Soil Biology & Biochemistry 42 (2010) 1816–1824

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains

Emily B. Hollister [a,*], Christopher W. Schadt [b], Anthony V. Palumbo [b], R. James Ansley [c], Thomas W. Boutton [a]

[a] Department of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843-2138, USA
[b] Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[c] Texas AgriLife Research, Vernon, TX 76384, USA

ARTICLE INFO

Article history:
Received 19 March 2010
Received in revised form
8 June 2010
Accepted 29 June 2010
Available online 14 July 2010

Keywords:
Woody plant encroachment
Soil microbial diversity
Functional diversity
Mesquite
GeoChip

ABSTRACT

In the southern Great Plains (USA), encroachment of grassland ecosystems by *Prosopis glandulosa* (honey mesquite) is widespread. Mesquite encroachment alters net primary productivity, enhances stores of C and N in plants and soil, and leads to increased levels of soil microbial biomass and activity. While mesquite's impact on the biogeochemistry of the region is well established, it effects on soil microbial diversity and function are unknown. In this study, soils associated with four plant types (C_3 perennial grasses, C_4 midgrasses, C_4 shortgrasses, and mesquite) from a mesquite-encroached mixed grass prairie were surveyed to in an attempt to characterize the structure, diversity, and functional capacity of their soil microbial communities. rRNA gene cloning and sequencing were used in conjunction with the GeoChip functional gene array to evaluate these potential differences. Mesquite soil supported increased bacterial and fungal diversity and harbored a distinct fungal community relative to other plant types. Despite differences in composition and diversity, few significant differences were detected with respect to the potential functional capacity of the soil microbial communities. These results may suggest that a high level of functional redundancy exists within the bacterial portion of the soil communities; however, given the bias of the GeoChip toward bacterial functional genes, potential functional differences among soil fungi could not be addressed. The results of this study illustrate the linkages shared between above- and belowground communities and demonstrate that soil microbial communities, and in particular soil fungi, may be altered by the process of woody plant encroachment.

1. Introduction

Land use and land cover changes frequently alter key aspects of ecosystem structure and function, affecting above- and belowground processes and contributing to global change (Houghton and Goodale, 2004; Jarnagin, 2004; Foley et al., 2005; Berthrong et al., 2009). One of the most widespread of these changes is that of woody plant encroachment into grassland ecosystems (Van Auken, 2000; Archer et al., 2001; Jackson et al., 2002; Liao et al., 2006). The encroachment of *Prosopis glandulosa* Torr (honey mesquite) into the grasslands of the southern Great Plains (USA) has contributed to extensive changes in the productivity and biogeochemistry (Archer et al., 2001; Asner et al., 2003) of the region. While the effects of mesquite encroachment have been shown to vary and can be altered by precipitation regime (Jackson et al., 2002), mesquite encroachment typically leads to enhanced levels of above- and belowground plant biomass (Hibbard et al., 2001; Asner et al., 2003), and altered rates of net primary productivity (Hughes et al., 2006). Mesquite, a species capable of symbiotic nitrogen fixation, establishes extensive root networks (Heitschmidt et al., 1988; Hibbard et al., 2001), creates zones of carbon- and nitrogen-enriched soil directly beneath its canopy (Tiedeman and Klemmedson, 1973; Dai et al., 2006), and contributes to the accumulation of biochemically recalcitrant compounds in the soil, including cutin and suberin, as well as the syringyl and vanillyl forms of lignin (Boutton et al., 2009).

In other ecosystems, above- and belowground communities have been shown to be linked to one another through strong feedback interactions (Wardle et al., 2004; Ehrenfeld et al., 2005;

* Corresponding author. Present address: Department of Soil and Crop Sciences, Texas A&M University, 370 Olsen Blvd, College Station, TX 77843-2474, USA. Tel.: +1 979 845 5695; fax: +1 979 845 0456.
E-mail addresses: ehollister@tamu.edu (E.B. Hollister), schadtcw@ornl.gov (C.W. Schadt), palumboav@ornl.gov (A.V. Palumbo), r-ansley@tamu.edu (R. James Ansley), boutton@tamu.edu (T.W. Boutton).

0038-0717/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2010.06.022

Kardol et al., 2007), and changes in plant community composition frequently alter the biomass and activity, composition, and/or functional capacity of their associated soil microbial communities (Smalla et al., 2001; Wallenstein et al., 2007; Marchante et al., 2008; Berthrong et al., 2009). Soil microbial biomass and activity typically increase following mesquite encroachment into Great Plains grasslands and the desert ecosystems of the southwestern USA (McCulley et al., 2004; Schade and Hobbie, 2005). However, the degree to which these increases are also accompanied by changes in the biodiversity and/or functional capacity of the soil microbial community remains unclear.

In recent years, microarray technology has emerged as a powerful tool for the detection and analysis of gene abundance and expression patterns, including those of functional genes. Microarrays provide a specific, sensitive, high-throughput tool through which thousands of genes can be detected simultaneously (Gentry et al., 2006; Loy et al., 2006). Functional gene arrays (FGA) are microarrays that contain probes for functional genes, such as those that might be involved in biogeochemical cycling and other environmental processes (Wu et al., 2001). The FGA used in this study is known as the GeoChip [version 2.0, (He et al., 2007)]. It is composed of 50-mer oligonucleotide probes and contains over 24,000 gene probes, representing more than 10,000 genes and 150 functional groups known to be involved to carbon, nitrogen, phosphorus and sulfur cycling, as well as the degradation of organic contaminants and metal reduction/resistance (He et al., 2007).

The purpose of this study was to characterize the structure and potential functional capacity of soil bacterial and fungal communities occurring in association with the four dominant vegetation types of a mesquite-encroached mixed grass prairie ecosystem of the southern Great Plains (i.e., mesquite, C_3 perennial grasses, C_4 midgrasses, and C_4 shortgrasses). Given the dramatic changes to soil C and N pools that typically occur following mesquite encroachment, we hypothesized that both the soil bacterial and fungal communities under mesquite would differ significantly from those associated with the grassland vegetation types. More specifically, we expected that the mesquite microbial community would harbor increased abundances of nitrogen-fixing and/or ammonia oxidizing bacteria, as well as enhanced levels of *Basidiomycota* and other fungi associated with the degradation of lignocellulose and other recalcitrant carbon compounds. We also hypothesized that the soil microbial community under mesquite would have a functional gene profile that differed significantly from those associated with the grasses, and we expected that the greatest differences in functional gene profiles would be found with respect to genes involved in ammonia oxidation, nitrification, and the metabolism of complex or recalcitrant carbon compounds.

2. Materials and methods

2.1. Study site

This study was conducted in a southern Great Plains mixed grass prairie ecosystem, located near Vernon, TX (33°51′20″ N, 99°26′50″ W). It was carried out in a series of replicated ($n = 3$), ungrazed sites (1–6 ha each) which contained patches of each of the four major plant functional types that dominate the vegetation of this region (further description below). Mean annual precipitation at this location is 665 mm, and the mean annual air temperature is 16.1 °C, with monthly average extremes ranging from 36 °C in the summer to −2.5 °C in the winter (Ansley et al., 1990).

