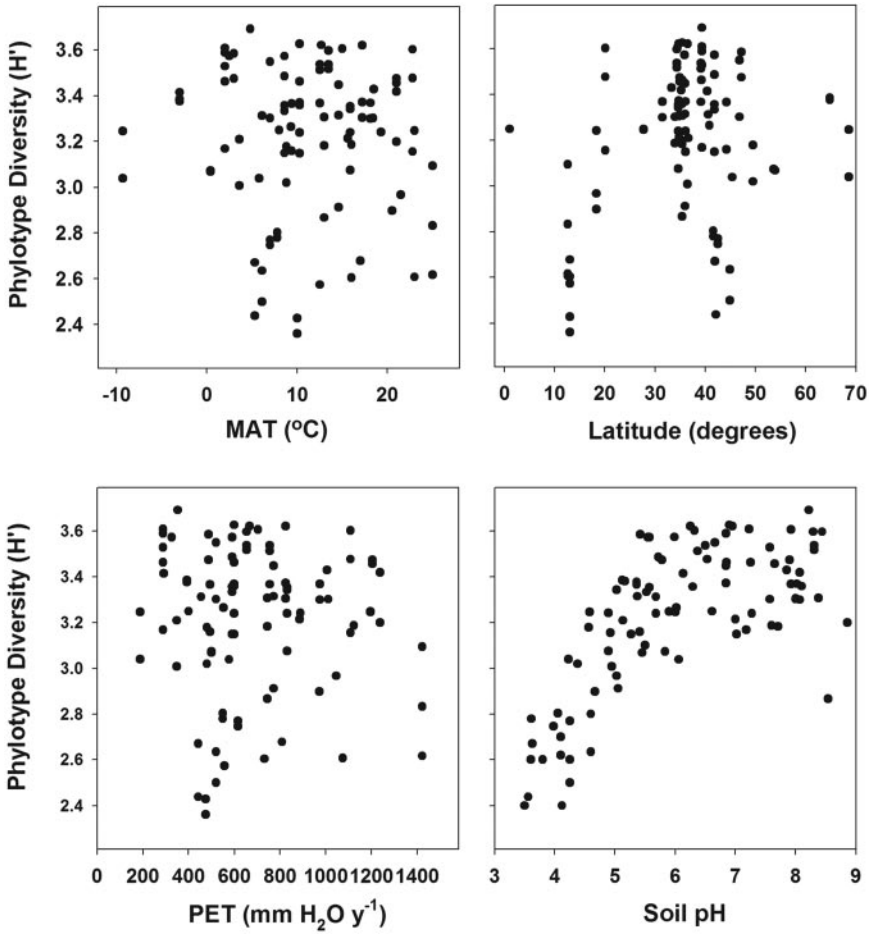

Fig. 2. Relationships between phylotype diversity (Shannon index) and MAT, latitude, PET, and soil pH (as in Fig. 1*A*). MAT, PET, and latitude are typically good predictors of animal and plant diversity at the continental scale (17, 18). In contrast, soil pH is the best predictor of bacterial diversity. The r^2 values for the MAT, latitude, PET, and pH regressions are 0.01, 0.16, 0.02, and 0.70, respectively (see Table 1 for complete regression statistics).

