Ecology, 96(12), 2015, pp. 3374–3385

Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession

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Abstract. Although we understand the ecological processes eliciting changes in plant community composition during secondary succession, we do not understand whether co-occurring changes in plant detritus shape saprotrophic microbial communities in soil. In this study, we investigated soil microbial composition and function across an old-field chronosequence ranging from 16 to 86 years following agricultural abandonment, as well as three forests representing potential late-successional ecosystems. Fungal and bacterial community composition was quantified from ribosomal DNA, and insight into the functional potential of the microbial community to decay plant litter was gained from shotgun metagenomics and extracellular enzyme assays. Accumulation of soil organic matter across the chronosequence exerted a positive and significant effect on fungal phylogenetic β-diversity and the activity of extracellular enzymes with lignocellulolytic activity. In addition, the increasing abundance of lignin-rich C_4 grasses was positively related to the composition of fungal genes with lignocellulolytic function, thereby linking plant community composition, litter biochemistry, and microbial community function. However, edaphic properties were the primary agent shaping bacterial communities, as bacterial β-diversity and variation in functional gene composition displayed a significant and positive relationship to soil pH across the chronosequence. The late-successional forests were compositionally distinct from the oldest old fields, indicating that substantial changes occur in soil microbial communities as old fields give way to forests. Taken together, our observations demonstrate that plants govern the turnover of soil fungal communities and functional characteristics during secondary succession, due to the continual input of detritus and differences in litter biochemistry among plant species.

Key words: Cedar Creek Reserve, Minnesota, USA; metagenomics; microbial community assembly; old field; secondary succession; soil.

Introduction

Ecological succession describes changes in biotic community composition following a disturbance (Connell and Slatyer 1977). Although mechanisms causing changes in plant community composition during secondary succession have received much attention over several decades (e.g., Grime 1979, Tilman 1988), we do not understand the extent to which saprotrophic soil microbial communities change during secondary succession. It is well understood that, during the decay of an individual leaf, microbial communities change as biochemical constituents of detritus are differentially depleted over time (Voříšková and Baldrian 2013). It is plausible that changes in plant community composition and litter biochemistry during secondary succession could elicit a similar effect on the composition and function of soil microbial communities. For example, plant species differ in the production and biochemical composition of detritus; therefore, changes in plant community composition during secondary succession modify the nature of organic substrates entering soil (Paul and Clark 1996). If soil microorganisms possess ecological trade-offs analogous to other organisms (Martiny et al. 2012), then changes in the availability of growth-limiting substrates (i.e., plant detritus) for saprotrophic metabolism should cause soil microbial communities to change during the course of secondary succession due to competitive displacement (Bardgett et al. 2005). Therefore, changes in plant community composition during secondary succession should be paralleled by concomitant changes in soil microbial communities.

Plant litter is biochemically heterogeneous, composed of simple organic molecules (e.g., saccharides, amino acids, and nucleic acids), cellulose, and hemicellulose, as well as polyphenolic compounds such as lignin. Whereas bacteria and molds are competitive at extracting energy from mono- and polymeric sugars (Hudson 1968), the ability to degrade lignin is mainly conserved in the fungal subkingdom Basidiomycota (Baldrian 2006). Because a limited number of microorganisms are capable of degrading this polyphenolic molecule, lignin

Manuscript received 29 January 2015; revised 29 May 2015; accepted 15 June 2015. Corresponding Editor: S. D. Allison.
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in plant detritus regulates both decay rate and microbial community composition (Osono 2007, McGuire et al. 2010, Talbot et al. 2011). In a well-studied example of grassland plant succession, an increase in leaf litter lignin indicates a decline in resource availability for saprotrophic microorganisms (Knops and Tilman 2000, Quested et al. 2007) and provides a plausible mechanism by which plants may drive concomitant changes in soil microbial communities. If lignified plant detritus increases during secondary succession, then it should favor organisms with the physiological capability to metabolize these substrates, thereby altering the composition and function of soil microbial communities as secondary succession progresses.

While it is well established that microbial biomass, respiration, and net N mineralization increase during secondary succession (Zak et al. 1990, Waldrop et al. 2006), advances in molecular ecology enable us to characterize microbial composition and function across temporal scales. Recent evidence revealed that soil bacterial communities are influenced by geologic substrate age, soil properties, and plant community composition (Nemergut et al. 2007, Jangid et al. 2013). Despite their key functions in soil C and N cycling, our understanding of changes in fungal communities during plant succession lags farther behind (Jumpponen 2003, Cutler et al. 2014). Inasmuch, we have a limited understanding of the mechanisms that might link changes in plant and soil microbial communities across temporal and spatial extents.

To test these ideas, we investigated bacterial and fungal composition and functional potential across a series of nine old fields, ranging from 16 to 86 years since agricultural abandonment, as well as three adjacent forests representing potential late-successional ecosystems. We hypothesized that changes in soil microbial community composition and functional characteristics across the old-field chronosequence are structured by increasing soil organic matter content, a proxy for plant litter abundance, as well as root lignin content. If the succession of microbial communities is related to shifts in plant functional traits that influence the biochemistry of plant detritus, we expect that microbial functional potential will co-vary with microbial communities across the chronosequence. We quantified fungal and bacterial richness, as well as phylogenetic β-diversity in an established grassland chronosequence with well-documented changes in plant community composition. Additionally, shotgun metagenomics and extracellular enzyme assays enabled us to quantify the functional potential of saprotrophic communities to degrade components of plant detritus.