Soils at these sites are classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series, with 0–1% slope (Soil Survey Staff, 2007). These clay loam surface soils (0–10 cm) have a particle size distribution of 32% sand, 52% silt, and 16% clay, and their pH values range from 7.0 to 7.2 (Ansley et al., 2006).

The plant community is comprised of four major functional groups, each differing in growth form, phenology, productivity (Ansley et al., 2004), and tissue chemistry (Levang-Brilz and Biondini, 2003). The herbaceous layer is a mixture of C_3 perennial grasses, C_4 midgrasses, and C_4 shortgrasses, as well as occasional forbs, and the over story is dominated by mesquite. *Nassella leucotricha* (Trin. & Rupr.) Pohl (Texas wintergrass) is the dominant C_3 perennial grass, and the dominant C_4 grasses include the midgrasses *Panicum obtusum* Kunth (vine mesquite) and *Sporobolus compositus* (Poir.) Merr. (meadow dropseed), as well as *Buchlöe dactyloides* Nutt. (buffalograss), a stoloniferous shortgrass species.

2.2. Soil sampling

In August 2005, soil cores were collected from two different patches of each vegetation type within each of the replicate plots described above ($n = 6$ cores vegetation type^{-1} among all replicate plots). Surface litter was gently brushed away and cores of mineral soil (2.5 cm diameter) were collected to a depth of 10 cm. Soil samples were stored on ice in the field and then at −20 °C upon return to the laboratory. Prior to nutrient analysis and DNA extraction, each core was weighed, and cores were combined to create three composite soil samples per plant functional type ($n = 12$ overall, each representing a single composite sample vegetation type^{-1} plot^{-1}). In order to quantify root biomass, an additional 4 soil cores per plant functional type were collected within each of the three replicate plots. These were collected as described above and were stored at 4 °C until analysis.

2.3. Soil organic carbon, soil total nitrogen, bulk density, and root biomass

Subsamples of each of the soil composites were saved for soil organic carbon (SOC) and nitrogen analysis and the quantification of soil bulk density. The subsamples used for SOC and soil total nitrogen analysis were dried for 48 h at 60 °C, passed through a 2-mm sieve to remove larger organic materials, pulverized in a centrifugal mill (Angstrom, Inc., Bellville, MI, USA), and analyzed for C and N concentrations with a Carlo Erba NA-1500 CHN analyzer (Carlo Erba Strumentazione, Milan, Italy). These surface soils have a neutral pH and tested negative for the presence of $CaCO_3$ (data not shown). As such, output from their direct analysis yielded % SOC. A second subsample of each soil composite was weighed both before and after drying at 105 °C for 24 h to determine gravimetric water content, which was utilized to correct whole soil core weights for bulk density. Root biomass was quantified from individual soil cores using a hydropneumatic elutriation system (Smucker et al., 1982) equipped with a 410 μm screen (Gillison's Variety Fabrication, Inc., Benzonia, MI, USA). Roots were dried at 60 °C for 48 h and then weighed.

Differences in bulk density, SOC, soil total nitrogen, and root biomass in the soils beneath each of the plant functional groups were analyzed using analysis of variance (ANOVA) (SPSS v 12.0.2, SPSS Inc, Chicago, IL, USA). Values were log-transformed where necessary to meet assumptions of normality and equality of variance. Post-hoc analysis was conducted using the Bonferroni procedure, and p-values ≤ 0.05 were considered to represent significant differences.

2.4. DNA extraction and purification

Soil samples collected beneath each plant functional group were combined in equal parts from each of the three replicate sampling

sites to create a single composite soil sample for each vegetation type, and the DNA extracted from each of these composites samples was used for clone library construction. Additional DNA was extracted from each of the 12 soil samples (4 vegetation types × 3 replicate plots), individually, for microarray analysis. DNA was extracted from soil samples using previously described methods (Zhou et al., 1996), purified using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA), and quantified with an ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE, USA).

2.5. Clone libraries

2.5.1. Bacterial 16S rRNA and fungal ITS/LSU amplification

The 16S rRNA gene was PCR amplified from community DNA samples using the general bacterial primers 27F and 1492R (Lane, 1991). Each 25 μl reaction contained 100 ng template DNA, 50 mM KCl, 30 mM Tris (pH 8.3), 4.0 mM $MgCl_2$, 25 μg bovine serum albumin, 200 μM each dNTP, 0.1 μM each primer, and 1.25 U *Taq* polymerase. Thermocycling was conducted in a GeneAmp PCR System 9700 (PerkinElmer Applied Biosystems, Norwalk, CT, USA) under the following conditions: (i) initial denaturation at 95 °C for 1 min; (ii) 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, 30 s; and (iii) a final extension at 72 °C for 7 min.

A portion of the fungal rRNA gene, containing the entire internal transcribed spacer (ITS) region as well as a portion of the large ribosomal subunit (LSU), was PCR amplified from community DNA samples using fungal-specific primers ITS9 (Egger, 1995) and LSU 1221R (Schadt et al., 2003). Each 25 μl reaction contained 200 ng template DNA, 50 mM KCl, 30 mM Tris (pH 8.3), 4.25 mM $MgCl_2$, 25 μg bovine serum albumin, 200 μM each dNTP, 0.5 μM each primer, and 1.875 U *Taq* polymerase. Thermocycling was conducted in an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: (i) initial denaturation at 95 °C for 2 min; (ii) 30 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 55 s, and extension at 72 °C for 1 min and 45 s (+2 s/cycle); and (iii) a final extension at 72 °C for 10 min.

In order to minimize PCR biases and artifacts, for both the 16S and ITS/LSU amplifications, five replicate amplifications were performed for each sample, their products combined for downstream use, and the number of PCR cycles was limited to 30.

2.5.2. Cloning and sequencing

The replicate PCR products from each original sample were pooled and then visualized on a 1.2% (w/v) low melting point agarose gel. Bands of approximately 1.5 and 2.0 kb in size were excised from the gel for the 16S and ITS/LSU PCR products, respectively, and they were purified using a QiaQuick gel extraction kit (Qiagen, Germantown, MD, USA). Purified PCR products were then cloned using a TOPO TA PCR-2.1 cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's directions, except that the use of the salt solution was omitted. Transformants containing the 16S and ITS/LSU PCR products were selected, 10 uL of cells were then suspended in 10 mM Tris (pH 8.0) and lysed at 99.9 °C for 15 min. Vector primers M13F and M13R were used to amplify the inserts according to the cloning kit manufacturer's protocol (Invitrogen).

