7-2017

The interacting roles of climate, soils, and plant production on soil microbial communities at a continental scale

M. P. Waldrop

J. M. Holloway

D. B. Smith

M. B. Goldhaber

Rebecca E. Drenovsky
John Carroll University, rdrenovsky@jcu.edu

See next page for additional authors

The interacting roles of climate, soils, and plant production on soil microbial communities at a continental scale

M. P. Waldrop,[1,8] J. M. Holloway,[2] D. B. Smith,[2] M. B. Goldhaber,[2] R. E. Drenovsky,[3] K. M. Scow,[4] R. Dick,[5] D. Howard,[6] B. Wylie,[6] and J. B. Grace[7]

[1]*U.S. Geological Survey, Menlo Park, California 94025 USA*
[2]*U.S. Geological Survey, Denver, Colorado 80225 USA*
[3]*Department of Biology, John Carroll University, University Heights, Ohio 44118 USA*
[4]*Land, Air & Water Resources, University of California – Davis, Davis, California 95616 USA*
[5]*Environmental and Natural Resources, Ohio State University, Columbus, Ohio 43210 USA*
[6]*U.S. Geological Survey, EROS Data Center, Sioux Falls, South Dakota 57030 USA*
[7]*U.S. Geological Survey, Lafayette, Louisiana 70506 USA*

Abstract. Soil microbial communities control critical ecosystem processes such as decomposition, nutrient cycling, and soil organic matter formation. Continental scale patterns in the composition and functioning of microbial communities are related to climatic, biotic, and edaphic factors such as temperature and precipitation, plant community composition, and soil carbon, nitrogen, and pH. Although these relationships have been well explored individually, the examination of the factors that may act directly on microbial communities vs. those that may act indirectly through other ecosystem properties has not been well developed. To further such understanding, we utilized structural equation modeling (SEM) to evaluate a set of hypotheses about the direct and indirect effects of climatic, biotic, and edaphic variables on microbial communities across the continental United States. The primary goals of this work were to test our current understanding of the interactions among climate, soils, and plants in affecting microbial community composition, and to examine whether variation in the composition of the microbial community affects potential rates of soil enzymatic activities. A model of interacting factors created through SEM shows several expected patterns. Distal factors such as climate had indirect effects on microbial communities by influencing plant productivity, soil mineralogy, and soil pH, but factors related to soil organic matter chemistry had the most direct influence on community composition. We observed that both plant productivity and soil mineral composition were important indirect influences on community composition at the continental scale, both interacting to affect organic matter content and microbial biomass and ultimately community composition. Although soil hydrolytic enzymes were related to the moisture regime and soil carbon, oxidative enzymes were also affected by community composition, reflected in the abundance of soil fungi. These results highlight that soil microbial communities can be modeled within the context of multiple interacting ecosystem properties acting both directly and indirectly on their composition and function, and this provides a rich and informative context with which to examine communities. This work also highlights that variation in climate, microbial biomass, and microbial community composition can affect maximum rates of soil enzyme activities, potentially influencing rates of decomposition and nutrient mineralization in soils.

Key words: *continental scale; soil biota; soil enzymes; structural equation model.*

Introduction

Understanding the biogeography of soil microbial communities is of great societal and scientific importance due to the myriad of ecosystem services provided by soil biota. Global scale patterns of microbial biogeography in the terrestrial environment have been well studied (Zak et al. 1994, Fierer et al. 2009, Vogel et al. 2009, Decaëns 2010, Nemergut et al. 2011) and efforts continue with new synthesis efforts such as the Earth Microbiome Project (Gilbert et al. 2014), the Global Soil Biodiversity Assessment Initiative, the International Soil Metagenome Sequencing Consortium, and others (Vogel et al. 2009). The field of microbial biogeography has the potential not only to help answer fundamental ecological questions, but may also assist scientists and land managers to utilize microbial communities to increase plant productivity, reduce plant disease and enhance soil health (Kourtev et al. 2002, Drenovsky et al. 2009, Griffin et al. 2009, Decaëns 2010, Wu et al. 2011). Improved understanding of the biogeography of

Manuscript received 29 August 2016; revised 28 March 2017; accepted 5 April 2017. Corresponding Editor: Steven D. Allison.

[8] E-mail: mwaldrop@usgs.gov

soil microbial communities may also advance spatially explicit biogeochemical models grounded in microbial ecology (Wallenstein and Hall 2011, Wieder et al. 2013).

Some of the first continental scale studies of soil microorganisms focused on microbial abundance, which showed that soil microbial communities are a consistent fraction (~2%) of the soil organic carbon (OC), and are strongly regulated by the composition and productivity of the aboveground plant community (Zak et al. 1994). More recent continental scale studies of microbial community composition and diversity have shown that there is a strong biogeography to soil microbial community composition (Drenovsky et al. 2009, Decaëns 2010, Wu et al. 2011). Although the fundamental controls of any biological community include selection, genetic drift, dispersal, and random mutation (Hanson et al. 2012), here we focus primarily on the selection pressures produced by the ecological systems. The fundamental factors affecting community composition include climate, land use, and soil edaphic properties such as OC, pH, and nutrient status. These factors affect soil microbial community composition at local, regional and continental scales (Griffiths et al. 1998, Fierer and Jackson 2006, Lauber et al. 2008, Angel et al. 2009, Fierer et al. 2009, Vogel et al. 2009, Decaëns 2010, Nemergut et al. 2011, Brockett et al. 2012). Microbial communities may also be structured in part by soil mineral composition and degree of weathering, as seen across soil chronosequence and mineral studies (Kourtev et al. 2002, Moore et al. 2010, Hemkemeyer et al. 2014), but this has received much less attention.

In a great majority of studies to date, the relationships between microbial community composition and soil properties are described using univariate statistical procedures, with conclusions based on the selection of a few variables from sets of highly correlated predictors. Within this analysis framework, a change in one or a few dominant factors are presumed to control community composition, and there is little discussion of how certain factors may be acting either directly or indirectly on microbial communities (Angel et al. 2009, Auguet et al. 2009, Fierer et al. 2009, Vogel et al. 2009, Decaëns 2010, Nemergut et al. 2011). Realistically, microbial communities exist within an ecosystem of interacting influences of climate, plant communities, and soil resources. These components themselves are also interrelated, complicating our ability to directly quantify relationships among multiple interacting ecosystem properties and soil microbial communities. However, we can explore relationships among multiple ecosystem components including microbial communities using structural equation modeling (SEM). Structural equation modeling allows us to graphically represent the strength of the interacting relationships present within soil ecological systems (Mitchell 1992, Grace et al. 2010, Jonsson and Wardle 2010). In so doing, we can identify the relative importance of direct and indirect processes influencing microbial communities and their activities. Structural equation modeling has been used in the past to explore the relationships between climate, soils, plant communities, and soil microbial communities in arctic, dryland, and global analyses (Siciliano et al. 2014, Tedersoo et al. 2014, Maestre et al. 2015). The common thread among these studies is that climate (primarily precipitation) indirectly affects soil microbial alpha diversity (e.g., local richness) through impacts on organic matter available to the microbial communities. Beta diversity (species composition) was impacted by soil pH when large gradients in pH were examined. However, these studies typically avoided heavily managed lands, and there was no examination of the linkages between variation community composition and soil processes.