Methods

Study sites

We studied soil microbial communities in an old-field chronosequence located at the Cedar Creek Ecosystem Science Reserve (Bethel, Minnesota, USA; see Plate 1). This landscape was formed following the Wisconsin glacial retreat (ca. 12 000 BP); mean annual temperature is 6°C with annual precipitation of 66 cm. Nine fields were selected from 21 potential sites (Appendix B: Table B1; Knops and Tilman 2000), all occurring on soils derived from sandy glacial outwash. Soil in the 86-yr-old field was an Alfic Udipsamment; whereas, soils in the remaining fields and forests were Typic Udipsamments (Grigal et al. 1974). Plant communities across the old-field chronosequence primarily consisted of C_3 and C_4 grasses (Appendix B: Table B2), as well as forbs and legumes. Dominant C_3 grasses included *Poa pratensis* and *Agropyron repens*; whereas, *Andropogon gerardi*, *Schizachyrium scoparium*, and *Leptoloma cognatum* represented prevalent C_4 grasses. Dominant forbs in the old fields included *Rudbeckia serotina* and *Rumex acetosella*, and *Lespedeza capitata* represented the prevalent legume. We also sampled three forests in this landscape, including an oak savanna (OS), an upland pin oak forest (UPO; *Quercus ellipsoidalis*), and a northern hardwood (NH) forest, residing on the same soil parent material as the old fields. Differences in plant community composition between late-successional ecosystems primarily result from fire frequency and its influence on soil N availability (Zak et al. 1990).

Soil sampling

In each old field, we collected soil cores (3 cm diameter) to a depth of 5 cm from 20 randomly selected locations across two previously established transects (Knops and Tilman 2000). This produced 20 soil cores for each site, which totaled 240 soil cores for the old field and forest sites. In the forests, we established two parallel 40-m transects and randomly sampled at 10 points along each transect. Within each old field and forest, soil samples were composited and sieved (2 mm) to homogenize soil and remove roots. A 2-g subsample was removed for enzyme analysis and was stored at 4°C (Burns et al. 2013). Remaining soil samples were immediately frozen and transported to the University of Michigan (Ann Arbor, Michigan, USA) for molecular analyses.

Environmental characteristics

Soil and root characteristics were quantified in old fields and forests; whereas, aboveground plant biomass was quantified in the nine old fields (Appendix B: Table B1). A soil subsample from each old field and forest was sent to the University of Wisconsin Soil Laboratories (Verona, Wisconsin, USA) to quantity soil pH, organic matter (SOM), and total N. Briefly, soil pH was quantified in deionized water using a pH meter (ThermoFischer Scientific, Waltham, Massachusetts, USA). Organic matter was determined using a CNS2000 Analyzer (Leco, St. Joseph, Minnesota). Total soil N was measured colorimetrically following digestion in concentrated H_2SO_4 (Lachet Instruments, Loveland, Colorado, USA). Root biochemistry was characterized

by lignin, cellulose, hemicellulose, and total N (Goering and Van Soest 1970, Van Soest et al. 1991); details of these analyses are presented in Appendix A. In old fields, biomass of each plant species was measured by clipping and drying all aboveground plant material from designated plots. We assigned plant species to C_3 grasses, C_4 grasses, forbs, legumes, sedges, and woody plants, and calculated relative dominance of each functional group. A species list of C_3 and C_4 grasses and their relative dominances at each old field can be found in Appendix B: Table B2.

DNA extraction and community analysis

Using a PowerMax Soil DNA Isolation Kit (MoBio, Carlsbad, California, USA), total DNA was extracted from three replicate samples, removed from composite soil samples collected in each old field and forest. DNA was extracted from 5 g of soil and stored at −80°C until we initiated metagenome sequencing and PCR amplification. Targeted amplification of bacterial and fungal ribosomal genes was performed to characterize the composition of soil microbial communities. We analyzed fungal composition by targeting the 28S gene using primers LROR_F (5′-CCGCTGAACTTAAGCATAT CAATA-3′; Amend et al. 2010) and LR21 (5′-ACTTC AAGCGTTTCCCTTT-3′; Hopple and Vilgalys 1994). Fungal primers were selected to preferentially amplify Basidiomycota, organisms largely responsible for lignin metabolism in soil. We expected that changes in the biochemical constituents of plant detritus across the chronosequence would structure the composition of Basidiomycetes. To quantify bacterial community composition, the 16S ribosomal gene was targeted using primers 27f (5′-AGAGTTTGGATCMTGGCTCAG-3′) and 519r (5′-GWATTACCGCGGCKGCTG-3′; Lane 1991). Details of PCR protocols can be found in Appendix A.

Sequencing was performed on a PacBio-RS II system (Pacific Biosciences, Menlo Park, California, USA) utilizing circular consensus technology, which can generate 99.5–99.9% sequence accuracy for DNA fragments ranging from 150 to 500 base pairs (bp; Travers et al. 2010). PCR products were purified and quantified using MinElute PCR (Qiagen, Venlo, Netherlands) and PicoGreen dsDNA kits (ThermoFisher Scientific, Waltham, Massachusetts, USA). Two barcoded samples, pooled in equimolar concentration, were multiplexed per SMRT chip. Twelve SMRT chips were sequenced at the University of Michigan Sequencing Facility. Sequences were processed in Mothur using established pipeline procedures (Schloss et al. 2011). Fungal and bacterial sequences were each rarefied to 5919 sequences per site, according to the site that yielded the fewest number of sequences, to ensure equal sampling across all old fields and forests. Operational taxonomic units (OTUs) were clustered at 97% (16S) and 99% (28S) sequence similarity according to established procedures to target species designations of bacteria and fungi (Porter et al. 2008, Schloss et al. 2011). Sequencing coverage of fungal and bacterial communities was estimated using Good's coverage estimator (Good 1953). OTU taxonomic identity was determined using the Ribosomal Database Project (RDP) classifier, and species richness was assessed using the Chao1 estimator (Chao 1984, Wang et al. 2007). To calculate phylogenetic β-diversity, phylogenetic trees were constructed using FastTree 2 (Price et al. 2010), followed by calculation of weighted UniFrac distance between pairs of old fields and forests (Lozupone et al. 2006). Sequences were uploaded to the Sequence Read Archive under study Accession No. SRP045834.