The M13 PCR products were purified using Montage Multiscreen filter plates (Millipore, Billerica, MA, USA) and were prepared for sequencing with a Big Dye v3.1 sequencing kit (Applied Biosystems). Primers 27F, 515F, 519R, and 1100R (Lane, 1991) were used to create overlapping sequence reads for the bacterial clones, and primers ITS9 and ITS4 (White et al., 1990) and LROR (Rehner and Samuels, 1994) were used to create overlapping reads for the fugal clones. Sequencing was performed on an ABI 3730 sequencer (Applied Biosystems) at Oak Ridge National Laboratory and at the Arizona Research Laboratories, Division of Biotechnology, Genomic Analysis and Technology Core Facility (http://gatc.arl.arizona.edu).

The bacterial 16S sequences generated here were submitted to GenBank under accession numbers EU051919 through EU052211, and the fungal ITS/LSU sequences were submitted under accession numbers EU489881 through EU490184.

2.5.3. Estimating diversity and richness, comparing community structure

Sequence data were quality checked using Q_{20} scores, compiled, and manually edited in Sequencher (GeneCodes, Ann Arbor, MI, USA). Those 16S contigs that were shorter than 623 bp in length or failed to assemble were discarded from further analysis, as were any fungal contigs that did not contain the full ITS region as well as the first 455 bp of the LSU. Clone sequences were checked for chimeras using Bellerophon (Huber et al., 2004). 16S sequences meeting the minimum length requirement described above were used for this analysis, but due to the difficulties associated with aligning the ITS region and the requirement for Bellerophon to work off of a sequence alignment, only the LSU was used for chimera detection among the fungal clones. Sequences that were identified as potentially chimeric were excluded from further analysis. The Classifier function of the Ribosomal Database Project (Wang et al., 2007) was used for broad taxonomic characterization of the bacterial clone libraries, and BLAST searches (Altschul et al., 1990) of both the ITS and LSU regions versus GenBank were used for taxonomic characterization of the fungal libraries. Those fungal sequences that produced strong matches to non-fungal sequences (e.g. ciliates, algae) were also removed from the data set.

Clone libraries were further characterized to determine the degree of diversity present within, and the degree of similarity occurring between, the microbial communities present under each plant type. 16S sequences were used for all bacterial analyses and statistical tests. However, among the fungi, the ITS sequences were used to delineate fungal operational taxonomic units (OTUs) and calculate their associated diversity indices (Taylor et al., 2008), and LSU sequences were used to construct phylogenetic trees and conduct the parsimony test among the fungal communities.

In separate analyses, 16S and LSU sequences were aligned using Clustal X (Thompson et al., 1997) and trimmed to a common length. Distance matrices were constructed in PHYLIP (Felsenstein, 2004) using the DNA-DIST function and the Jukes-Cantor evolution model.

OTUs, defined at the 97% similarity level, were identified using MOTHUR v1.10 (Schloss et al., 2009) for the 16S sequences, and CAP3 (Huang and Madan, 1999) was used to cluster the ITS sequences at the same similarity level (Taylor et al., 2008). MOTHUR was also used to calculate diversity indices, richness estimates, and rarefaction values, based upon the presence and abundance of OTUs in each of the clone libraries. The Shannon (H') and Simpson ($1/D$) indices were used to establish relative rankings of diversity levels among clone libraries, and Chao I was used to provide estimates of species richness that might be expected if more exhaustive sampling were to occur. Nonmetric multidimensional scaling (NMDS) of the communities, based upon their OTU composition, was carried out using the PAST software program (Hammer et al., 2001) and the Bray–Curtis similarity coefficient in order to visualize relative similarities and differences among the bacterial and fungal communities.

The parsimony test, as implemented by MOTHUR, was used to assess whether differences in the structure of the vegetation-based microbial communities, as inferred by phylogenetics, could be attributed to random variation (i.e., by chance) or some other factor (e.g. changes in vegetation). Sequence data was pooled across clone

libraries to create community-wide phylogenetic trees for the 1) bacterial 16S and 2) fungal LSU data sets, and trees were constructed using the DNA-ML (maximum likelihood) function in PHYLIP (Felsenstein, 2004). MOTHUR's parsimony function was used to generate both a parsimony score and a random probability distribution for each of the phylogenetic trees. P-values of less than 0.05 were considered to be statistically significant. Analogous to an ANOVA, pairwise comparisons of individual clone libraries were performed only if significant differences were detected at the whole community level first.

2.6. GeoChip (version 2.0) functional gene array

2.6.1. Amplification and labeling

Rolling circle amplification of soil community DNA samples was performed using a TempliPhi 500 amplification kit (Amersham Biosciences, Piscataway, NJ, USA) (Wu et al., 2006). Amplification was confirmed by visualizing products on a 1% agarose gel under UV light, and the amplified DNA was fluorescently labeled with Cy5-dUTP using a random priming method (2004). Labeled DNA products were then transferred immediately to a QIAquick PCR purification column (Qiagen) and purified according to the manufacturer's instructions. Cy5-dUTP dye incorporation was quantified and evaluated by measuring absorption (A_{260}, A_{280}, and A_{650}) of labeled samples using ND-1000 spectrophotometer (Nanodrop Technology). Following purification and quantification, samples were concentrated using a vacuum centrifuge (Savant Instruments Inc, Holbrook, NY, USA) and stored at −20 °C until hybridization.

2.6.2. Hybridization

All hybridizations were carried out in duplicate, and each of them was prepared and processed independently. Microarray hybridization, incubation, and post-hybridization washing were carried out as described by Rhee et al. (2004) with the following modifications as suggested by Wu et al. (2006): (a) An alternate hybridization solution (containing 50% formamide; 5X SSC (1X SSC is equal to 150 mM NaCl plus 15 mM trisodium citrate); 0.10% SDS; 4 μg unlabeled herring sperm DNA; 0.33 μl 1 mM spermidine; and ddH_2O in a total volume of 40 μl) was used in place of the hybridization solution described by the authors; and (b) 1.6 μl of RecA (490 μg ml^{-1}) were added to each hybridization mixture just prior to the application of samples to the arrays.

Following incubation, each microarray slide was removed from its hybridization chamber, and its cover slip was removed by immersion in a pre-warmed (50 °C) washing buffer (1× SSC and 0.01% SDS). Arrays were washed in a series of buffers of decreasing stringency, beginning with 5 min at 50 °C in a fresh aliquot of the buffer above, a second washing in 0.1× SSC and 0.1% SDS for 10 min at room temperature, and a final series of 4 washes in 0.1× SSC for 1 min at room temperature with gentle shaking. Arrays were dried using compressed N_2, followed by centrifugation at 500×g in a clean, padded slide box, at room temperature.

2.6.3. Image processing and data analysis

A ScanArray Express microarray analysis system (Perkin–Elmer, Wellesley, MA, USA) was used to scan the microarrays at a resolution of 10 μm. Scans were processed using the ImaGene 6.0 software package (BioDiscovery, El Segundo, CA, USA). The mean hybridization signal intensity for each spot was quantified, and local background signals were subtracted from the signal of each spot. Poor-quality hybridization spots were automatically flagged by ImaGene, and additional spots were removed based upon their signal to noise ratio (SNR). SNR was calculated as (mean signal intensity − mean background intensity)/standard deviation of the background (Wu et al., 2006), and spots with SNR values <2 were excluded from further analysis (Loy et al., 2002).