Microbial community composition and soil function can be linked through the analysis of exo-enzymatic activity (EEA), which controls processes such as decomposition and nutrient release from soil organic matter (Sinsabaugh 1994). Enzymatic activity has been used in both conceptual and explicit models to predict, for example, nutrient limitation effects on C cycling, temperature acclimation of microbial communities, and soil carbon storage (Waldrop and Firestone 2004, Allison et al. 2007, Sinsabaugh et al. 2008, Wieder et al. 2013). Many of the same factors that influence microbial community composition also affect EEA. Globally, soil enzyme activities are tied to substrate availability, pH, precipitation, and microbial nutrient demand (Sinsabaugh et al. 2008), but some evidence suggests that EEA is also affected by the size and composition of the soil microbial community (Waldrop et al. 2004, Sinsabaugh et al. 2008, Drenovsky et al. 2009, Decaëns 2010, Wu et al. 2011, Burns et al. 2013). Thus while our objective is to produce a comprehensive model of the ecological factors that directly and indirectly structure microbial communities across multiple land use and soil types, we also hypothesize that patterns of potential microbial activity (enzyme activities) are affected by community composition in addition to soil edaphic variables.

We explored variation in microbial community composition and enzyme activity at the continental scale using soils sampled along two transects across the U.S. (Smith et al. 2009; Fig. 1). As background descriptive statistics, we examined the relationships between microbial communities and categorical variables including land use and soil order that are presented in Appendix S1. We then investigated the direct and indirect environmental controls on microbial biomass, microbial community composition, and soil enzyme activity using SEM, with particular emphasis on relationships among climate, soil properties, and plant productivity.

Methods

Soil sampling

We sampled two continental-scale transects including a N-S transect extending from northern Manitoba to the U.S.-Mexico border near El Paso, Texas, and an E-W

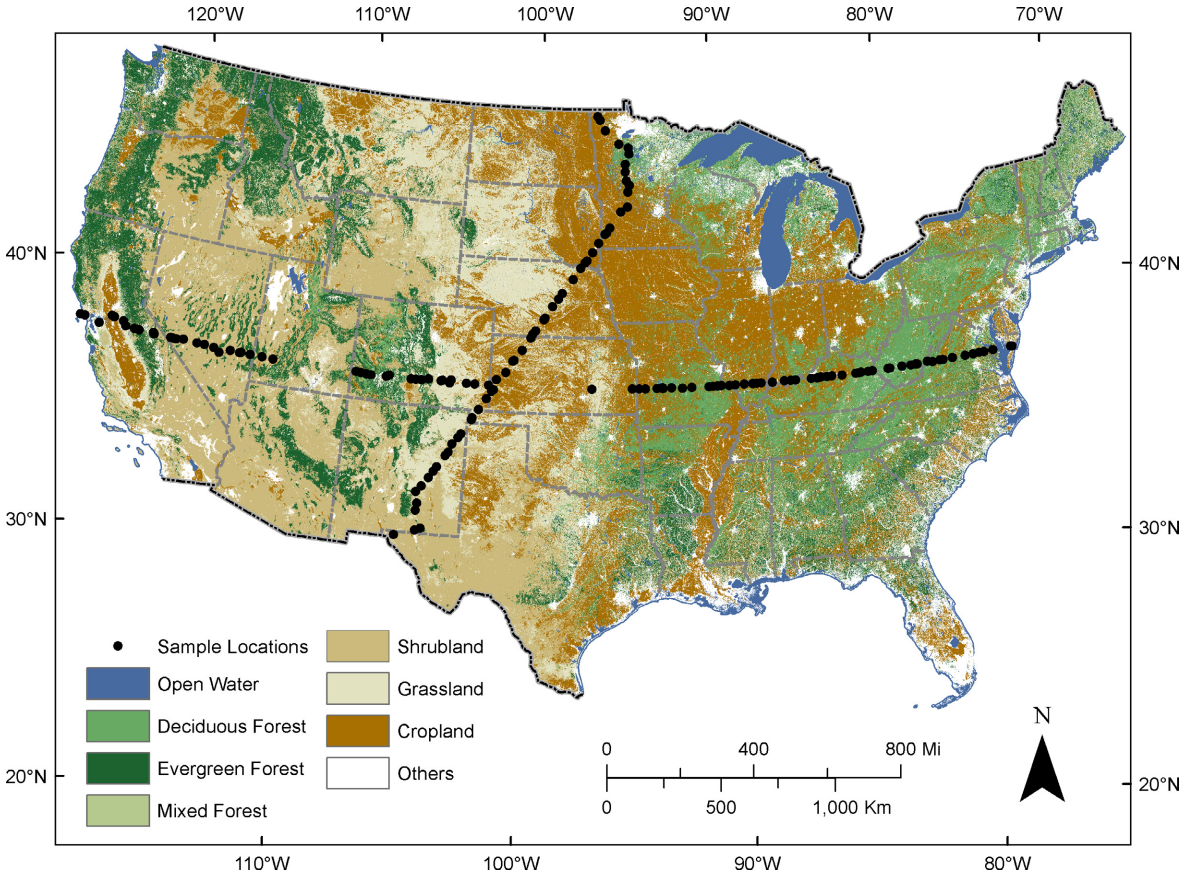

FIG. 1. Locations of the samples taken for the Geochemical Landscapes Project. Samples for microbial and geochemical analysis were taken at ~40 km intervals along both longitudinal and latitudinal gradients from A horizon soils. Land cover map was taken from the 2011 version of the National Land Cover Database (Homer et al. 2007) which was re-sampled from the original 30 m resolution to 250 m resolution using the majority land cover. [Color figure can be viewed at wileyonlinelibrary.com]

transect that follows along the 38th parallel from the Pacific coast north of San Francisco to the Atlantic coast in Maryland (Fig. 1). Transects cross multiple climatic, physiographic, land use, geologic, pedologic, and ecological boundaries. Each transect was divided into 40-km segments. For each segment, a 1-km wide latitudinal strip was randomly selected; within each strip, a potential sample site was selected from the most representative landscape within the most common soil type (Smith et al. 2009). Areas surrounding power plants, roadways, buildings, and other unusual landscape features were avoided. Mineral soil horizons (A horizon) were sampled in the summer and fall of 2004. Soils for microbial characterization were sampled using sterile techniques. The A horizon from within one 60 × 60 cm quadrant was either sampled directly using a sterile stainless steel trowel, or a shovel or auger if the A horizon was deep. Samples were homogenized in a sterile glass or stainless steel bowl and placed in a sterile 50-mL centrifuge tube. The tube was sealed in a zip lock plastic bag for storage and was immediately frozen or refrigerated for a maximum of 3 d before being shipped and stored at −20°C prior to analysis. Soils for physical and chemical analysis were taken concurrently, air-dried and sieved to 2 mm. A total of 265 samples were sampled across the two continental gradients. Of these, 185 were analyzed for phospholipid fatty acid (PLFA) and soil chemical and physical analysis, and a smaller subset of 108 for enzyme activities. Descriptive land cover and land use data were collected through on site observation and soil order was determined by using latitude and longitude comparison to USDA databases. All climate, soil chemical, mineralogical, and microbial data were organized by soil order and land use and are presented in the Appendix S1.