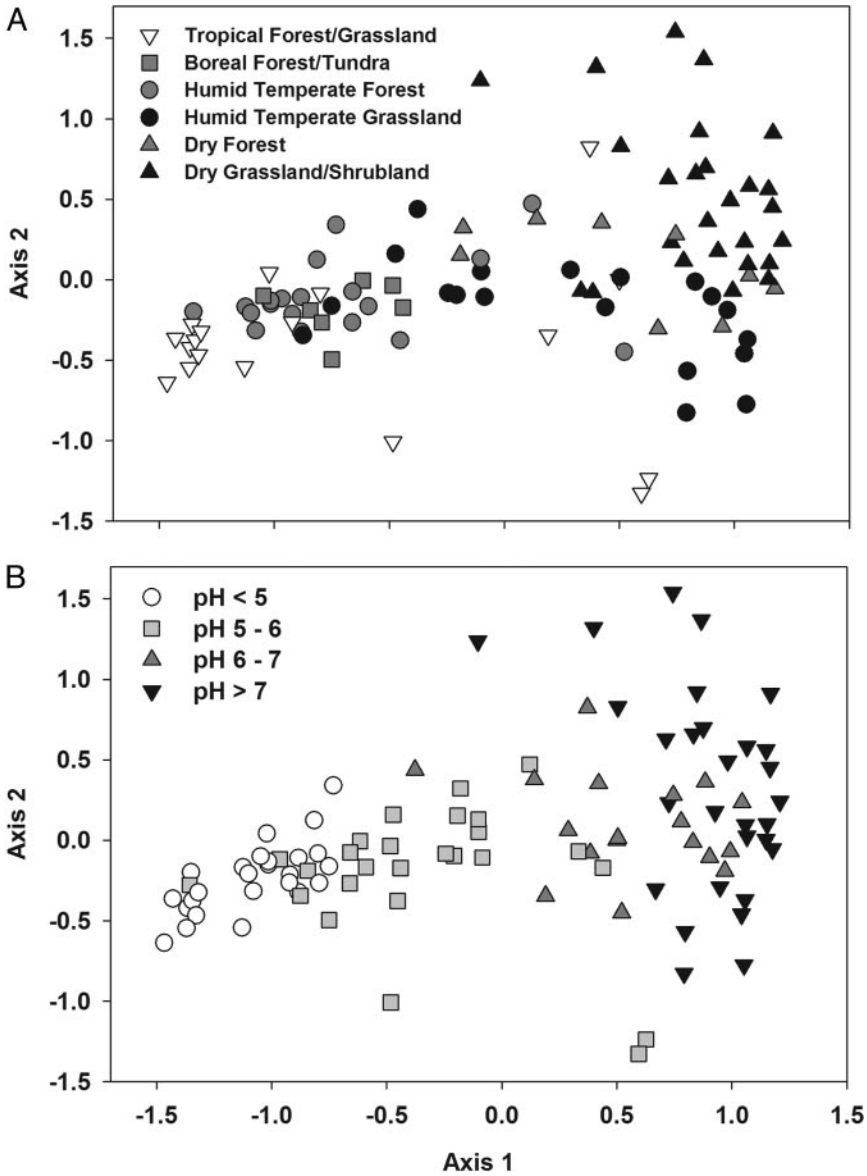

Fig. 3. Nonmetric multidimensional scaling plots of soil bacterial communities, showing the relative differences in community composition. Plots *A* and *B* are identical except that *A* is overlayed with general ecosystem type and *B* is overlayed with pH category. The r^2 values between ordination distance and distance in the original space are 0.73 and 0.16 for axis 1 and axis 2, respectively. Soil pH explains 83% of the variability along the primary axis, axis 1 (see text). The addition of more than two dimensions did not lead to a significant increase in the explanatory power of the ordination. The Sorensen distance metric (44) was used to quantify the similarity between phylotype patterns.

composition at local scales (13), soil pH was a better predictor of community structure at the continental scale (Table 2). The strong correlation between soil pH and microbial community structure could be a result of soil pH integrating a number of other individual soil and site variables. However, we would also expect soil pH to be an independent driver of soil bacterial diversity, because the intracellular pH of most microorganisms is usually within 1 pH unit of neutral (26). Moreover, any significant deviation in environmental (extracellular) pH should impose stress on single-celled organisms. The stress of residing in suboptimal pH environments has been shown to have a significant effect on the overall diversity and composition of microbial communities in a range of terrestrial and aquatic environments (27–29).

The degree of similarity between soil bacterial communities was largely unrelated to geographic distance. For example, forest soils from the Northeast U.S., Northwest U.S., boreal, and tropical regions had bacterial communities that were relatively similar in composition. Once the soil environmental variables listed in Table 2 were taken into account, geographic distance was found to be a poor predictor of the degree of similarity in bacterial communities ($r_{Mantel} = 0.13$, $r^2 = 0.02$, $P = 0.15$), suggesting that soils with similar environmental characteristics have similar bacterial communities regardless of geographic distance. We estimated the relationship between the number of unique taxa (phylotypes) and area sampled using the distance-decay approach (14, 30, 31). Our estimated z value, the rate of turnover of unique phylotypes across space, is 0.03 (95% confidence interval: 0.02 to 0.04, $P < 0.001$). This value is similar to the z values reported for other microbial groups (14, 31) and much lower than z values commonly reported for plant and animal taxa (32). As suggested in refs. 14 and 31, the low z values reported for microbial taxa may be related, in part, to the discontinuous nature of the microbial

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Table 2. Pearson correlations between the ordination score of the first axis of the nonmetric dimensional scaling ordination (which explains 73% of the variance in the original data) and key soil and site characteristics

Variable	r	r^2
Vegetation type	−0.62	0.38
MAT	0.09	0.01
SMD	0.73	0.54
Organic C, %	−0.51	0.26
C:N ratio	−0.50	0.25
Silt + clay, %	−0.04	0.01
pH	0.91	0.83
CMR	−0.44	0.20

Vegetation type is a binary variable (forest/nonforest). SMD, soil moisture deficit; CMR, potential C mineralization rate.

habitats surveyed and the relatively low taxonomic resolution of the rDNA-based methodology used in this study. Nevertheless, our results provide strong evidence that environmental factors, such as soil pH, are more important than geographic distance in influencing the continental-scale spatial structuring of microbial communities at higher taxonomic levels. In the soil environment, the distribution and structure of bacterial communities can largely be understood in terms of habitat properties alone.