Signal intensity values were normalized by mean, and outliers were removed according to He et al. (2007). Data from each of the technical replicates were combined and gene detection was considered successful if a positive hybridization signal for a given probe occurred across both of the technical replicates. Normalized signal intensity values were then averaged across technical replicates and used in downstream analysis. The scope of this study is limited to the functional genes contained in the "A probe" portion of the array (summarized in Table 4) which differs from that reported in He et al. (2007) in that it lacks the additional multiple probe variants for each gene, but rather contains the single "best" probe in terms of predicted specificity for each gene, that are replicated on each array slide.

ANOVA was used to compare signal intensities and gene abundances across vegetation types (SPSS v 12.0.2). False discovery rate detection [FDR, (Benjamini and Hochberg, 1995)] was used to evaluate post-hoc comparisons, and p-values smaller than their corrected FDR thresholds were considered to represent significant differences.

Analysis of similarity (ANOSIM) was used to compare the similarity of functional gene expression patterns across vegetation types. Analogous to ANOVA, ANOSIM compares the mean difference of ranks between groups and within them, generating the statistic R (Clarke, 1993). Values of R range from −1 to +1, with negative values and values near 0 indicating similarity among samples and values approaching +1 indicating a strong dissimilarity among samples. Bray–Curtis dissimilarity values were calculated among samples (Legendre and Legendre, 1998), replicate samples were grouped by vegetation type, and ANOSIM was conducted using the PAST software program (Hammer et al., 2001). And, finally, NMDS was performed as described above (for the OTU analysis) in order to visualize relative similarities and differences of the communities based upon their functional gene detection profiles.

3. Results

3.1. Soil characteristics

Soils sampled under mesquite were characterized by a lower bulk density than those associated with the various grass types (Table 1). Given this difference, the SOC and total N data associated with these soils are presented here on a g m^{-2} basis. Soils associated with mesquite contained significantly more root biomass, and despite having lower bulk densities, the mesquite soils also contained significantly more SOC, and total N than did those sampled under the C_4 mid- or shortgrasses ($p < 0.05$) (Table 1). The soils occurring under the C_3 perennial grasses contained root biomass, SOC, and total N at levels that were intermediate in value to, and not significantly different from, the mesquite and C_4 grass samples. SOC and total N content were tightly coupled with one another in these soils, thus, no significant differences were found with respect to soil C/N ratios.

Table 1
Site characteristics, including root biomass, and soil organic carbon (SOC) content and total soil nitrogen (total N) content in surface soils (0–10 cm), mean ± standard error. Significant differences are indicated by differing letters within each column.

Vegetation type	Root biomass (g dry weight m^{-2})	SOC (g C m^{-2})	Total N (g N m^{-2})	C/N ratio
Mesquite	1953 ± 238^{a}	1415 ± 64^{a}	140 ± 6^{a}	10.12 ± 0.16
C_3 perennial grass	1843 ± 201^{ab}	1305 ± 89^{ab}	129 ± 8^{ab}	10.11 ± 0.14
C_4 midgrass	1579 ± 209^{b}	1148 ± 52^{b}	115 ± 5^{b}	10.08 ± 0.15
C_4 shortgrass	1440 ± 102^{b}	1074 ± 34^{b}	107 ± 3^{b}	10.02 ± 0.14

Table 2
Summary of sequence library sizes, operational taxonomic units (OTUs), and their diversity and richness estimates. OTUs were defined as sequences sharing ≥97% similarity and served as the basis for the Shannon, Simpson, and Chao I calculations.

Plant functional type	Library size	OTUs identified	Shannon (H')	Simpson ($1/D$)	Chao I estimated richness
(A) Bacteria					
Mesquite	83	71	4.20	226.87	341
C_3 perennial grass	54	47	3.80	178.87	184
C_4 midgrass	75	54	3.84	75.00	162
C_4 shortgrass	83	68	4.14	170.15	245
(B) Fungi					
Mesquite	78	44	3.49	32.64	122
C_3 perennial grass	89	46	3.48	29.67	104
C_4 midgrass	88	38	3.19	16.86	57
C_4 shortgrass	85	40	3.38	27.89	47

3.2. Bacterial and fungal community composition

A total of 295 bacterial sequences were produced, with individual clone libraries containing 54 to 83 sequences and 47 to 71 unique OTUs each (Table 2). Associated diversity statistics and richness estimators largely reflected differences in library size, with the bacterial community associated with mesquite containing greater diversity and estimated richness than the C_3 perennial grass, C_4 midgrass, or C_4 shortgrass communities (Table 2). Although the clone libraries captured a wide variety of taxa, their associated rarefaction curves did not reach saturation (Fig. 1A), suggesting that a substantial portion of the soil bacterial community was not captured by our sequencing efforts.

Phylum-level characterization of the 16S clones showed that the libraries were largely composed of individuals representing the *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria*. These four phyla accounted for 95% of library membership, and the remaining 5% included members of the *Gemmatimonadetes*, *Bacteriodetes*, *Cyanobacteria*, and *Nitrospira* (Fig. 2A). Despite the degree of similarity that the communities shared with one another, their separation, based upon OTU composition and community structure can be seen in the NDMS plot in Fig. 3A. The mesquite and C_3 perennial grass communities were very distinct from one another, but the C_4 grasses clustered together.

An additional 340 fungal sequences were produced, resulting in clone libraries that ranged in size from 78 to 89 individuals (Table 2) and contained 38 to 46 unique OTUs each. The fungal communities that occurred under mesquite and C_3 perennial grass appeared to harbor greater levels of richness and diversity than those occurring beneath the C_4 mid- and shortgrasses. At the phylum-level, the fungal libraries were dominated by members of the *Ascomycota* (42–76%), composed to a lesser degree by members of the *Zygomycota* (9–31%) and *Basidiomycota* (9–24%), and contained few *Chytridiomycota* (0–2%) (Fig. 2B). Like the bacterial communities, NMDS of the fungal community OTUs resulted in the distinct separation of the mesquite and C_3 perennial grass communities and clustering among the C_4 grasses (Fig. 3B). Unlike the bacterial communities, however, the rarefaction curves produced for the fungal sequence libraries appear to be closer to leveling off (Fig. 1B).

3.3. Analysis of community structure

Analysis of community differences using the phylogeny-based parsimony test did not detect significant differences ($p = 0.078$) in phylogenetic structure of the bacterial community (Table 3). It did, however, detect significant differences with respect to the fungal communities (Table 3). Pairwise comparisons of each of the fungal clone libraries showed that the mesquite soil fungal community harbored a phylogenetic structure that was significantly different from those associated with the with C_3 perennial grasses ($p = 0.037$), C_4 midgrasses ($p = 0.033$), and C_4 shortgrasses ($p = 0.041$). None of the fungal communities associated with each of the grass types was significantly different from one another.