Chemical and mineralogical data

Chemical and mineralogical data collected from each site include organic and inorganic carbon, pH, quantitative mineralogy, and total concentration of 47 elements including S, P, Ca, Mg, and K (Garrett et al. 2009, Smith et al. 2009). Total carbon and nitrogen were determined on dried ground samples by pyrolysis (Carlo Erba NA 1500 CHN analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA)). Carbonate was determined as CO_2 by coulometric titration on a sample

treated with 2 N $HClO_4$ (Brown et al. 2002), with an lower limit of detection of 0.05%. Organic carbon was calculated as the difference between total and carbonate carbon. Major elements, including S, P, Ca, Mg, and K, were analyzed by ICP-MS on sample digested in a mixture of HCl, HNO_3, $HClO_4$ and HF (Briggs and Meier 2002). Soil mineralogy including the percentage content of plagioclase, potassium feldspar, and total clay minerals, was determined by quantitative X-ray diffraction (XRD) analysis (Eberl and Smith 2009).

Climate

Climate data from each site were collected by spatially joining the transect point sites to precipitation polygons and temperature points from the closest location with data available. The precipitation and temperature values are from NRCS (Natural Resources Conservation Service) PRISM Project (PRISM Climate Group 2006); 30 yr average annual precipitation for the conterminous U.S. (1961–1990). The temperature values are 30 yr averages (1971–2000).

Plant production

The eMODIS Normalized Difference Vegetation Index (NDVI) was used as a proxy for plant productivity, as it has been shown to correlate with photosynthetically active vegetation and daytime CO_2 uptake (Tucker et al. 1985, Wylie 2003, Pettorelli et al. 2005). Normalized Difference Vegetation Index was calculated from average value composites at the resolution of 250 m for all of 2004 (Jenkerson et al. 2010). Composites were put into annual time series of 52 bands for each annual multi-band image. These temporal time series of NDVI were then temporally smoothed to replace temporary drops in NDVI associated with residual cloud cover in some of the weekly NDVI composites (Swets et al. 1999).

Microbial community composition

Phospholipid fatty acid analysis was used to quantify soil microbial community composition because it is a relatively rapid and quantitative screening method for describing microbial communities in soil and is correlated with microbial biomass (White and Ringelberg 1997). Phospholipids are integral components of cell membranes and are metabolized rapidly when a cell dies in soil; therefore, they can be used to quantify living microbial groups including fungi, Gram+ and Gram− bacteria, and actinomycetes. Classification of PLFAs is based chain length, degree of unsaturation, and substituted groups (e.g., methyls, hydroxyls, and cylopropane rings; White and Ringelberg 1997).

Phospholipid fatty acids were extracted from triplicate subsamples of soil in a chloroform/methanol/phosphate buffer (1:2:0.8 v/v/v) with the volume of phosphate buffer adjusted for existing soil–water content. Soils were shaken for 2 h, centrifuged, and the supernatant was decanted into separatory funnel. Soils were re-extracted with an additional extract solution, vortexed, shaken for 30 min, centrifuged, and the second supernatant was added to the first. The supernatants were combined with a PO_4 buffer and $CHCl_3$, shaken, and the phases were separated overnight. The $CHCl_3$ layer was decanted and dried under N_2 at 32°C. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns conditioned with $CHCl_3$, with neutral lipids and glycolipids eluted with $CHCl_3$ and acetone and polar lipids eluted with methanol and air dried under N_2. Polar lipids were subjected to mild alkaline methanolysis to form fatty methyl esters. Extracts were prepared with hexane containing the 19:0 lipid as an internal standard and analyzed by gas chromatography (Hewlett Packard 6890, Agilent Technologies Inc., Santa Clara, California, USA) using a 25 m Ultra 2 (5%-phenl)-methyl polysiloxane column (J&W Scientific, Agilent Technologies Inc., Santa Clara, California, USA). Peaks were identified using FAME standards and MIDI peak identification software (MIDI, Inc., Newark, Delaware, USA; Bossio and Scow 1998). Of the 137 identified peaks, a group of 23 PLFAs were selected to evaluate microbial community structure based on presence in 75% of samples analyzed at 1 mol% or greater. Individual PLFAs used to address microbial community structure were converted to mol% before being incorporated into principal components analysis (PCA) for data reduction using covariances among the data. We used the sum of total extracted PLFAs (nmol/g) as an index of total soil microbial biomass (Bååth and Anderson 2003). On a subset of fifteen samples, the biomass of microbial carbon was also estimated using the chloroform fumigation direct-extraction technique (Vance et al. 1987).

Enzyme activities

Activities of β-glucosidase, arylsulfatase, phosphatase, and *N*-acetylglucosaminidase (NAGase) were determined during short-term incubations on 1 g of air-dried soil. The respective *p*-nitrophenyl (pnp) substrates used in these four assays were 5 mmol/L pnp-β-glucoside, 5 mmol/L pnp-sulfate, 5 mmol/L pnp-phosphate, and 2 mmol/L pnp-β-*N*-acetylglucosaminidase. For β-glucosidase activity the buffer was a pH 6 modified universal buffer (MUB) and pH 12 *tris*-hydroxy aminomethane (THAM) was added to stop the hydrolysis reaction and to deprotonate the pnp, producing a yellow color measured at 420 nm (Tabatabai 1994). For the other pnp assays, 50 mmol/L pH 5.8 acetate buffer was used and NaOH stopped the reaction and developed product color. Controls for both substrate (substrate + buffer) and soil (soil + buffer) were also included and subtracted from the assay measurement. β-glucosidase and arylsulfatase were analyzed in duplicate at 37°C for 1 h. Phosphatase and NAGase activities were assayed for 2–4 h at 22°C in a 96 well plate format allowing for eight analytical replicates per sample. In the 96 well plate

format, one g air-dried soil sample was added to 100 mL acetate buffer (pH 5.0; 50 mmol/L), and homogenized on a stir plate. About 100 μL soil homogenate was added to 100 μL of buffered substrate. Plates were placed in the dark and not shaken. At the end of the assay, 100 μL of reaction product was pipetted to a new clear plate containing NaOH to end the reaction and develop the color. Color was measured at 405 nm on a Biotek spectrometer (Biotek, Winooski, Vermont, USA) after developing a pnp standard curve.

Phenol oxidase and peroxidase enzyme assays were measured using the 96 well plate format at 22°C. About 5 mmol/L L-DOPA was used as the substrate in 50 mmol/L acetate buffer. Peroxidase assay included an aliquot of 0.3% H_20_2 solution in each assay well. Assays were incubated overnight and measured 450 nm on a Biotek spectrometer after 100 μL was transferred to a new clear plate (Miller and Dick 1995, Waldrop et al. 2010). A standard curve was developed by oxidizing known quantities of L-DOPA and measuring its absorbance.