Shotgun metagenomic analysis

To gain insight into the genetic potential of soil microbial communities to degrade plant detritus, sequences from shotgun metagenomes were assigned to functional genes involved in the decay of cellulose, chitin, galactose-containing oligosaccharides, lignin, pectin, starch, and xylan. Twelve libraries, from the nine old fields and three late-successional forests, were multiplexed and sequenced on four lanes of a HiSeq Illumina instrument (Illumina, San Diego, California, USA), with 150 bp reads. Bacterial sequences were assigned to 20 genes in seven substrate categories (Appendix A: Table A1) using the SEED model within MG-RAST (Meyer et al. 2008). Due to the overwhelming dominance of bacterial sequences in the MG-RAST databases, fungal genetic potential was analyzed following the creation of nine gene databases and six substrate categories (Appendix A: Table A2) created collectively from the Carbohydrate Active Enzyme database, Peroxibase, the Functional Gene Repository, and NCBI reference sequences (Fawal et al. 2013, Fish et al. 2013, Lombard et al. 2013, Tatusova et al. 2014). Using these resources, the taxonomic assignment of DNA sequences enabled the creation of fungal gene databases separate and distinct from bacteria. For each metagenome, abundance of genes involved in the decay of each substrate category (e.g., cellulose, lignin, xylan) was calculated following assignation of metagenome sequences to functional gene databases using the Blastn algorithm (Altschul et al. 1990). Bacterial and fungal gene assignment required 60% minimum sequence homology, E-value cut-off value of 1×10^{-5}, minimum alignment 40 bp, and gene assignments were standardized to the number of sequences with predicted functions (sensu Fierer et al. 2012). To investigate factors contributing to site differences in the composition of fungal and bacterial genes involved in the decay of the seven substrate categories, two Euclidean distance matrices were calculated. Metagenomes can be publicly accessed in the MG-RAST database via Project ID 5588.

Extracellular enzyme analysis

To calculate potential enzyme activity of soil communities, extracellular enzyme assays were conducted in

96-well plates, allowing between eight and 16 technical replicates per sample. To measure activity of β-1,4-glucosidase, cellobiohydrolase, β-xylosidase, and N-acetyl-β-glucosaminidase, we used 200 μmol/L methylumbellyferyl-linked substrates (Saiya-Cork et al. 2002). A 25-mmol/L L-dihydroxy-phenylalanine substrate was used to assay phenol oxidase and peroxidase activity; 25 μL H_2O_2 (0.12%) was included to assay peroxidase activity. Further details can be found in Appendix A. Enzyme activities were expressed as $\mu mol \cdot h^{-1} \cdot g^{-1}$. Phenol oxidase and peroxidase activity were combined for a single measure of lignolytic enzyme potential, and enzyme activities were square-root transformed. To quantify site variation in enzyme potential, a Euclidean distance matrix was calculated according to pairwise dissimilarity in the activity of five enzyme categories.

Statistical analysis

Univariate and multivariate statistics were employed to understand the factors contributing to changes in microbial community composition and functional potential across the old-field chronosequence. To identify changes in environmental characteristics during secondary succession, we used linear regression to explore the relationships between site age and SOM, soil N, and pH. We also quantified the relationship between the relative dominance of C_3 and C_4 grasses and time since agricultural abandonment, as well as root lignin : N. Permutational multivariate analysis of variance (PerMANOVA) tested whether differences in plant functional composition (i.e., the relative dominance of C_3 grasses, C_4 grasses, etc.) occurred between youngest and oldest abandoned fields. Regression analysis quantified relationships between fungal OTU richness and SOM, as well as bacterial OTU richness and soil pH. To visualize variation in soil microbial community composition, functional gene assemblages, and enzyme potential across old fields and forests, we employed principal coordinate analysis (PCoA). Further, Pearson correlation coefficients were calculated to quantify correlations between principal coordinate axes and individual taxa, gene abundances, and enzyme activity. To identify factors contributing to fungal and bacterial phylogenetic β-diversity between old fields, we quantified the relationship between principal coordinate axes of UniFrac ordinations and site age, SOM, soil pH, and C_4 grass relative dominance. Further, redundancy and distance-based redundancy analysis (RDA, db-RDA) determined the extent to which variation in microbial community composition and functional potential across the old-field chronosequence was related to *z*-transformed environmental variables (Legendre and Anderson 2006). To understand whether changes in the soil microbial community corresponded to variation in functional characteristics across the old-field chronosequence, Mantel tests quantified correlations between fungal and bacterial UniFrac site comparisons and Euclidean differences in functional gene assemblages and enzyme potential using the vegan package (Oksanen et al. 2013) in R (R Development Core Team 2013). Assumptions of linearity were verified prior to regression analysis. PerMANOVA determined significance of vectors after 9999 permutations, and forward-stepping selection determined the model significantly describing variation in microbial response variables ($\alpha < 0.05$; results were marginally significant at $\alpha < 0.10$) with the lowest AIC value. Statistical tests were conducted using the vegan package in R.

Results

Environmental characteristics

Soil and root analysis confirmed that soil properties across the old-field chronosequence changed through time (Appendix B: Table B1). Soil organic matter ($r^2 = 0.58$; $P = 0.018$) and soil N ($r^2 = 0.67$; $P = 0.007$) increased with site age; whereas, no relationship occurred between old-field age and soil pH ($r^2 = 0.13$; $P = 0.32$). No linear relationships occurred between the relative dominance of plant functional groups (e.g., C_4 grasses, forbs, etc.) and site age ($P = 0.16$–0.43). However, PerMANOVA revealed that the relative dominance of plant functional groups in fields comprising the early portion of our chronosequence (16–52 yr) was significantly different from the oldest fields (61–86 yr); wherein, a higher representation of C_3 grasses and forbs, as well as fewer C_4 grasses, occurred in the youngest old fields (pseudo-$F_{2,9} = 9.05$; $P = 0.024$). Further, lignin : N was positively correlated with C_4 relative dominance ($r^2 = 0.74$; $P = 0.003$). Taken together, these results indicate that the increased abundance of C_4 grasses across the old-field chronosequence occurred concomitantly with an increase in root lignin : N, evidence for a progressive change in litter biochemistry during secondary succession.