3.4. Functional gene detection and community profile comparisons

Approximately 500 GeoChip probes were successfully detected across all samples, representing genes and gene families from each of the functional groups that were included on the microarray (Table 4). Genes involved in organic contaminant degradation were detected with the greatest abundance, followed by those related to metal resistance, nitrogen cycling, and the degradation of carbon compounds and strongly reflect the number of probes, from each category, that are contained within the array (He et al., 2007). No statistically significant differences were found among samples with respect to: (a) the number of gene probes detected; (b) their aggregate signal intensities; or (c) the number of gene probes detected relative to their taxonomic origin (Table 5). Likewise, additional analysis of functional gene profiles via ANOSIM also failed to detect significant differences among our four soil microbial

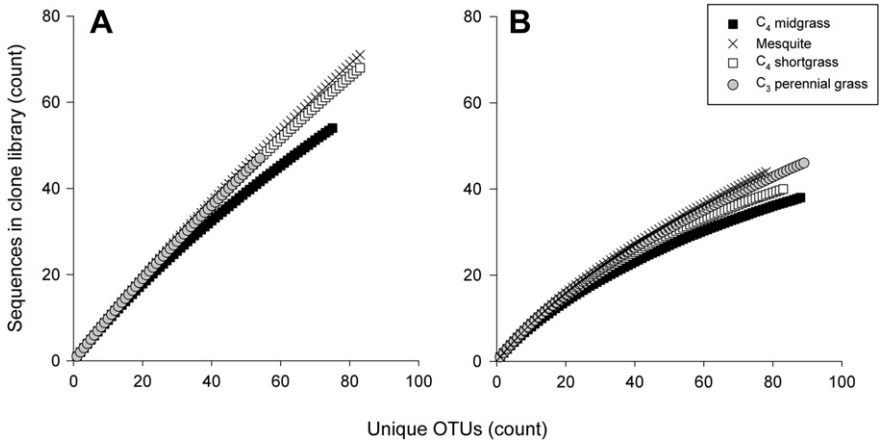

Fig. 1. Rarefaction analysis of the A) bacterial 16S rRNA and B) fungal ITS gene sequences from soils associated with each of our four prairie vegetation types. The total number of sequences per library is plotted against the number of unique OTUs encountered within the same library. OTUs were defined at the 97% similarity level.

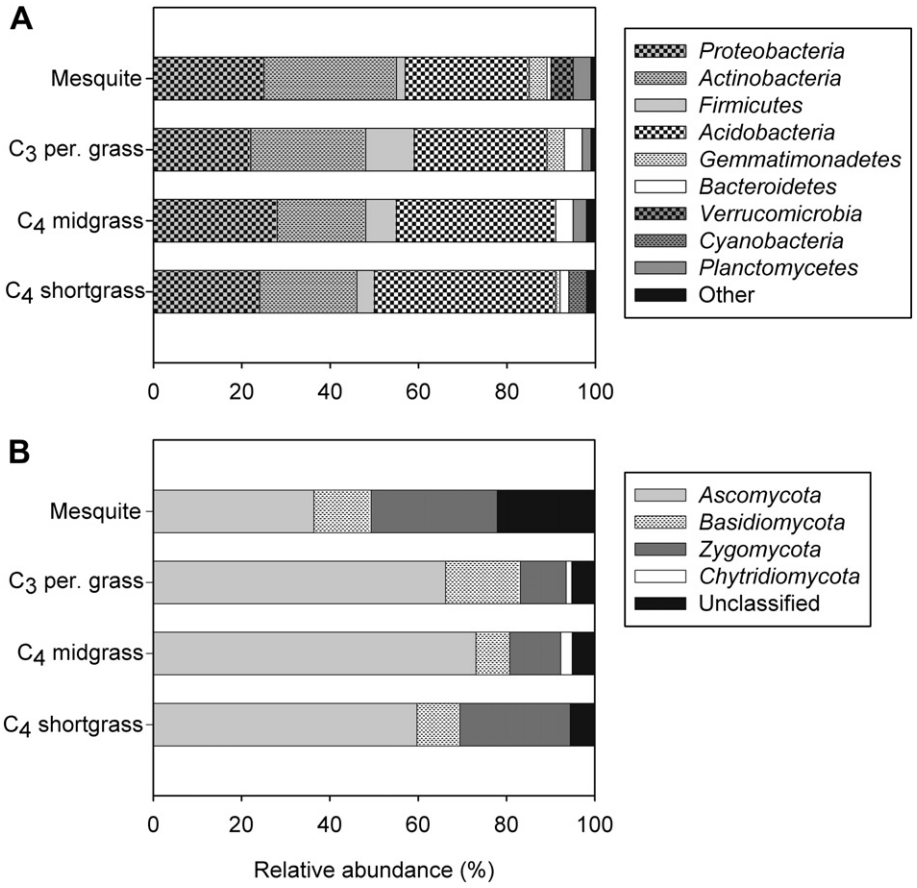

Fig. 2. Distribution and relative abundance of microbial taxonomic groups in mixed grass prairie soils based upon the analyses of A) bacterial and B) fungal rRNA gene clone libraries.

communities with respect to their functional gene diversity or overall gene abundance patterns (results not shown). Overlap among the communities' gene abundance patterns is illustrated in the NDMS plot shown in Fig. 4.

4. Discussion

The ability of individual plant species, or assemblages, to support unique microbial communities has been demonstrated in a number of ecosystems (Anderson et al., 2003; Callaway et al., 2004; Hawkes et al., 2005; Wallenstein et al., 2007), and it is often attributed to differences in productivity rates, nutrient use efficiency, and the quantity and quality of different plants' organic inputs to the soil. Thus, the increases in ANPP, root biomass, SOC and soil total N that typically occur following mesquite encroachment (Archer et al., 2001; Hughes et al., 2006; Boutton et al., 2009) could be expected to alter microbial community composition, diversity, and/or function. Our study identified shifts in community composition; however, concurrent shifts in potential functional capacity were not detected and suggest the possibility that the communities described here, or some portion of them, may be functionally redundant with one another.