Data analyses

Comparisons among soil orders and land uses were made using ANOVA and Tukey–Kramer post hoc tests ($P < 0.05$), after normalizing the data, using the JMP 9.0 statistical program (SAS Institute, Cary, North California, USA). Principal components analysis was used to reduce the number of variables within the microbial, chemical, mineralogical, and enzyme databases before using them within the structural equation model. This was done in order to create a few aggregate variables that were representative of each dataset and could be interpreted in light of the most important trends within each dataset. The PCA was conducted on covariances because units and ranges of values were similar within each database. Details of the analyses are given in *Results*.

We constructed an initial structural equation meta-model (Grace et al. 2010) for evaluation based upon a priori knowledge to represent a general hypothesis of the effects of large-scale major environmental drivers on microbial communities (Fig. 2). This initial model was designed to allow us to evaluate the full set of measured variables related to climate, plant productivity, and soil organic and inorganic properties. We considered the inclusion of latent variables to represent climate, soil inorganic, and soil organic variables, though these were eventually omitted as they were unnecessary to the model. Variables were log transformed to meet assumptions of homogenous variances and normal distributions. We used the SEM package (version 3.1-3, John Fox, McMaster University, Canada) in R to estimate parameters and test model-data consistency. The final model was obtained by removing non-significant variables and paths and adding additional paths as recommended by modification indices (Grace et al. 2010, 2012). We compared models using chi-square difference tests and AIC_c comparisons to arrive at the final best fitting model.

Results

Microbial PLFA data were reduced from relative abundance data to a single new variable representing community composition using PCA. This first single PC (PC1) explained 40% of the PLFA dataset, and was strongly influenced by several fungal and Gram− biomarkers (see

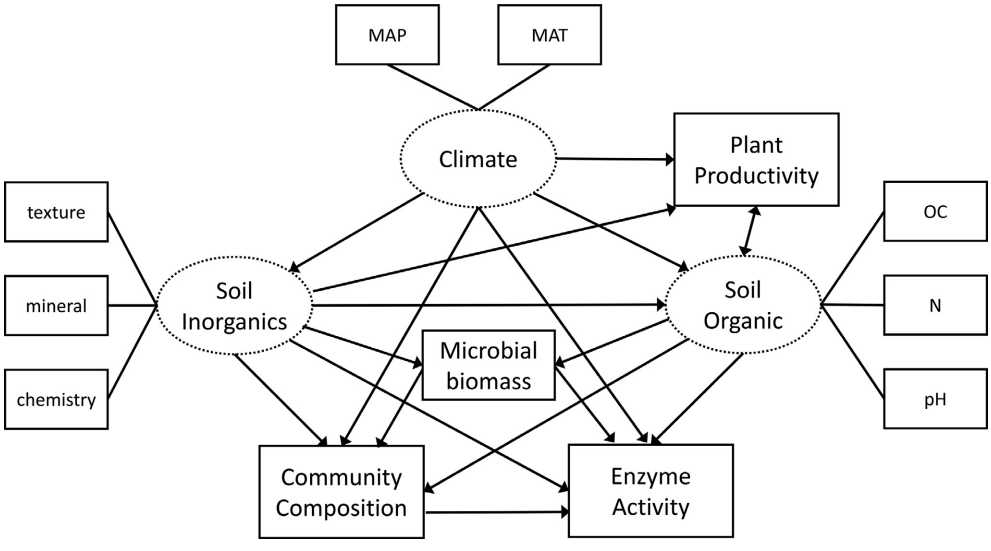

FIG. 2. Initial meta-model designed to represent possible relationships between climate, plant productivity, soils, and microbial community composition and function. Ovals represent general theoretical constructs that encompass more than one measured variable. Lines represent univariate relationships between variables. This initial hypothesized model was then modified during multiple iterations of the SEM model through additions or subtractions of variables and relationships to provide the most robust model based upon the AIC criterion.

Appendix S1: Table S1). Thus increasingly positive values for PC1 indicate increasing relative abundance of fungi and some Gram− bacteria.

From the soil element concentration (geochemical) dataset, PCA resulted in the first variable, explaining 51% of the variability, to be most strongly defined by Ca (eigenvector [v] = 0.94) and carbonate (v = 0.26). This new eigenvector was termed "$CaCO_3$". The second principal component of the geochemical database explained another 22% of the variance, was described by iron (v = 0.53) and magnesium (v = 0.77), and was termed "FeMg" (see Appendix S1: Table S2).

Principal components analysis of the soil mineral composition dataset revealed the first principal component, explaining 59% of the database, was dominantly quartz (v = 0.89) and was ignored because quartz is highly resistant to chemical weathering, and thus is commonly distributed in soils. The second principal component (explaining 14%) was potassium feldspar (v = −0.42), plagioclase (v = −0.47), and 2:1 clays (v = 0.55). This XRD variable was termed "Weathering" due to the fact that during the course of weathering k-feldspars and plagioclase, largely derived from granitic rock, will be weathered out and 2:1 clays will form (see Appendix S1: Table S3).

The PCA analysis of the soil enzyme data produced two new variables. The first principal component, incorporating 43% of the variability in the dataset, included β-glucosidase (v = 0.43), arylsulfatase (v = 0.52), and phosphatase (v = 0.50), and we termed this eigenvector "hydrolytic enzymes". The second, incorporating 19% of the variability in the dataset contained phenol oxidase + peroxidase (v = 0.59), and *N*-acetyl-glucosaminidase (v = −0.62), and we termed this second principal component "oxidative enzymes" (see Appendix S1: Table S4).

Soil chemical, mineralogical, microbial, and enzymatic data were analyzed by one way ANOVA followed by Tukey–Kramer post-hoc tests with land use and soil order as fixed effects (Appendix S1).

Structural equation model

The most parsimonious model for predicting microbial community composition and soil enzyme activities at the continental scale included precipitation, plant productivity, pH, soil mineralogy, microbial biomass, and organic C as the primary exogenous and endogenous variables (Fig. 3). The final χ^2 of the model was 23 (df = 19, P = 0.24), indicating good model-data consistency. All parameters within the model interactions are