Taxonomic and phylogenetic composition of microbial communities

We used taxonomic and phylogenetic analyses to investigate environmental factors contributing to the turnover of the soil microbial community during secondary succession. Analysis of 71 028 non-chimeric fungal sequences resulted in 1851 unique sequences, with an average sequence length of 385 bp. A total of 1754 OTUs were identified at 99% 28S sequence similarity. Representing 88% of total sequences, primers preferentially amplified Basidiomycota (Edwards and Zak 2010), with Agaricales comprising the most abundant order. In decreasing order, the remaining OTUs were classified as *Incertae sedis* (4.9%), Chytridiomycota (3.0%), Glomeromycota (2.6%), Blastocladiomycota (0.72%), and Ascomycota (0.70%). Good's coverage estimates for 28S sequencing ranged from 0.76 to 0.85 across sites, indicating that sites were relatively well sampled. Analysis of 71 028 bacterial sequences resulted in 4968 unique sequences and 3407 bacterial OTUs clustered at 97% similarity, ranging from 440 to 530 bp in length. Despite identical sequencing effort, Good's coverage estimates for

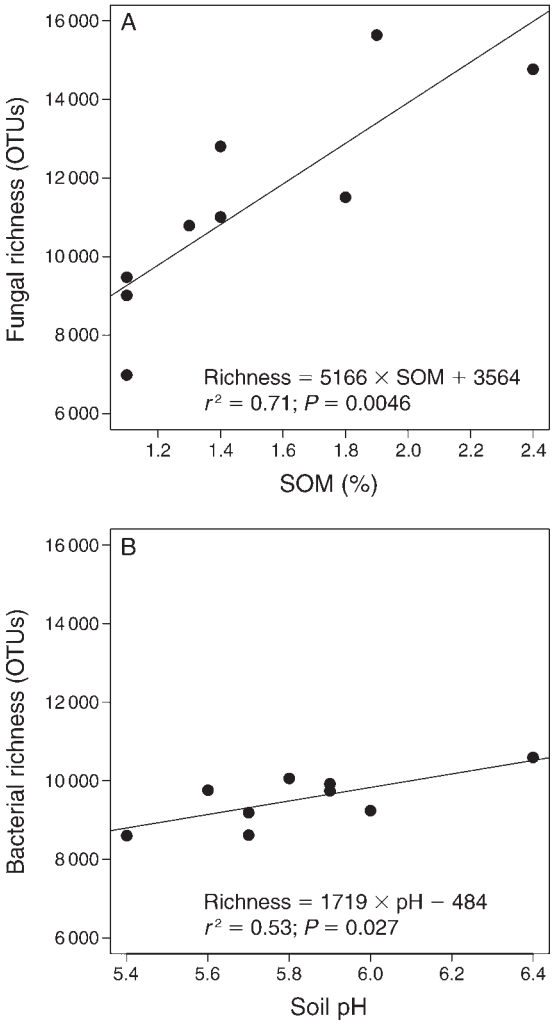

FIG. 1. Linear regression analysis of estimated (A) fungal and (B) bacterial operational taxonomic unit (OTU) richness. Estimated fungal OTU richness significantly ($P < 0.05$) increased with soil organic matter (SOM) content; whereas, bacterial OTU richness significantly increased with soil pH. OTUs were clustered at 99% (28S) and 97% (16S) sequence similarity. OTU richness was calculated by the Chao1 estimator.

bacteria were lower than fungi, ranging from 0.59 to 0.76 among sites. With decreasing abundance, the majority of bacterial OTUs fell into the phyla of Acidobacteria (26%), Proteobacteria (21%), Actinobacteria (20%), Bacteriodetes (6.1%), Gemmatimonadetes (3.8%), Planctomycetes (3.2%), and Verrucomicrobia (2.9%).

Fungal OTU richness estimates, calculated by the Chao1 indicator, ranged from 6990 to 15 635 OTUs, with highest richness occurring in the 61-yr old field and lowest in the youngest site (16 yr). Conversely, highest bacterial richness occurred in the youngest field (16 yr; 10 594 OTUs), whereas the oldest field had the lowest bacterial richness (86 yr; 8604 OTUs). Bacterial richness of forest sites ranged between 6033 and 8394 OTUs in the UPO and NH forests, respectively. Fungal OTU richness of the old fields was positively related to soil organic matter content (Fig. 1A; $r^2 = 0.71$; $P = 0.0046$); whereas, bacterial OTU richness was positively related to soil pH across the old fields (Fig. 1B; $r^2 = 0.53$; $P = 0.027$).

Taxonomic analysis of fungal and bacterial communities revealed changes in microbial composition across the old-field chronosequence, as well as distinct compositional shifts between old fields and late-successional forest ecosystems (Appendix C: Fig. C1). Of the 10 most abundant fungal families, those unclassified were negatively correlated to site age ($r^2 = 0.48$; $P = 0.037$) and the proportion of sequences assigned to Clavariaceae increased ($r^2 = 0.59$; $P = 0.016$). When comparing old fields to late-successional forests, the proportion of sequences classified as Russalaceae (36.3% ± 3.7% vs. 0.90% ± 0.88%, forest vs. old field, respectively) and Theleophoraceae (8.40% ± 1.02% vs. 0.27% ± 0.23%) were significantly higher in forests relative to old fields. Of the most abundant bacterial families, the proportion of sequences assigned to Acidobacteria Gp1 ($r^2 = 0.44$; $P = 0.053$) and Gemmatimonaceae ($r^2 = 0.44$; $P = 0.052$) negatively correlated to site age, albeit both relationships were marginally significant. Further, Acidobacteria Gp1 and Acidobacteria Gp2 decreased with increasing soil pH ($r^2 = 0.50$–0.62; $P < 0.010$); whereas, Chitinophagaceae and Gemmatimonadaceae increased with pH ($r^2 = 0.48$–0.53; $P = 0.0070$–0.013). Further, the proportion of 16S sequences classified as Acidobacteria Gp2 was higher in forests (6.5% ± 2.2%) than old fields (2.1% ± 0.29%); whereas, the relative abundance of Gemmatimonadaceae was lower in forests (2.1% ± 0.21%) than old fields (4.4% ± 0.41%).