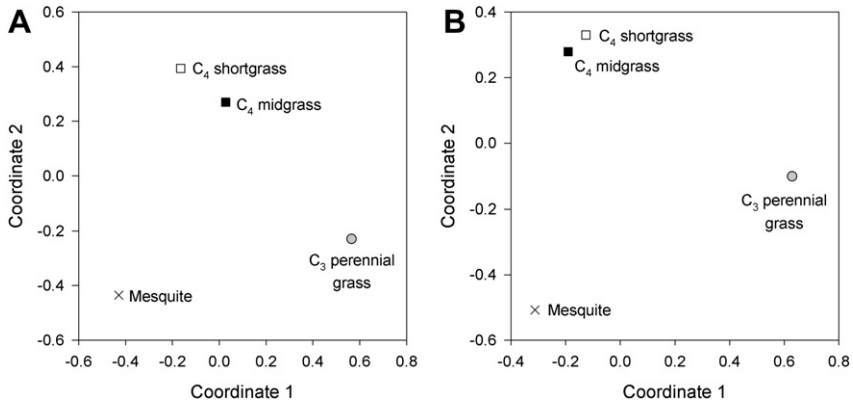

Fig. 3. Nonmetric multidimensional scaling (NMDS) of the A) bacterial and B) fungal communities based upon their OTU composition shows a distinct separation of the mesquite and C_3 perennial grass communities and clustering of the communities associated with the C_4 grasses.

Table 3
Parsimony scores and their significance for the (A) bacterial and (B) fungal communities as computed by TreeClimber. Significance values indicate the likelihood of differences in community structure among the clone libraries being due to random evolution (null hypothesis) versus evolutionary forcing (i.e. perturbation, vegetation change).

Vegetation pair	Parsimony score	Significance
(A) Bacteria		
All bacterial samples	146	0.078
(B) Fungi		
All fungal samples	135	0.045
Mesquite − C_3 perennial grass	37	0.037
Mesquite − C_4 midgrass	36	0.033
Mesquite − C_4 shortgrass	35	0.041
C_3 perennial − C_4 midgrass	44	0.063
C_3 perennial − C_4 shortgrass	41	0.056
C_4 midgrass − C_4 shortgrass	43	0.086

The soil bacterial community associated with mesquite was more diverse and had a greater estimated richness than those associated with any of the other plant functional types we surveyed. Although these differences did not lead to statistically significant findings with the parsimony test, it is expected that deeper sequencing of these communities might lead to the detection of such differences. We consider this a likely possibility, given that the *p*-value of the community-wide test was close to being statistically significant ($p = 0.078$), the separation observed among the communities based upon their OTU composition was fairly distinct (Fig. 3A), and our Chao I richness estimates (Table 2) and rarefaction analysis (Fig. 1A) suggest that a substantially high level of richness remained uncaptured by our sequence libraries.

In contrast, we did find statistically significant differences among the fungal communities. The fungi associated with mesquite were significantly different from each of the other fungal communities that we surveyed. Contrary to our hypothesis, mesquite associated soils did not harbor increased abundances of *Basidiomycota* or other well-characterized lignin degraders. Rather, mesquite soils contained increased abundances of *Zygomycota* (specifically *Mortierella* spp.) and unclassified fungi, but fewer *Ascomycota* (Fig. 2B) relative to the soils associated with the other plant functional types.

Similar shifts in the abundance of *Zygomycota* and *Ascomycota* have been reported in the comparison of shrub-encroached and tussock soils in the Arctic tundra (Wallenstein et al., 2007). Members of the *Zygomycota* are commonly characterized as *r*-selected species and are sometimes referred to as "sugar" fungi (Poll et al., 2009). They are commonly present at both early and late stages of decomposition (Rayner and Boddy, 1988), and they are thought to metabolize simple sugars and the by-products of lignin degradation (Allison et al., 2009). It is likely that their response to woody plant establishment, both here and in Wallenstein et al. (2007), is related to overall increases in substrate availability resulting from increased productivity among the woody species (Weintraub and Schimel, 2005; Hughes et al., 2006), rather than increased concentrations of recalcitrant carbon compounds specifically.

Despite the differences that were detected with respect to fungal community composition and the strong suggestion that differences among the bacteria might be detected with additional sequencing, no differences were found with respect to the soil communities' potential functional gene capacity. Functional gene probes variants were detected in similar numbers across all samples, and their signal intensities occurred at similar levels across all samples, too (Table 5). These results suggest that the soil microbial communities examined here may share a high degree of functional redundancy with one another regardless of their origin.

Table 4
Summary of functional gene array content ("A" probes) and the distribution of probes successfully detected among the soil microbial communities characterized in this study.

Gene functional group	Number of genes on the array	Number of gene probes	Probes detected in this study
Carbon degradation	980	1014	56
Carbon fixation	376	428	14
Methane cycle	303	303	13
Nitrogen cycle	1988	2027	82
Sulfur cycle	627	646	20
Metal reduction/resistance	1610	1933	128
Organic remediation	2774	2774	178
Total (including others not specifically listed)	8658	9170	494

Table 5
Results of ANOVA tests evaluating the effects of aboveground vegetation type on belowground microbial community functional gene expression. Using false discovery rate control for our p-values, significant results would be indicated by cases in which the *p*-value was smaller than the critical value. No test yielded statistically significant results.

Response Variable	Significance			
	Number of probes detected		Summed signal intensity	
	(*p*-value)	(critical value)	(*p*-value)	(critical value)
Gene functional group				
Carbon degradation	0.175	0.031	0.168	0.027
Carbon fixation	0.143	0.023	0.551	0.050
Methane cycle	0.246	0.042	0.224	0.035
Nitrogen fixation	0.221	0.038	0.168	0.027
Nitrification	0.037	0.004	0.082	0.008
Nitrogen reduction	0.210	0.035	0.286	0.042
Sulfur cycle	0.326	0.050	0.320	0.046
Organic degradation	0.150	0.027	0.136	0.019
Metal resistance/reduction	0.112	0.012	0.150	0.023
Gene probe origin				
Archaea	0.126	0.015	0.059	0.004
Bacteria	0.133	0.019	0.133	0.015
Eukaroyta	0.310	0.046	0.229	0.038
Lab clone/unclassified	0.066	0.008	0.101	0.012

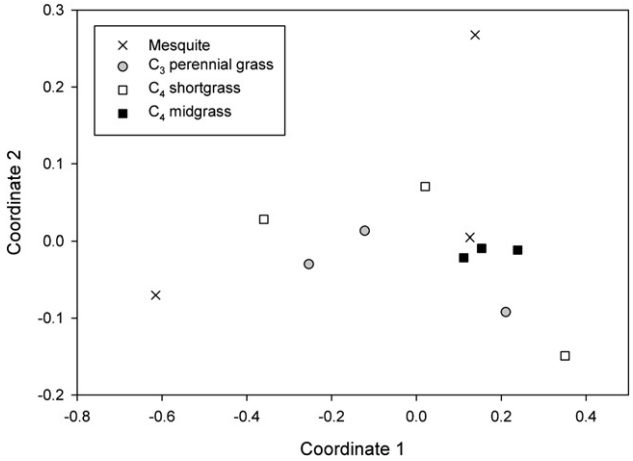

Fig. 4. Nonmetric multidimensional scaling (NMDS) of the replicate soil communities based upon gene probe detection with the GeoChip functional gene array. With the exception of the close clustering of the C_4 midgrass communities, the distribution of the communities suggests a high degree of overlap based upon potential functional gene capacity.