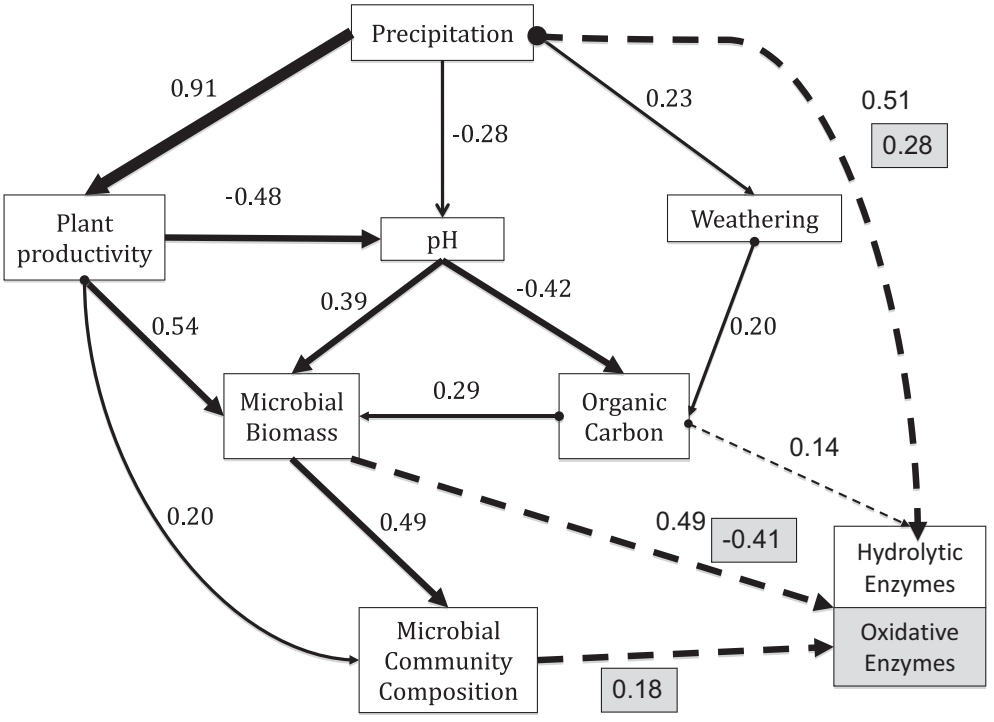

FIG. 3. Final structural equation model regarding the interactive controls of microbial community composition and soil enzyme activities. All relationships are statistically significant (P < 0.05). Numbers near lines indicate the parameter estimate (correlation coefficient) and its relationship (+ or −). The thickness of the arrow increases with the size of the correlation coefficient. Solid lines indicate the model without enzyme activities and dashed lines indicate additional model parameters and correlations with enzymes included. R^2 for endogenous variables were: hydrolytic enzymes (0.76), oxidative enzymes (0.16), soil carbon (0.25), plant productivity (0.83), pH (0.57), microbial biomass (0.22), and community composition (0.34). Statistics for non-enyzme model: Model AIC = 50, χ^2 = 12, P = 0.21, df = 9; For model including enzymes: Model AIC = 74 χ^2 = 23; P = 0.24, df = 19. R^2 for non-enzyme variables did not change when enzymes were included.

well supported. An SEM model without the inclusion of soil enzymes was also evaluated, as it allowed for a greater number of observations. The structure and path coefficients of this "no enzyme" model did not differ from the final model with enzymes included.

Plant productivity and microbial biomass were the most influential direct controls on community composition within the model. Precipitation played an indirect role in the model by controlling microbial community composition through its effects on plant productivity (+), soil pH (−), and soil OC concentrations (+) (Fig. 3). Soil pH was indirectly related to microbial community composition through its effect on soil OC content (−) and microbial biomass (+). Interestingly, although soil OC concentration increases as soil pH declines, microbial biomass and pH are positively related such that the ratio of microbial biomass to soil OC is lowest at low pH and increases as soils become more alkaline (Fig. 4). Soil hydrolytic enzymes were found to be a function of precipitation, soil OC, and microbial biomass, but a portion of the enzyme pool, made up of oxidative and chitin-degrading enzymes, was found to also be a function of microbial community composition (Fig. 3). Several parameters were not important in the final SEM model and were excluded. These included mean annual temperature, soil texture, carbonates, soil nutrients, and latitude and longitude.

Discussion

Soil microbial communities and their activities exist within a changing and interacting set of climatic, biotic, and edaphic properties of ecosystems. Thus to understand microbial communities is to understand how multiple factors within ecosystems also interact. For example, as microbial communities respond to climate change, it will be important to understand how plants and soils may also change, and what factors microbial communities are directly responding to (e.g., available soil C, which may be relatively rapid) or indirectly responding to (e.g., soil weathering or changes in climate, which could be much slower). We find SEM to be helpful in aiding our ability to investigate the direct and indirect influences that control microbial communities. Our selected model shows that climate, specifically mean annual precipitation, can be viewed as the ultimate "distal" control (sensu; Firestone and Davidson 1989) of microbial community composition through its effects on plant productivity and soil edaphic properties such as pH and soil OC. Soil pH and OC, in turn, are strong determinants of microbial biomass and community composition. Climate, pH, and OC have long been shown to be important factors controlling soil microbial community composition at local, regional and continental scales, likely because of their influence on the types and quantity of carbon compounds available to soil microbial communities (Angel et al. 2009, Drenovsky et al. 2009, Brockett et al. 2012). However, it is interesting to note that MAP and not MAT was the strong climatic influence on microbial community composition and enzyme potentials, despite the well-known temperature sensitivity of soil microbial communities.

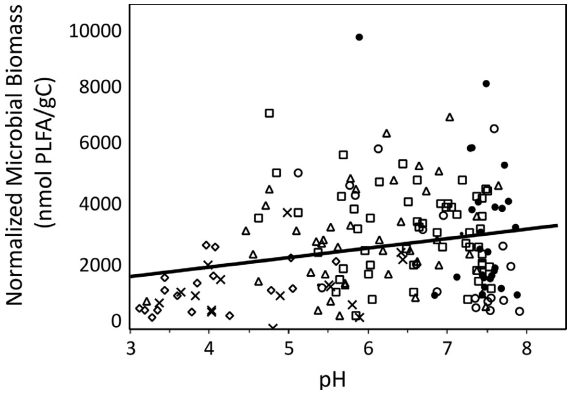

Fig. 4. Relationship between soil pH and microbial biomass (as PLFA) per unit soil organic carbon. The declining spread of the relationship with soil pH indicates that resources decline to the soil microbial community as soils become more acidic ($P < 0.05$, $R^2 = 0.06$). Markers represent soil order. Alfisol = △, Aridisol = ●, Entisol = ○, Inceptisol = X, Mollisol = □, Ultisol = ◇.

Plant productivity, microbial biomass, and OC were the most important "proximal" controls on microbial community composition and enzyme activities. Positive relationships among microbial biomass, OC, and enzyme activities has been observed in a variety of studies and provide evidence that microorganisms are largely carbon limited in soils (Zak et al. 1994). The importance of soil OC in structuring soil microbial community composition has been observed, although it is often secondary to the strong influence of soil pH, which is discussed in more detail below (Griffiths et al. 1998, Lauber et al. 2008, Fierer et al. 2009). What is unique about our statistical model is that rather than relying on only soil OC concentration as an explanatory variable, we also utilized microbial biomass and plant productivity metrics. Microbial biomass and plant productivity are likely a better reflection of the pool of available C substrates for microbial consumption than total SOM, much of which is inaccessible to the microbial community. Thus by using the additional metrics of C resource availability to the microbial community, we found that these metrics were important direct controls on community composition that has not been as widely examined.

Interestingly, we found that soil %N and C/N ratios were not important contributors to the SEM model, despite the strong relationship between these metrics and microbial community composition (particularly fungal/bacterial ratios) observed at large continental scales (Fierer et al. 2009). In our analysis, microbial community composition is largely affected by the relative abundance of fungi (see Appendix S1: Table S1), and thus would be expected to show this similar pattern.