Phylogenetic analysis of fungal and bacterial communities confirmed taxonomic trends in community turnover observed across the old-field chronosequence and forests. Calculated from weighted UniFrac distance, site comparisons in fungal phylogenetic distance varied from 30% to 78%, and bacterial pairwise differences ranged from 11% to 32%. Visualized in ordination space, phylogenetic differences between fungal communities formed three distinct clusters (Fig. 2A), whereby forests communities were differentiated from old fields on PCo1. The youngest old fields (16–52 yr) clustered from the oldest old fields (61–86 yr) along PCo2; linear regression revealed that PCo2 was inversely correlated with site age (Appendix C: Fig. C2; $P = 0.002$), SOM ($P = 0.001$), and C_4 grass relative dominance ($P = 0.016$). Bacterial communities in forests also differed from the old fields (Fig. 2B), as demonstrated by separation across PCo1. Interestingly, forest bacterial communities did not cluster as tightly as those for forest fungal communities. Bacterial phylogenetic β-diversity between old fields resulted in variation across PCo2, although neither ordination axis varied with site age.

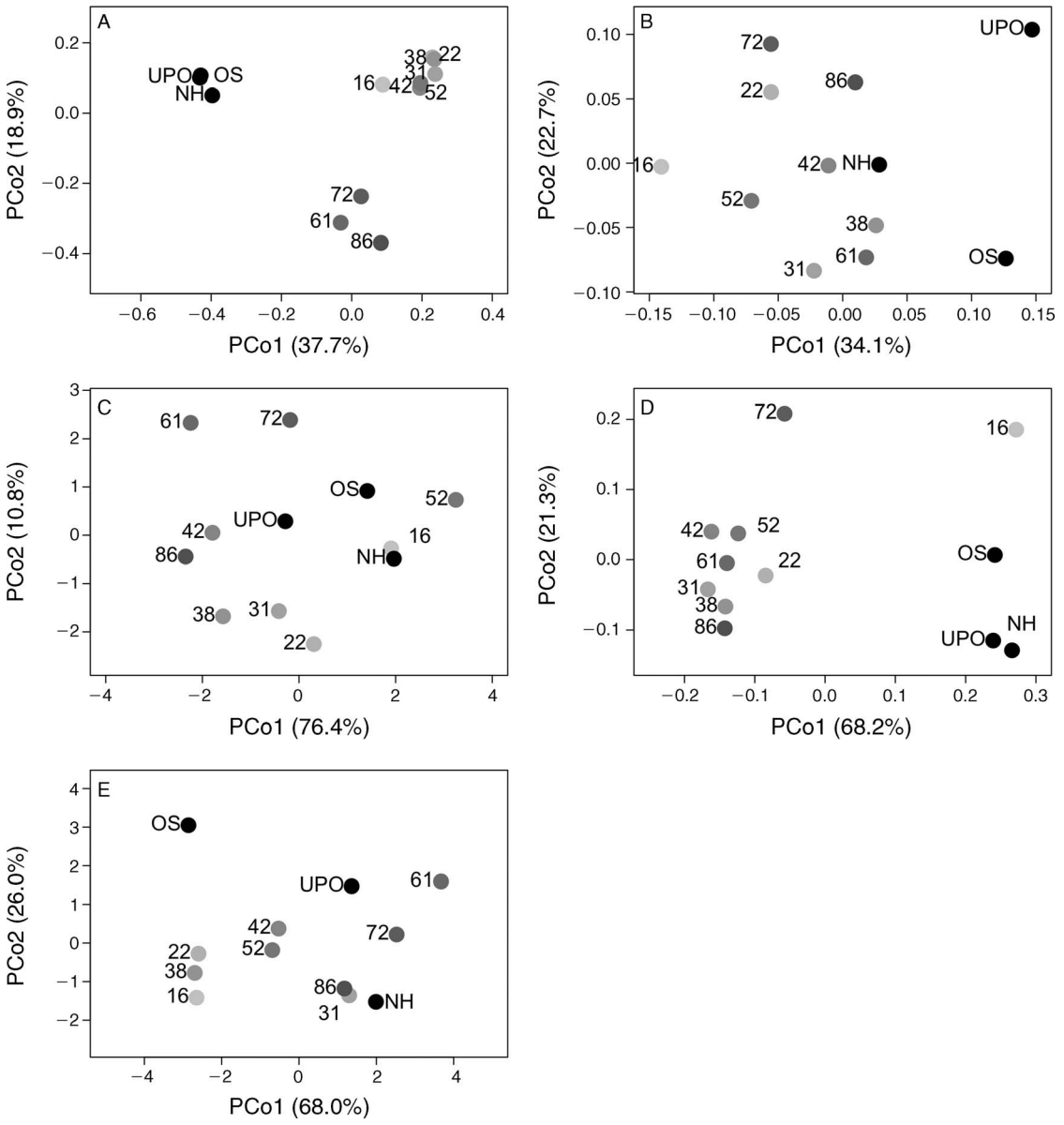

FIG. 2. Principal components analysis (PCoA) of (A) fungal phylogenetic composition, (B) bacterial phylogenetic composition, (C) fungal functional gene composition, (D) bacterial functional gene composition, and (E) soil enzyme potential. Phylogenetic distances were calculated by the weighted UniFrac distance metric. The Euclidean distance metric was used to calculate pairwise site differences in functional characteristics. Numbers indicate old-field age (years) and gray shading represents a gradient of field age, with young fields in light gray and older fields in dark gray. Three forested sites (northern hardwoods [NH], oak savanna [OS], and upland pin oak [UPO; *Quercus ellipsoidalis*]) are in black.

Factors structuring soil microbial β*-diversity*

We employed redundancy analysis to test the hypothesis that changes in the production and biochemistry of plant detritus shaped soil microbial communities across the successional chonosequence. SOM, soil pH, root cellulose, root lignin, and relative dominance of C_3 and C_4 grasses were included in the global model to account for variation in phylogenetic β-diversity in the nine old fields (Appendix C: Table C1). Results revealed that SOM best explained fungal phylogenetic β-diversity, accounting for 30% of the variation in pairwise UniFrac distance across the old-field chronosequence. Bacteria phylogenetic β-diversity responded to variation in soil pH, which accounted for 22% of the variation in UniFrac distance (Fig. 3A).