While functional redundancy is thought to be commonplace in soil microbial communities (O'Donnell et al., 2005), and the results of this study suggest that, at a minimum, a core group of function genes can be found across all of the communities we characterized, we put the interpretation of full functional redundancy forward with caution, as: a) the results of our microarray study are based upon the detection of gene probes within pools of DNA, rather than actively expressed pools of RNA, and b) despite the large number of probes contained within the GeoChip, it is not all-encompassing and may have been limited in its ability to detect all potential changes in our soil communities.

As ecosystems undergo succession, their microbial communities often become increasingly diverse and contain greater proportions of fungi relative to their earlier stages of development (Bardgett et al., 2005). This has been demonstrated in cases of primary succession (e.g. at the forefront of receding glaciers) and secondary succession (e.g. in grasslands following agricultural abandonment), and microcosm experiments have shown that increased fungal diversity may lead to more efficient degradation of recalcitrant substances (Setälä and McLean, 2004). Such shifts have been demonstrated in studies of soil fungal communities from other aggrading shrub- and woodland ecosystems (Anderson et al., 2003; Wallenstein et al., 2007), and while our study did not address bacterial versus fungal abundance specifically, we did observe significant changes in the structure of the soil fungal community with mesquite encroachment.

Succession-associated changes in community structure are often attributed to the increased quantities and complexity of organic matter that accrue in the soil as succession proceeds (Bardgett et al., 2005). It is anticipated that woody plant-imposed and succession-related changes in fungal community structure would also result in changes to soil community function that would provide the community with an enhanced ability to metabolize and degrade complex carbon substrates. Unfortunately, given that: a) the majority of the gene probes used in the design of the GeoChip were of bacterial (~98%), rather than fungal origin (He et al., 2007), b) nearly all of fungal probes on the array originated from laboratory studies of cultured organisms; and c) one of the major responses of the fungal communities to mesquite establishment was an increase in the relative abundance of unclassified fungal OTUs, it is likely that this version of the GeoChip may have been limited in its ability to detect changes in fungal portion of our soil communities, particularly that which is uncultured or undescribed. It is anticipated that the development of an FGA which places greater emphasis on functional genes of fungal origin and from uncultivated or undescribed organisms (Edwards et al., 2008), might provide greater insight into the function of this soil microbial community than did this version of the GeoChip.

5. Conclusion

Woody plant encroachment imparts substantial changes to the structure and function of grasslands and grassland-like ecosystems. The results of this study provide new information regarding the effects of mesquite on ecosystem structure, illustrate the linkages shared between above- and belowground communities, and demonstrate that soil microbial communities, and in particular soil fungi, may be altered by the process of woody plant encroachment. Although we were unable to assess the functional significance of such a fungal community shift, it appears that the functional profile of the bacterial portion of these soil communities has remained relatively stable. Future research directed more specifically at the fungal portion of this community will be likely to yield valuable insights in to the functional significance of woody plant induced community changes.

Acknowledgements

This research was supported by the USDA/CASMGS Program, and E.B. Hollister was supported by the U.S. Department of Energy Global Change Education Program's Graduate Research Environmental Fellowship, by NASA Headquarters under an Earth Systems Science Fellowship Grant, and by an NSF Doctoral Dissertation Improvement Grant. The authors would like to thank Gene Wickham, Sanghoon Kang, Dawn Klingeman, Lee Gunter, Jennifer Reeve, Emily Martin, Heidi Mjelde, Betty Kramp, Mike Castellano, Darrin Moore, Lisa Alexander, and Kirk Jessup for their assistance with this study. Additional thanks are extended to Terry Gentry for the use of his laboratory space and equipment; Michael Thon for the use of his lab's computing resources; and to two anonymous reviewers, whose suggestions helped to improve this manuscript.

References

Allison, S., LeBauer, D., Rosario Ofrecio, M., Reyes, R., Ta, A.-M., Tran, T., 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology and Biochemistry 41, 293–302.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410.

Anderson, I.C., Campbell, C.D., Prosser, J.I., 2003. Diversity of fungi in organic soils under a moorland – Scots pine (*Pinus sylvestris* L.) gradient. Environmental Microbiology 5, 1121–1132.

Ansley, R.J., Jacoby, P.W., Cuomo, G.J., 1990. Water relations of honey mesquite following severing of lateral roots: influence of location and amount of subsurface water. Journal of Range Management 43, 436–442.

Ansley, R.J., Pinchak, W.E., Teague, W.R., Kramp, B.A., Jones, D.L., Jacoby, P.W., 2004. Long-term grass yields following chemical control of honey mesquite. Journal of Range Management 57, 49–57.

Ansley, R.J., Boutton, T.W., Skjemstad, J.O., 2006. Soil organic carbon and black carbon storage and dynamics under different fire regimes in temperate mixed-grass savanna. Global Biogeochemical Cycles 20, GB3006. doi:3010.1029/2005GB002670.

Archer, S.R., Boutton, T.W., Hibbard, K.A., 2001. Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schultze, E.D., Harrison, S.P., Heimann, M., Holland, E.A., Lloyd, J., Prentice, I.C., Schimel, D. (Eds.), Global Biogeochemical Cycles in the Climate System. Academic Press, San Diego, CA, pp. 115–137.

Asner, G.P., Archer, S., Hughes, R.F., Ansley, R.J., Wessman, C.A., 2003. Net changes in regional woody vegetation cover and carbon storage in Texas drylands, 1937–1999. Global Change Biology 9, 316–335.

VanAuken, O.W., 2000. Shrub invasions of North American semiarid grasslands. Annual Review of Ecology and Systematics 31, 197–215.

Bardgett, R.D., Yeates, G.W., Anderson, J.M., 2005. Patterns and determinants of soil biological diversity. In: Bardgett, R.D., Usher, M.B., Hopkins, D.W. (Eds.), Biological Diversity and Function in Soils. Cambridge University Press, Cambridge, UK, pp. 100–118.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Socitey 57, 289–300.

Berthrong, S.T., Schadt, C.W., Pineiro, G., Jackson, R.B., 2009. Afforestation alters the composition of functional genes in soil and biogeochemical processes in South American grasslands. Applied and Environmental Microbiology 75, 6240–6248.

Boutton, T., Liao, J., Filley, T., Archer, S., 2009. Belowground carbon storage and soil dynamics accompanying woody plant encroachment in a subtropical Savanna. In: Lal, R., Follett, R. (Eds.), Soil Carbon Sequestration and the Greenhouse Effect. Soil Science Society of America, Madison, WI, pp. 181–205.

Callaway, R.M., Thelen, G.C., Rodriguez, A., Holben, W.E., 2004. Soil biota and exotic plant invasion. Nature 427, 731–733.

Clarke, K.R., 1993. Nonparametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117–143.

Dai, X., Boutton, T.W., Hailemichael, M., Ansley, R.J., Jessup, K.E., 2006. Soil carbon and nitrogen storage in response to fire in a temperate mixed-grass savanna. Journal of Environmental Quality 35, 1620–1628.