However, in addition to various forest, grassland, and shrubland soils, our study also includes cropland soils, which are absent from Fierer et al. (2009). Agricultural soils contained moderate to high levels of fungal biomass despite having low C/N ratios (see Appendix S1: Table S5), a feature that may have reduced the strength of the relationship between soil C/N and our metric of microbial community composition. Additionally, the soil C/N ratio covaried with NDVI ($r = 0.49$), and thus including plant productivity in the SEM reduced the importance of soil C/N.

The NDVI-generated index of plant productivity was a central component of the SEM and allowed for a similar plant productivity metric to be compared from hundreds of sites at the continental scale within the same season. Few large scale studies have included remotely sensed plant productivity to provide data relative to changes in soil communities, though it is used in plant and animal ecological studies (Bailey et al. 2004, Pettorelli et al. 2005). Given that microbial communities and soil edaphic properties are so strongly tied to plant inputs, studies of microbial biogeography would benefit from remotely sensed measures of plant productivity, as they likely are a better indicator of belowground resource flux than is plant biomass or even plant community composition (Hamada et al. 2014).

Soil pH was an central factor in the SEM model, which is consistent with results of other large scale studies of microbial community composition and soil enzyme activities (Fierer and Jackson 2006, Sinsabaugh et al. 2008, Fierer et al. 2009). However, our study found that pH is not a direct driver of community composition, but rather contributes indirectly. Multiple processes in soils are a function of pH, from cation exchange to ATP membrane transport. However, the SEM suggests that pH also has strong effects on carbon resource supply to the microbial community. As soils become more alkaline, the ratio of microbial biomass to soil OC concentrations increase, indicating that the size of the bioavailable C pool may have increased (Fig. 4), which, from what we know from the SEM model, could drive changes in microbial community composition. In the SEM model, soil pH is a function of plant productivity and precipitation, but we were surprised that variables representing soil weathering and soil parent material were not important additional contributors to the model (especially as they affect pH), perhaps because they co-vary with the included variables. As reflected by its central role in the SEM model, soil pH is a master variable in many biogeochemical processes (e.g., organic matter hydroloysis, acid-base reactions in soil pore waters) and integrates several ecosystem-scale variables, including climate, microbial biomass, soil carbon, and plant productivity. It is likely for these reasons that pH has been observed to be such an important driver of soil microbial community composition.

The SEM model also revealed that soil mineralogy has an indirect influence on microbial community composition, through its effect on stabilizing soil organic matter. Few studies have shown relationships between mineral composition and microbial community composition (Moore et al. 2010), perhaps because mineral composition may not vary strongly at local or regional scales but also because soil mineralogy may not have a strong direct influence on community composition. At the large spatial gradients such as the continental transects utilized in this study, large variations in soil mineralogy are present, and the indirect influence on community composition could be observed through mineral composition influence on soil OC (Masiello et al. 2004, Rasmussen et al. 2005). It is important to highlight that the SEM model supports the idea that the mineral composition of soils is a direct stabilizing force on the long term stability of soil OC (Doetterl et al. 2015). Precipitation acts indirectly on soil OC through effects on weathering, soil pH, and plant productivity, and is an important component of ecosystem C dynamics globally (Carvalhais et al. 2015). The low ratio of PLFA/soil C in low pH/high carbon soils (Fig. 4), could cause the low rates of soil respiration per unit C in high C soils observed elsewhere (Doetterl et al. 2015), and a positive feedback to C stability in some systems.

Microbial community influences on soil enzyme activities is important because different microbial groups, most specifically fungi and bacteria, may differ in the types and amount of enzymes they produce, as well as their regulating factors (Sinsabaugh 2010, Strickland and Rousk 2010). Thus a greater understanding of the composition of soil microbial communities could presumably be of value in predicting soil enzyme potentials, which are often the rate limiting step in decomposition processes (Sinsabaugh et al. 2008, Sinsabaugh 2010). Soil enzyme activity is a product of pools of enzymes physically located on the soil matrix, so called 'abiontic enzymes, as well as those directly associated with intact microbial cells (Skujiņš and Burns 1976). Thus the enzyme potential measurements we make reflect both long-term microbial production of enzymes and the conditions that promote the stabilization of those produced enzymes in the soil matrix. The SEM model also supports the idea that soil enzymes are stabilized in the soil matrix and yet are also a reflection of the extant microbial community. Hydrolytic and oxidative enzyme activities were explained largely by microbial biomass, and less so by organic C or our "mineral weathering" variable, where many enzymes would likely be sorbed. This pattern may be reflective of the fact that microbial cells are the source of the most active enzymes and the location of most rapid C turnover, and enzymes sorbed to SOM and mineral surfaces play a minor role. Interestingly, although the composition of the soil microbial community was unrelated to hydrolytic activities, oxidative enzyme activities and enzymes involved in chitin degradation were related to microbial community composition. The microbial community metric PC1 was strongly influenced by the soil fungal biomarkers, which

can produce greater quantities of oxidative enzymes for lignin degradation and NAGase for chitin degradation (Sinsabaugh 2010, Strickland and Rousk 2010, Kluber et al. 2011). Thus, these data support the idea that although hydrolytic enzyme activities are largely controlled by the composition and abundance of C substrates, pH, and precipitation, oxidative and chitin degrading enzymes may also be influenced by variation in fungal abundance and/or composition. Given that new microbial-based carbon cycling models indicate that enzyme kinetics may be critical for understanding fate of carbon in soils globally (Wieder et al. 2013), it becomes increasingly important to understand what biological, edaphic, and climatic variables influence the enzymatic properties of soils.

This project integrates several large geochemical and microbial datasets across broad land use categories; however, there are several limitations to the collected data. First, PLFA biomarkers represent broad functional microbial groups and do not provide the taxonomic resolution present in 16S and 18S pyrosequencing approaches. Also, several variables that may have been important contributors to the model were not available, including soil orthography or elevation changes, seasonality, and land use history.

Although SEM presents an integrative model of hypothesized drivers controlling microbial communities, it does not present the entire picture. This study is primarily focused on large-scale patterns in microbial community composition, and only one-third of the variability in community could be explained by our model. At the local and regional scale, influences such as recent site history, land use, and plant community composition likely play an important role in structuring communities (Martiny et al. 2006, Drenovsky et al. 2009). Indeed, although the PLFA method has been a robust fingerprinting technique for comparing microbial communities among plant communities, soils, land uses, and climates (Bååth and Anderson 2003, Drenovsky et al. 2004, Brockett et al. 2012, Welc et al. 2012), we could not strongly differentiate microbial communities by land use or soil type (see Appendix S1). But given the large area covered by our transects, and the variability at that scale, perhaps that is not a surprising result. Additionally, fundamental ecological processes such as dispersal limitation, mutation, and drift were not considered in our study, and could help to explain some of the remaining variation in community composition (Petraitis et al. 1996, Martiny et al. 2011, Hanson et al. 2012).