Factors structuring microbial functional potential

Microbial functional characteristics, quantified as the relative abundance of functional genes involved in litter decay and the potential activity of extracellular enzymes

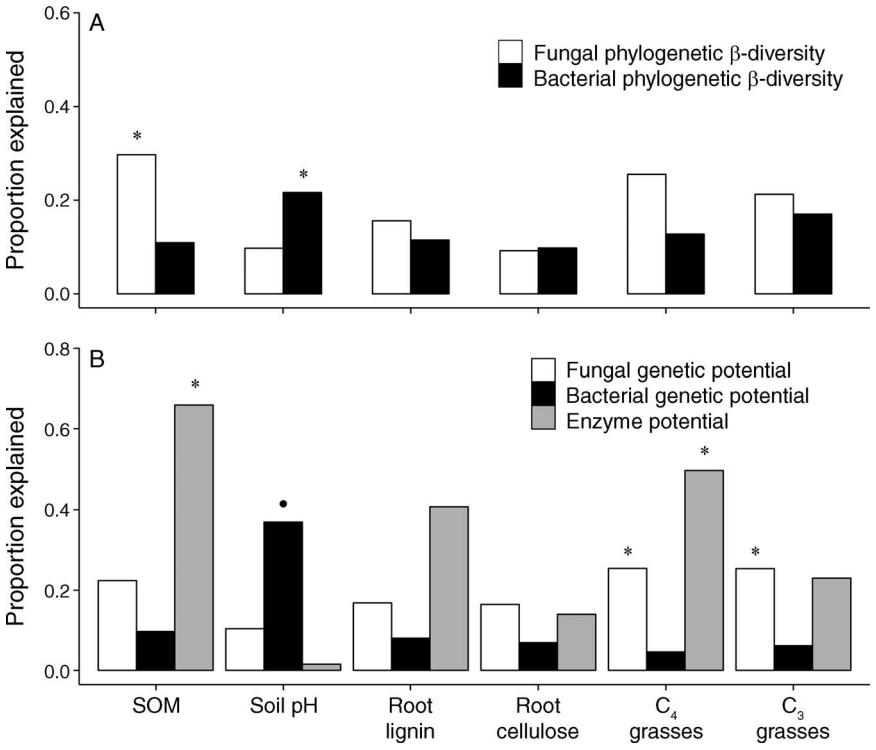

FIG. 3. Variation explained in microbial (A) phylogenetic β-diversity and (B) functional potential by individual variables included in redundancy analysis across the old-field chronosequence. Phylogenetic distances were calculated by the weighted UniFrac distance metric. The Euclidean distance metric was used to calculate pairwise site differences in functional characteristics. SOM represents soil organic matter content and relative dominance was calculated as proportion of total biomass; an asterisk denotes significance at $\alpha < 0.05$, a solid circle represents significance at $\alpha < 0.10$.

in soil, were hypothesized to increase with increases in resource availability (i.e., plant detritus production and biochemistry) during secondary succession. When considering individual gene categories, the relative abundance of fungal and bacterial genes encoding pectin degradation decreased with site age (Appendix C: Fig. C3; $r^2 = 0.54$–0.73; $P = 0.004$–0.028); whereas, no other substrate categories were related to time since agricultural abandonment ($P = 0.20$–0.53). With increasing soil pH across old fields, the relative abundance of bacterial genes involved in pectin degradation increased ($r^2 = 0.53$; $P = 0.026$) and bacterial lignolytic genes declined ($r^2 = 0.53$; $P = 0.025$). Total enzyme potential increased across the chronosequence ($r^2 = 0.47$; $P = 0.042$), a relationship that appeared to be driven by cellulolytic ($r^2 = 0.46$; $P = 0.046$) and xylanolytic ($r^2 = 0.87$; $P = 0.0003$) enzyme potential. Because we did not observe an increase in fungal or bacterial lignolytic gene abundance, or lignolytic enzyme potential with time since agricultural abandonment, these observations do not support the expectation for a greater microbial capacity to metabolize lignin as secondary succession progresses.

Multivariate comparisons of fungal genetic potential, bacterial genetic potential, and enzyme potential were calculated between sites using the Euclidean distance metric and ranged from 0.6% to 9.5%. PCoA visualizations of microbial functional characteristics across all 12 sites (Fig. 2C–E) illustrated that none of the forests served as functional end points for old-field succession. Fungal gene assemblages clustered in the center of ordination space in Fig. 2C, while old fields scattered across the full spectrum of PCo1 and PCo2. PCo1 correlated with fungal pectinolytic genes ($r = -0.89$; $P = 0.0001$) and fungal genes encoding the decay of galactose-containing oligosaccharides ($r = -0.72$; $P = 0.0071$). PCo2 was correlated to the relative abundance of fungal lignolytic genes ($r = -0.64$; $P = 0.025$) and fungal xylanolytic genes ($r = 0.74$; $P = 0.0056$). Bacterial gene assemblages of forest ecosystems were separated along PCo1 of Fig. 2D, an axis positively correlated with bacterial pectinolytic genes ($r = 0.75$; $P = 0.005$) and negatively correlated with bacterial lignolytic genes ($r = -1.00$; $P < 0.0001$). Separation of old fields along PCo2 was positively correlated with bacterial cellulolytic genes ($r = 1.00$; $P < 0.0001$) and negatively correlated with bacterial chitinolytic, xylanolytic, and starch-degrading genes ($r = -0.60$ to -0.92; $P = 0.0003$–0.041). Variation in enzyme potential along both PCoA axes of Fig. 2E indicated no predictable pattern of enzyme activity in the late-successional forests. PCo1 significantly correlated with an increase in β-1,4-glucosidase, cellobiohydrolase, N-acetyl-β-glucosaminidase, and β-1,4-xylosidase

PLATE 1. Aerial photograph of the Cedar Creek Ecosystem Science Reserve (Minnesota, USA) looking west across the northern part of our property. In the foreground are 27 experimental fields, and 24 more fields are apparent in the upper-left corner of the image. Photo credit: Jacob Miller.

($r = 0.60$–0.95; $P = 0.00001$–0.040). N-acetyl-β-glucosaminidase was also positively correlated with PCo2 of Fig. 2E ($r = 0.80$; $P = 0.0017$).