Edwards, I.P., Upchurch, R.A., Zak, D.R., 2008. Isolation of fungal cellobiohydrolase I genes from sporocarps and forest soils by PCR. Applied and Environmental Microbiology 74, 3481–3489.

Egger, K.N., 1995. Molecular analysis of ectomycorrhizal fungal communities. Canadian Journal of Botany-Revue Canadienne De Botanique 73, S1415–S1422.

Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system. Annual Review of Environment and Resources 30, 75–115.

Felsenstein, J., 2004. In: PHYLIP – Phylogeny Inference Package. University of Washington, Seattle, WA.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A.,

Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570–574.
Gentry, T.J., Wickham, G.S., Schadt, C.W., He, Z., Zhou, J., 2006. Microarray applications in microbial ecology research. Microbial Ecology 52, 159–175.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Paleoentologia Electronica 4, 1–9.
Hawkes, C.V., Wren, I.F., Herman, D.J., Firestone, M.K., 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8, 976–985.
He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., Zhou, J., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. The ISME Journal 1, 67–77.
Heitschmidt, R.K., Ansley, R.J., Dowhower, S.L., Jacoby, P.W., Price, D.L., 1988. Some observations from the excavation of honey mesquite root systems. Journal of Range Management 41, 227–231.
Hibbard, K.A., Archer, S., Schimel, D.S., Valentine, D.W., 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82, 1999–2011.
Houghton, R.A., Goodale, C.L., 2004. Effects of land use change on the carbon balance of terrestrial ecosystems. In: DeFries, R.S., Asner, G.P., Houghton, R.A. (Eds.), Ecosystems and Land Use Change. American Geophysical Union, Washington, D.C, pp. 85–98.
Huang, X.Q., Madan, A., 1999. CAP3: a DNA sequence assembly program. Genome Research 9, 868–877.
Huber, T., Faulkner, G., Hugenholtz, P., 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20, 2317–2319.
Hughes, R.F., Archer, S.R., Asner, G.P., Wessman, C.A., McMurtry, C., Nelson, J., Ansley, R.J., 2006. Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. Global Change Biology 12, 1733–1747.
Jackson, R.B., Banner, J.L., Jobbagy, E.G., Pockman, W.T., Wall, D.H., 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418, 623–626.
Jarnagin, S.T., 2004. Regional and global patterns of population, land use, and land cover change: an overview of stressors and impacts. GIScience and Remote Sensing 41, 207–227.
Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T., van der Putten, W.H., 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77, 147–162.
Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackenbrandt, E., Goodfellow, M. (Eds.), Nucelic Acid Techniques in Bacterial Systematics. John Wiley & Sons, Chichester, UK, pp. 115–157.
Legendre, P., Legendre, L., 1998. Numerical Ecology, xv, 853 pp. Elsevier, Amsterdam; New York.
Levang-Brilz, N., Biondini, M.E., 2003. Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains grasslands, USA. Plant Ecology 165, 117–144.
Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology & Biochemistry 38, 3184–3196.
Loy, A., Lehner, A., Lee, N., Adamczyk, J., Meier, H., Ernst, J., Schleifer, K.H., Wagner, M., 2002. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Applied and Environmental Microbiology 68, 5064–5081.
Loy, A., Taylor, M.W., Bodrossy, L., Wagner, M., 2006. Applications of nucleic acid microarrays in soil microbial ecology. In: Cooper, J.E., Rao, J.R. (Eds.), Molecular Techniques for Soil, Rhizosphere, and Plant Microorganisms. CABI Publishing, Wallingford, U.K, pp. 18–41.
Marchante, E., Kjøller, A., Struwe, S., Freitas, H., 2008. Invasive *Acacia longifolia* induce changes in the microbial catabolic diversity of sand dunes. Soil Biology and Biochemistry 40, 2563–2568.
McCulley, R.L., Archer, S.R., Boutton, T.W., Hons, F.M., Zuberer, D.A., 2004. Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85, 2804–2817.
O'Donnell, A.G., Colvan, S.R., Malosso, E., Supaphol, S., 2005. Twenty years of molecular analysis of bacterial communities in soils and what have we learned about function? In: Bardgett, R.D., Usher, M.B., Hopkins, D.W. (Eds.), Biological Diversity and Function in Soils. Cambridge University Press, Cambridge, UK, pp. 44–56.
Poll, C., Brune, T., Begerow, D., Kandeler, E., 2009. Small-scale diversity and succession of *fungi* in the detritusphere of rye residues. Microbial Ecology.
Rayner, A., Boddy, L., 1988. Fungal communities in the decay of wood. Advances in Microbial Ecology 10, 115–166.
Rehner, S., Samuels, G., 1994. Taxonomy and phylogeny of *Gliocladium* analyzed by large subunit rDNA sequences. Mycological Research 98, 625–634.
Rhee, S.K., Liu, X.D., Wu, L.Y., Chong, S.C., Wan, X.F., Zhou, J.Z., 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Applied and Environmental Microbiology 70, 4303–4317.
Schade, J.D., Hobbie, S.E., 2005. Spatial and temporal variation in islands of fertility in the Sonoran desert. Biogeochemistry 73, 541–553.
Schadt, C.W., Martin, A.P., Lipson, D.A., Schmidt, S.K., 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301, 1359–1361.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.
Setälä, H., McLean, M.A., 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139, 98–107.
Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology 67, 4742–4751.
Smucker, A.J.M., Mcburney, S.L., Srivastava, A.K., 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agronomy Journal 74, 500–503.
Soil Survey Staff, N. R. C. S., United States Department of Agriculture. 2007. Official soil series descriptions. http://soils.usda.gov/technical/classification/osd/index.html (accessed 11.04.07).
Taylor, D.L., Booth, M.G., Mcfarland, J.W., Herriott, I.C., Lennon, N.J., Nusbaum, C., Marr, T.G., 2008. Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. Molecular Ecology Resources 8, 742–752.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25, 4876–4882.
Tiedeman, A.R., Klemmedson, J.O., 1973. Effect of mesquite on physical and chemical properties of soil. Journal of Range Management 26, 27–29.
Wallenstein, M.D., McMahon, S., Schimel, J., 2007. Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. FEMS Microbiology Ecology 59, 428–435.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261–5267.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Weintraub, M.N., Schimel, J.P., 2005. Nitrogen cycling and the spread of shrubs control changes in the carbon balance of Arctic tundra ecosystems. BioScience 55, 408–415.
White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York, pp. 315–322.
Wu, L.Y., Thompson, D.K., Li, G.S., Hurt, R.A., Tiedje, J.M., Zhou, J.Z., 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. Applied and Environmental Microbiology 67, 5780–5790.
Wu, L.Y., Liu, X., Schadt, C.W., Zhou, J.Z., 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Applied and Environmental Microbiology 72, 4931–4941.
Zhou, J.Z., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Applied and Environmental Microbiology 62, 316–322.