In conclusion, SEM presents a way of visualizing how climate, soils, plants and microbial communities interact directly and indirectly at the continental scale. The patterns observed are supportive of what is currently known about microbial biogeography, and contribute new information about the strength of interactions among ecological variables in structuring microbial communities and their activities. This study underscores the importance of carbon resource availability and plant productivity in structuring soil microbial communities at the continental scale. It also highlights the central role soil pH plays in soil systems, both reflecting variation in climate and plant productivity, and affecting carbon resource supply to microbial communities. Finally, this work highlights that although hydrolytic and oxidative enzymes are a function of the microbial biomass, soil C, and precipitation, oxidative enzymes also seem to be affected by variation in community composition, in part due to changes in fungal abundance. Because soil enzymes are critical to carbon turnover, understanding variation in microbial community composition, particularly of fungi, could be used to modify potential rates of C cycling in mechanistic models. In future studies and synthesis activities, SEM has the potential to expand our understanding of the microbe-environment system (Bowker et al. 2010, Delgado-Baquerizo et al. 2013, Eisenhauer et al. 2015). Ideally, it would be used in combination with manipulative experiments (Wootton 1994, Gough and Grace 1999) to test hypotheses regarding which factors within a path diagram are vulnerable or resilient in response to climate or land use change.

Acknowledgments

This work was supported by the USDA Soil Processes grant 2007-35107-1782 as well as the USGS Geochemical Landscapes Program and the USGS Climate and Land Use R&D Program. We are grateful for the assistance all the researchers who assisted with soil sampling across the conterminous U.S. and Sabrina Sevilgen, Monica Haw, Cate Rychel, and Richard White III for laboratory assistance. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

Allison, S. D., T. Gartner, K. Holland, M. Weintraub, and R. L. Sinsabaugh. 2007. Soil enzymes: linking proteomics and ecological process. Pages 704–711 *in* C. J. Hurst, R. L. Crawford, J. L. Garland, D. A. Lipson, A. L. Mills, and L. D. Stetzenbach, editors. Manual of environmental microbiology, third edition.

Angel, R., M. I. M. Soares, E. D. Ungar, and O. Gillor. 2009. Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME Journal 4:553–563.

Auguet, J.-C., A. Barberan, and E. O. Casamayor. 2009. Global ecological patterns in uncultured archaea. ISME Journal 4:182–190.

Bååth, E., and T. H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35:955–963.

Bailey, S. A., M. C. Horner-Devine, G. Luck, L. A. Moore, K. M. Carney, S. Anderson, C. Betrus, and E. Fleishman. 2004. Primary productivity and species richness: relationships among functional guilds, residency groups and vagility classes at multiple spatial scales. Ecography 27:207–217.

Bossio, D., and K. Scow. 1998. Impacts of Carbon and Flooding on Soil Microbial Communities: Phospholipid Fatty Acid Profiles and Substrate Utilization Patterns. Microbial Ecology 35:265–278.

Bowker, M. A., F. T. Maestre, and C. Escolar. 2010. Biological crusts as a model system for examining the biodiversity–ecosystem function relationship in soils. Soil Biology and Biochemistry 42:405–417.

Briggs, P. H., and A. L. Meier. 2002. The determination of forty-two elements in geologic materials by inductively coupled plasma-mass spectrometry. *In* J. R. Taggart, Jr., editor. Analytical methods for chemical analysis of geologic and other materials, U.S. Geological Survey: U.S. Geological Survey Open-File Report 02-223, chap. I, p. I-1 through I-14.

Brockett, B. F. T., C. E. Prescott, and S. J. Grayston. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in Western Canada. Soil Biology and Biochemistry 44:9–20.

Brown, Z. A., C. Papp, E. Brandt, and P. Aruscavage. 2002. Carbonate carbon by coulometric titration. Pages 1–6. Open-File Report, U.S. Geological Survey.

Burns, R. G., J. L. DeForest, J. Marxsen, R. L. Sinsabaugh, M. E. Stromberger, M. D. Wallenstein, M. N. Weintraub, and A. Zoppini. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58:216–234.

Carvalhais, N., et al. 2015. Global covariation of carbon turnover times with climate in terrestrial ecosystems. Nature 514:213–217.

Decaëns, T. 2010. Macroecological patterns in soil communities. Global Ecology and Biogeography 19:287–302.

Delgado-Baquerizo, M., et al. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502:672–676.

Doetterl, S., et al. 2015. Soil carbon storage controlled by interactions between geochemistry and climate. Nature Geoscience 8:780–783.

Drenovsky, R. E., G. N. Elliott, K. J. Graham, and K. M. Scow. 2004. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. Soil Biology and Biochemistry 36:1793–1800.

Drenovsky, R. E., K. L. Steenwerth, L. E. Jackson, and K. M. Scow. 2009. Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecology and Biogeography 19:27–39.

Eberl, D. D., and D. B. Smith. 2009. Mineralogy of soils from two continental-scale transects across the United States and Canada and its relation to soil geochemistry and climate. Applied Geochemistry 24:1394–1404.

Eisenhauer, N., M. A. Bowker, J. B. Grace, and J. R. Powell. 2015. From patterns to causal understanding: structural equation modeling (SEM) in soil ecology. Pedobiologia 58:65–72.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences USA 103:626–631.

Fierer, N., M. S. Strickland, D. Liptzin, M. A. Bradford, and C. C. Cleveland. 2009. Global patterns in belowground communities. Ecology Letters 12:1238–1249.

Firestone, M. K., and E. A. Davidson. 1989. Microbiological basis of NO and N2O production and consumption in soil. Pages 7–21 *in* M. O. Andreae, and D. S. Schimel, editors. Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, Hoboken, New Jersey, USA.

Garrett, R. G., G. E. M. Hall, J. E. Vaive, and P. Pelchat. 2009. A water-leach procedure for estimating bioaccessibility of elements in soils from transects across the United States and Canada. Applied Geochemistry 24:1438–1453.

Gilbert, J. A., J. K. Jansson, and R. Knight. 2014. The Earth Microbiome project: successes and aspirations. BMC Biology 12:156.

Gough, L., and J. B. Grace. 1999. Effects of environmental change on plant species density: comparing predictions with experiments. Ecology 80:882–890.

Grace, J. B., T. M. Anderson, H. Olff, and S. M. Scheiner. 2010. On the specification of structural equation models for ecological systems. Ecological Monographs 80:67–87.

Grace, J. B., D. R. Schoolmaster Jr., G. R. Guntenspergen, A. M. Little, B. R. Mitchell, K. M. Miller, and E. W. Schweiger. 2012. Guidelines for a graph-theoretic implementation of structural equation modeling. Ecosphere 3:73.

Griffin, D. W., T. Petrosky, S. A. Morman, and V. A. Luna. 2009. A survey of the occurrence of Bacillus anthracis in North American soils over two long-range transects and within post-Katrina New Orleans. Applied Geochemistry 24:1464–1471.

Griffiths, B. S., K. Ritz, N. Ebblewhite, and G. Dobson. 1998. Soil microbial community structure: effects of substrate loading rates. Soil Biology and Biochemistry 31:145–153.

Hamada, Y., J. A. Gilbert, P. E. Larsen, and M. J. Norgaard. 2014. Toward linking aboveground vegetation properties and soil microbial communities using remote sensing. Photogrammetric Engineering & Remote Sensing 80:311–321.

Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. H. Martiny. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nature Reviews Microbiology 10:497–506.

Hemkemeyer, M., G. J. Pronk, K. Heister, I. Kögel-Knabner, R. Martens, and C. C. Tebbe. 2014. Artificial soil studies reveal domain-specific preferences of microorganisms for the colonisation of different soil minerals and particle size fractions. FEMS Microbiology Ecology 90:770–782.

Homer, C., J. Dewitz, J. Fry, M. Coan, N. Hossain, C. Larson, N. Herold, A. McKerrow, J. N. VanDriel, and A. J. Wickham. 2007. Completion of the 2001 National Land CoverDatabase for the Conterminous United States. Photogrammetric Engineering and Remote Sensing 73:337–341.

Jenkerson, C. B., T. K. Maiersperger, and G. L. Schmidt. 2010. eMODIS: A user-friendly data source. Open File Report 1055, U.S. Geological Survey.

Jonsson, M., and D. A. Wardle. 2010. Structural equation modelling reveals plant-community drivers of carbon storage in boreal forest ecosystems. Biology Letters 6:116–119.

Kluber, L. A., J. E. Smith, and D. D. Myrold. 2011. Distinctive fungal and bacterial communities are associated with mats formed by ectomycorrhizal fungi. Soil Biology and Biochemistry 43:1042–1050.

Kourtev, P. S., J. G. Ehrenfeld, and M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166.

Lauber, C. L., M. S. Strickland, M. A. Bradford, and N. Fierer. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40:2407–2415.

Maestre, F. T., et al. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Sciences 112:15684–15689.

Martiny, J. B., J. A. Eisen, K. Penn, S. D. Allison, and M. C. Horner-Devine. 2011. Drivers of bacterial β-diversity depend on spatial scale. Proceedings of the National Academy of Sciences USA 108:7850–7854.

Martiny, J. B. H., et al. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102–112.

Masiello, C. A., O. A. Chadwick, J. Southon, M. S. Torn, and J. W. Harden. 2004. Weathering controls on mechanisms of carbon storage in grassland soils. Global Biogeochemical Cycles 18:GB4024.

Miller, M., and R. P. Dick. 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biology and Biochemistry 27:1161–1166.

Mitchell, R. J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modelling. Functional Ecology 6:123–129.

Moore, J., J. L. Macalady, M. S. Schulz, A. F. White, and S. L. Brantley. 2010. Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. Soil Biology and Biochemistry 42:21–31.

Nemergut, D. R., et al. 2011. Global patterns in the biogeography of bacterial taxa. Environmental Microbiology 13: 135–144.

Petraitis, P. S., A. E. Dunham, and P. H. Niewiarowski. 1996. Inferring multiple causality: the limitations of path analysis. Functional Ecology 10:421–431.

Pettorelli, N., J. O. Vik, A. Mysterud, J.-M. Gaillard, C. J. Tucker, and N. C. Stenseth. 2005. Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends in Ecology & Evolution 20:503–510.

PRISM Climate Group. 2006. PRISM climate data. Oregon State University.

Rasmussen, C., M. S. Torn, and R. J. Southard. 2005. Mineral assemblage and aggregates control carbon dynamics in a California Conifer Forest. Soil Science Society of America Journal 69:1711.

Siciliano, S. D., et al. 2014. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. Soil Biology and Biochemistry 78:10–20.

Sinsabaugh, R. S. 1994. Enzymic analysis of microbial pattern and process. Biology and Fertility of Soils 17:69–74.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42:391–404.

Sinsabaugh, R. L., et al. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252–1264.

Skujiņš, J., and R. G. Burns. 1976. Extracellular enzymes in soil. CRC Critical Reviews in Microbiology 4:383–421.

Smith, D. B., L. G. Woodruff, R. M. O'Leary, W. F. Cannon, R. G. Garrett, J. E. Kilburn, and M. B. Goldhaber. 2009. Pilot studies for the North American Soil Geochemical Landscapes Project – site selection, sampling protocols, analytical methods, and quality control protocols. Applied Geochemistry 24:1357–1368.

Strickland, M. S., and J. Rousk. 2010. Considering fungal: bacterial dominance in soils – methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42:1385–1395.

Swets, D. L., B. C. Reed, J. D. Rowland, and S. E. Marko. 1999. A weighted least-squares approach to temporal NDVI smoothing. Proceedings of the 1999 ASPRS Annual Conference. ASPRS publications, Portland, Oregon, USA.

Tabatabai, M. A. 1994. Methods of soil analysis part 2. Microbiological and biochemical properties. Pages 775–833 *in* R. W. Weaver, P. S. Bottomly, and J. S. Angle, editors. Soil enzymes. SSSA, Madison, Wisconsin, USA.

Tedersoo, L., et al. 2014. Fungal biogeography. Global diversity and geography of soil fungi. Science 346:1256688.

Tucker, C. J., T. E. Goff, and J. Townshend. 1985. African landcover classification using satellite data. Science 227:369–375.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703–707.

Vogel, T. M., P. Simonet, J. K. Jansson, P. R. Hirsch, J. M. Tiedje, J. D. van Elsas, M. J. Bailey, R. Nalin, and L. Philippot. 2009. TerraGenome: a consortium for the sequencing of a soil metagenome. Nature Reviews Microbiology 7:252.

Waldrop, M. P., and M. K. Firestone. 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235–248.

Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36:1443–1451.

Waldrop, M. P., K. P. Wickland, R. White III, A. A. Berhe, J. W. Harden, and V. E. Romanovsky. 2010. Molecular investigations into a globally important carbon pool: permafrost-protected carbon in Alaskan soils. Global Change Biology 16:2543–2554.

Wallenstein, M. D., and E. K. Hall. 2011. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109:35–47.

Welc, M., E. K. Bünemann, A. Fließbach, E. Frossard, and J. Jansa. 2012. Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. Soil Biology and Biochemistry 49:184–192.

White, D. C., and D. B. Ringelberg. 1997. Utility of the signature lipid biomarker analysis in determining the in situ viable biomass, community structure, and nutritional/physiologic status of deep subsurface microbiota. Pages 119–136 *in* A. S. Penny, and D. L. Haldeman, editors. The microbiology of the terrestrial deep subsurface. CRC Press, New York, New York, USA.

Wieder, W. R., G. B. Bonan, and S. D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change 3:909–912.

Wootton, J. T. 1994. Predicting direct and indirect effects: an integrated approach using experiments and path analysis. Ecology 75:151–165.

Wu, T., E. Ayres, R. D. Bardgett, D. H. Wall, and J. R. Garey. 2011. Molecular study of worldwide distribution and diversity of soil animals. Proceedings of the National Academy of Sciences USA 108:17720–17725.

Wylie, B. 2003. Calibration of remotely sensed, coarse resolution NDVI to CO2 fluxes in a sagebrush–steppe ecosystem. Remote Sensing of Environment 85:243–255.

Zak, D. R., D. Tilman, R. R. Parmenter, C. W. Rice, F. M. Fisher, J. Vose, D. Milchunas, and C. W. Martin. 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75:2333–2347.