Multivariate redundancy analysis investigated the environmental factors that contributed to changes in functional potential of the soil microbial community across the old-field chronosequence (Fig. 3B; Appendix C: Table C1). Forward-stepping model selection revealed that C_4 grass relative dominance accounted for a large proportion of the variation in fungal functional gene composition (26%), providing support for the hypothesis that changes in plant communities structure the genetic capacity of fungal communities to decompose detritus. Considering Euclidean dissimilarities between bacterial gene assemblages, soil pH was marginally significant and accounted for 36% of variation across the old-field chronosequence. Supporting the hypothesis that plant succession influences the physiological attributes of soil microbial communities via changes in resource availability, SOM best accounted for variation in extracellular enzyme potential, capturing 66% of Euclidean differences in activity across old fields.

Link between community composition and functional potential

Mantel correlations tested the hypothesis that fungal and bacterial β-diversity were linked to variation in microbial functional potential across the old-field chronosequence. Analyses revealed fungal UniFrac distance was correlated with extracellular enzyme potential ($r = 0.44$; $P = 0.019$) and marginally related to site differences in the relative abundance of fungal genes, calculated by the Euclidean distance metric ($r = 0.33$; $P = 0.067$). We found a significant correlation between bacterial UniFrac distance and variation in bacterial functional gene composition ($r = 0.60$; $P = 0.002$), indicating that bacterial genetic potential was linked to bacterial phylogenetic relationships. However, we observed no relationship between bacterial UniFrac distance and extracellular enzyme potential ($P = 0.36$).

DISCUSSION

Our observations support the hypothesis that changes in fungal communities parallel changes in plant communities across the old-field chronosequence; whereas, edaphic properties appear to be a more important environmental filter for soil bacteria. Evidence consistent with these ideas comes from the fact that changes in SOM across the old-field chronosequence accounted for increased fungal OTU richness and fungal phylogenetic β-diversity. Secondly, concomitant changes in fungal gene assemblages with C_4 grass relative dominance indicate that changes in plant community composition across the old-field chronosequence selected fungi according to their physiological capacity to metabolize organic substrates in plant detritus. Moreover, an increasing root lignin : N ratio with C_4 grass dominance across the old-field chronosequence indicates that litter biochemistry is an important selective force underlying fungal community turnover. While soil bacteria were not sensitive to changes in plant communities, soil pH accounted for differences in bacterial richness, phylogenetic β-diversity, and the composition of bacterial genes

mediating litter decay. Lastly, corresponding changes in fungal functional gene assemblages and enzyme activity across the chronosequence provide evidence for the link between composition and function in soil microbial communities. Inasmuch, our observations partially support our overall hypothesis that saprotrophic microbial communities in soil are structured by changes in the biochemical composition of detritus as plant composition changes during secondary succession.

Plant-driven changes in resource availability shape fungal communities and their capacity to metabolize plant detritus

During old-field succession, changes in resource availability appear to structure the community composition of soil fungi. Increasing fungal OTU richness paralleled SOM accumulation (Fig. 1), suggesting that a greater number of fungal taxa were able to meet minimum resource requirements as resource availability increased over successional time (Tilman 1980, Waldrop et al. 2006). Because the late-successional forests had lower fungal OTU richness relative to the oldest fields, a unimodal relationship may exist between successional time and fungal richness due to the declining heterogeneity of plant detritus in later stages of secondary succession. A reduced range of organic substrates entering soils in late-successional forests, the result of increasing dominance of one or a few tree species, may limit the number of ecological niches to be filled by saprotrophic fungi (Zak et al. 2003, Meier and Bowman 2008). Secondly, variation in SOM content during old-field succession accounted for fungal phylogenetic turnover from youngest to oldest fields (Fig. 3), indicating that fungal community membership successively shifted with greater resource availability (Tscherko et al. 2004). Consistent with our observations, the correlation between fungal β-diversity and increasing soil C and N across a glacial chronosequence (Zumsteg et al. 2012) points to resource availability as an ecological driver of fungal succession. Further, the idea that saprotrophic soil fungi possess ecological trade-offs that influence their competitiveness for access to growth-limiting substrates is consistent with our observations. However, evaluating this expectation requires a detailed understanding of the physiological capabilities of individual fungal taxa in our study as well as interspecific interactions (Boddy 2000).

As a result of plant community turnover during old-field succession, changes in the abundance and biochemical composition of plant detritus appear to shape the functional potential of the fungal community to degrade organic substrates. For example, overall enzyme potential increased with SOM, suggesting that the accumulation of plant detritus through time supported a more active microbial community (Tscherko et al. 2004). This may be the result of an increase in fungal OTU richness or an increase in microbial biomass (Zak et al. 1990, Waldrop et al. 2006). Changes in plant litter biochemistry also appeared to structure fungal functional characteristics, as the relative dominance of C_4 grasses predicted temporal changes in the composition of fungal genes mediating litter decay. This link between plant functional groups and litter biochemistry is supported by our results, increased root lignin : N ratios with C_4 grass relative dominance, as well as reports of increased organic matter C:N with C_4 grass biomass (Knops and Tilman 2000, Quested et al. 2007).

Due to the increased dominance of C_4 grasses across the old-field chronosequence, we hypothesized that a greater abundance of lignified plant detritus provided a plausible force driving the competitive displacement of fungal taxa (Osono 2007, Voříšková and Baldrian 2013). Counter to expectations, root lignin content did not significantly account for changes in fungal gene assemblages across the old-field chronosequence, nor were increases in lignolytic gene abundance or enzyme potential observed. Instead, collective changes in plant litter biochemistry may be more important in structuring the capacity of the fungal community to metabolize plant detritus. For example, the observed decline in fungal and bacterial pectinolytic gene abundance across the chronosequence suggests that differences in pectin content among plant species during secondary succession could have consequences for the metabolic capacity of the soil microbial community. Taken together, our results suggest that plants underlie compositional changes in soil microbial communities during secondary succession via the continual input of detritus, as well as differences in litter biochemistry among plant species (van der Wal et al. 2013, Cutler et al. 2014).