Here, we show that the structure of soil bacterial communities is not random at the continental scale and that the diversity and composition of soil bacterial communities at large spatial scales can largely be predicted with a single variable, soil pH. These results suggest that, to some degree, the large-scale biogeographical patterns observed in soil microorganisms are fundamentally distinct from those observed in well studied plant and animal taxa. Although the biogeography of microorganisms remains poorly understood, and many questions remain unanswered, a thorough integration of microbial ecology into the field of biogeography is likely to provide a more comprehensive understanding of the factors controlling the Earth's biodiversity and biogeochemistry.

Methods

Soil Collection. A total of 98 soil samples that were distinct with respect to soil and site characteristics were collected from a wide array of ecosystem types in North and South America (see Table 3). Only soils unsaturated for the majority of the year were examined. Soils were collected near the height of the plant growing season at each location. To examine whether seasonal variation was important, an additional set of soil samples was collected 6 months after the initial collection at a subset of sites. At each site, the upper 5 cm of mineral soil was collected from 5–10 locations within a given plot of ≈100 m^2 and composited into a single bulk sample. All soil samples were shipped to the University of California, Santa Barbara, within a few days of collection, where they were sieved to 4 mm, homogenized, and archived at −80°C.

Site and Soil Description. For sites in the U.S., climate information for each site was estimated from historical average data (1971–2000) provided by the National Oceanic and Atmospheric Administration. For sites outside the U.S., climate information was provided by researchers working at the individual sites. Average annual soil moisture deficit (in millimeters of H_2O per $year^{-1}$) was estimated as the sum of the differences between mean monthly PET and mean monthly precipitation. PET was estimated by using Thornthwaite's method with a correction for latitude (33). Soil pH, organic C concentrations, and particle size distributions were measured on each soil sample by using standard methods (see Table 3). Potential C mineralization rates were estimated by measuring the rates of CO_2 production over the course of a 50-d incubation at 20°C after adjusting all soils to 35% of water-holding capacity.

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) Analyses. To compare bacterial diversity and community structure across soils, we used a T-RFLP method. The method quantifies sequence variability in small-subunit (16S) ribosomal DNA extracted from soil, producing a DNA "fingerprint" for each bacterial community based on the length and abundance of unique phylotypes (restriction fragments) from each soil sample. Although sequence analysis of clone libraries provides more detailed phylogenetic information, the T-RFLP method is better suited for analyzing a large number of samples and for quantitatively detecting differences in the diversity and composition of highly complex soil bacterial communities (34–37). One limitation of the T-RFLP method is that it underestimates total bacterial diversity because the method resolves only a limited number of bands per gel (generally <100), and bacterial species can share phylotypes (37). However, the method does provide a robust index of bacterial diversity (35, 36, 38), and T-RFLP results are generally consistent with the results from clone libraries (39, 40).

For the T-RFLP procedure, DNA was extracted from 5–10 g (dry weight equivalent) of each soil sample by using the UltraClean Mega Soil DNA kit (MoBio Laboratories, Carlsbad, CA). DNA was further purified by using a Sepharose 4b column, as described in Jackson *et al.* (41), with DNA yields quantified by PicoGreen fluorometry (Molecular Probes). The HEX-labeled primer Bac8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and unlabeled primer Univ1492r (5′-GGTTACCTTGTTACGACTT-3′) (42) were used for amplification of bacterial 16S rDNA. Each 50-μl PCR mixture contained 1× HotStarTaq Master Mix (Qiagen), 0.5 μM each primer, 50 μg of BSA, and 50 ng of DNA. Each of the 35 PCR cycles consisted of 60 s at 94°C, 30 s at 50°C, and 60 s at 72°C. Products were combined from three PCRs per DNA sample and purified with a QiaQuick PCR purification kit (Qiagen, Valencia, CA). After size verification by agarose-gel electrophoresis, PCR products were digested in separate reactions by using HhaI and RsaI restriction enzymes (New England Biolabs). Digested DNA samples were separated by electrophoresis on an ABI Prism 3100 genetic analyzer using GENESCAN analysis software (Applied Biosystems). The analysis and standardization of the T-RFLP profiles was conducted as described in Dunbar *et al.* (37). Only those fragments in a particular sequencing sample between 50- and 600-bp in length that had a standardized fluorescence >4% of the total fluorescence for that sample were included in the analyses.

Data Analysis. We used T-RFLP data (phylotype length and square-root-transformed proportional abundance) (31) from both the RsaI and HhaI enzymes for ordination by nonmetric multidimensional scaling and the Mantel tests. These analyses were conducted in PC-ORD (43) by using the Sorensen distance metric (44), with Monte Carlo tests (1,000 randomized runs) to determine significance. All other statistical analyses were performed in SYSTAT (45). We used the Shannon index to estimate phylotype diversity, as recommended by Hill *et al.* (20). The phylotype–area relationship was estimated by using the distance-decay approach (14, 30, 31). Correlations between soil and site variables were examined by using linear regressions with a Pearson correction for multiple comparisons. We conducted partial Mantel tests using the method described in Horner-Devine *et al.* (30) to examine the correlation between geographic distance and the degree of similarity in bacterial community composition when soil characteristics are held constant.

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