Soil pH structures bacterial communities, and their genetic capacity to degrade organic material

Unlike fungi, bacterial OTU richness and phylogenetic β-diversity were influenced by changes in soil pH across the old-field chronosequence, indicating that edaphic factors are the primary agent structuring bacteria communities (Fierer and Jackson 2006, Rousk et al. 2010). Increasing soil pH resulted in greater OTU richness, results consistent with the idea that deviations in soil pH may introduce stress on single-celled organisms and limit the survival of taxa outside of their pH optimum (Kowalchuk et al. 2002, Fierer and Jackson 2006). Further, it is plausible that such a stress could alter competitive outcomes that drive bacterial community turnover. For example, changes in soil pH altered the abundance of Chitinophagaceae, Gemmatimonadaceae, and families assigned to Acidobacteria (Lauber et al. 2009, Bajerski and Wagner 2013). Providing support to this assertion, studies report that bacterial community turnover is structured by changes in geologic substrate age and soil pH, but are not sensitive to changes in plants across a successional sequence (Kuramae et al. 2011, Cutler et al. 2014). Yet, others observe that bacterial communities mirror changes in plant communities during succession (Mitchell et al. 2010). Because variation in soil

pH across the old-field chronosequence did not follow time since agricultural abandonment, our findings contrast observations of predictable changes in bacterial communities with successional time (Nemergut et al. 2007) and indicate that edaphic factors may alter the trajectory of bacterial succession.

Soil pH appears to also shape bacterial functional gene assemblages, indicating that consistent filters structure changes in bacteria community composition and genetic potential to degrade organic substrates. In addition to the altered abundance of genes involved in cellulose, pectin, and lignin decay, redundancy analysis provided evidence that soil pH has direct consequences on the bacterially mediated decay of organic substrates. It appears that soil bacteria are selected on account of pH tolerance, and corresponding changes in functional assemblages are the consequence of phylogenetically conserved traits in a changing bacterial community. However, further experimentation would be necessary to test this hypothesized mechanism.

Microbial community composition and functional potential: are there links?

Wide variation in phylogenetic dissimilarity between communities, yet a relatively narrow range of Euclidean differences in functional gene composition and enzyme potential, indicates the presence of a core set of metabolic capacities within fungal and bacterial communities (Burke et al. 2011, Talbot et al. 2014) and further suggests that soil microbial communities may exhibit a degree of functional overlap across the old-field chronosequence. Yet, significant correlations between variation in enzyme potential and fungal β-diversity support the idea that changes in fungal composition across the chronosequence have consequences for the capacity of the microbial community to decompose organic material. The lack of correspondence between bacterial β-diversity and enzyme potential indicates that bacteria comprise a relatively small fraction of the saprotrophic soil community in our chronosequence, because sandy, dry, and acidic soils favor fungi (Paul and Clark 1996, Zak et al. 2003). The marginally significant correlation between fungal function and composition suggests other functional traits may also be important in determining access to resources, such as growth efficiency, dispersal ability, and combative mechanisms of competition (Boddy 2000, Bissett et al. 2010). Quantifying a wider range of traits of bacterial and fungal communities is necessary to further understand the primary functional traits that shape soil microbial communities.

Forests as potential endpoints to the succession of microbial communities

Distinct differences between fungal and bacterial community composition beneath forests and old fields provides evidence that communities change substantially from the oldest old field (86 years) to late-successional forests (Fig. 2). The separation of fungal communities in forest ecosystems from those in old fields was driven by an increase in Russalaceae and Theleophoraceae, demonstrating the well-established trend toward increasing ectomycorrhizal associations in forests (Kranabetter et al. 2005, Twieg et al. 2007). While we expected to observe a gradual increase in ectomycorrhizal associations as grasslands gave way to forests, additional sampling of sites of early forest development would be necessary to test whether microbial communities in young forests would converge in composition and function as these forests age. The fungal gene assemblages beneath forests were largely nested within functional variation across the old-field chronosequence, providing further evidence that fungal communities have overlapping saprotrophic capabilities. Similarly, bacterial communities were also distinct in the late-successional forests, marked by an increase in Acidobacteria and lower abundance of Gemmatimonadaceae relative to old fields. However, blurred distinctions between old field and forest bacterial communities in ordination space may reinforce that bacteria are less sensitive to plant species differences (Cutler et al. 2014).

Conclusions

During secondary succession, we have demonstrated that plant-derived changes in litter biochemistry and abundance, hence substrate availability, underlie the turnover of soil fungal communities and their functional characteristics; whereas, bacterial communities respond to variation in soil pH. Distinct differences between old field and forest microbial communities further indicated that substantial turnover occurs during microbial succession, as old fields give way to forests in this landscape. We provide evidence that changes in fungal and bacterial communities have consequences for the genetic and enzymatic potential of the soil microbial community to harvest energy from organic substrates. Yet, smaller differences in the functional potential of saprotrophic bacterial and fungal communities relative to community turnover indicate some degree of overlapping functional abilities within soil communities, pertaining to the physiological capacity to metabolize plant detritus. Investigation of the relationship between genetic potential and gene regulation through meta-transcriptomics and meta-proteomics may be required to capture the mechanism linking microbial community composition and function. Nevertheless, our results indicate that changes in plant communities during secondary succession cause concomitant changes in the composition of fungal communities, which, in turn, have direct consequences for the decomposition of plant litter.

Acknowledgments

Research was made possible by support from the National Science Foundation's Graduate Research Fellowship Program and the School of Natural Resources & Environment at the University of Michigan. The authors thank Dave Tilman and Troy Mielke from the Cedar Creek Ecosystem Science Reserve,

as well as Rima Upchurch, Zac Freedman, and Karl Romanowicz for help in sampling and method development.

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Supplemental Material

Ecological Archives

Appendices A–C are available online: http://dx.doi.org/10.1890/15-0184.1.sm

Data Availability

Data associated with this paper have been deposited in the Sequence Reads Archive: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA259629/ and in the MG-RAST metagenomics analysis server: http://metagenomics.anl.gov/linkin.cgi?project=5588