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Research Article

RESEARCH ARTICLE

Woody plant encroachment, and its removal, impact bacterial and fungal communities across stream and terrestrial habitats in a tallgrass prairie ecosystem

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One sentence summary: Woody plant encroachment, and its restoration via mechanical removal, not only alter the edaphic environment, but also impact bacterial and fungal community dynamics.

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ABSTRACT

Woody plant encroachment has become a global threat to grasslands and has caused declines in aboveground richness and changes in ecosystem function; yet we have a limited understanding on the effects of these phenomena on belowground microbial communities. We completed riparian woody plant removals at Konza Prairie Biological Station, Kansas and collected soils spanning land–water interfaces in removal and woody vegetation impacted areas. We measured stream sediments and soils for edaphic variables (C and N pools, soil water content, pH) and bacterial (16S rRNA genes) and fungal (ITS2 rRNA gene repeat) communities using Illumina MiSeq metabarcoding. Bacterial richness and diversity decreased with distance from streams. Fungal richness decreased with distance from the stream in wooded areas, but was similar across landscape position while Planctomycetes and Basidiomycota relative abundance was lower in removal areas. Cyanobacteria, Ascomycota, Chytridiomycota and Glomeromycota relative abundance was greater in removal areas. Ordination analyses indicated that bacterial community composition shifted more across land–water interfaces than fungi yet both were marginally influenced by treatment. This study highlights the impacts of woody encroachment restoration on grassland bacterial and fungal communities which likely subsequently affects belowground processes and plant health in this ecosystem.

Keywords: woody encroachment; tallgrass prairie; land–water interface; bacterial communities; fungal communities

INTRODUCTION

Tree and shrub encroachment into grasslands and the subsequent conversion of prairies and grasslands into woodlands and forests have shifted the fundamental character of this biome in several locations (Van Auken 2000, 2009; Briggs *et al.* 2005). Causes of woody encroachment, or the increase in density of woody plant cover, vary by locality, but are generally related to an increase in grazing, low fire frequency and intensity, human disturbances (Van Auken 2000; Köchy and Wilson 2001), as well as regional climatic and edaphic conditions (Archer, Schimel and Holland 1995; Van Auken 2009). The transition from open grasslands to forested and shrubby woodlands alters ecosystem function that may not be easily reversible due to physiological advantages of woody plant species after recruitment (Ratajczak *et al.* 2011) or reduction in fire intensity at woody–grass interfaces (Engber *et al.* 2011; Ratajczak *et al.* 2011).

Woody encroachment significantly impacts both terrestrial and stream ecosystems, but processes governing these impacts

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may be substantially different between these ecosystem types. Across landscapes, woody encroachment has been associated with declines in plant species richness (Ratajczak, Nippert and Collins 2012). Further, encroachment increases soil and plant biomass C and N accrual (McKinley and Blair 2008), decreases soil CO_2 flux (Lett *et al.* 2004), leads to greater nutrient heterogeneity (Kleb and Wilson 1997) and increases annual net primary productivity in grasslands (Lett *et al.* 2004; Hughes *et al.* 2006). In riparian zones (the interface between the landscape and streams), the effect of greater density of woody vegetation indirectly leads to a reduction in in-stream algal biomass, particularly filamentous algae, and increases in stream ecosystem respiration rates (Riley and Dodds 2012) due to greater canopy cover and lower light availability. Reductions in autotrophic biomass and increases in respiration in woody encroached riparian zones likely alter resource quantity and quality for aquatic food webs (Whiting, Whiles and Stone 2011). The expansion of woody vegetation across grassland landscapes, and into riparian corridors, has large consequences for both ecosystem function (C and N cycling) and structure (plant and algae) across terrestrial and aquatic habitats.

Woody encroachment affects biogeochemical cycling, ecosystem function and autotrophic (plants and algae) diversity (Lett *et al.* 2004; McKinley and Blair 2008; Reisinger *et al.* 2013). However, less is known about the impact of woody encroachment, and its subsequent removal, on grassland bacterial and fungal community dynamics (but see Hollister *et al.* 2010; Yannarell, Menning and Beck 2014) which are likely linked to these ecosystem changes. Honey mesquite encroachment in the Great Plains region results in greater soil bacterial and fungal diversity and causes shifts in fungal community composition compared to C_3 or C_4 grass soils (Hollister *et al.* 2010). In Illinois hill prairies undergoing shrub encroachment, both bacterial and fungal community composition shifted across a forest-shrub-open prairie continuum; fungi responded more strongly to these changes in plant communities (Yannarell, Menning and Beck 2014). Thus, woody encroachment into prairie ecosystems changes microbial community structure and likely their activity in soils.

Restoration of riparian zones is a management practice to return an area to a pre-disturbance ecological state, functionally (e.g. sediment or N retention by creating forested buffers) (e.g. Osborne and Kovacic 1993; Hill 1996) and/or structurally (e.g. removal of invasive plant species) (Richardson *et al.* 2007). In the context of woody encroachment into grasslands, physical removal of woody vegetation may restore riparian areas and streams to their native grass-dominated state. This approach has proven successful in conifer dominated landscapes (Provencher *et al.* 2000; Jones *et al.* 2005), but less is known about how removal restorations in grassland riparian zones may affect both riparian and stream ecosystem dynamics. Other work in a tallgrass prairie ecosystem indicates that woody vegetation riparian removals return prairie streams to their native state functionally (Riley and Dodds 2012), and therefore may serve as a means of conserving grassland ecosystems.

Woody encroachment effects on soil-associated microbes (Hollister *et al.* 2010; Yannarell, Menning and Beck 2014) are documented, yet effects on streams which drain these landscapes are less known. Woody vegetation and its removal in tallgrass prairie may cause differential effects on microbial community dynamics of riparian soil versus sediments associated with the nearby stream ecosystem. We expected removal effects because within removal riparian soils, microbes will be impacted indirectly by multiple changes to the abiotic environment (e.g. increased temperature due to loss of canopy, reduction in leaf litter and C quantity) but also directly by removing woody plant species as many bacteria and fungi are tightly associated belowground with these plant species. Stream sediment microbes will also be impacted by removals, but as this habitat is not in close association with woody plants, the magnitude of these effects will likely be lesser (Riley and Dodds 2012). We hypothesized that (1) removal restorations will affect bacteria in both stream sediments and soils—bacteria in both habitats will differ in community composition between woody encroached and restoration habitats, but the shift in composition will be stronger for bacterial communities in terrestrial soils; (2) removal restorations will shift fungal community composition compared to woody encroached areas in soil habitats and not stream sediments as the majority of fungi are terrestrial. Fungal community composition will more strongly differ between removal and encroached soils compared to bacteria due to greater plant–fungi associations than plant-bacteria. Lastly, the presence of woody plants and herbaceous plants yields a variety of C sources (i.e. leaf litter biochemical composition, root exudates) (Wardle 2006), and host pathogenic, saprobic or mutualistic associations with microorganisms which will increase the number of microbial niches. We hypothesize that (3) removal restorations will lower bacterial and fungal richness and diversity in soils compared to woody encroached soils by reducing the diversity of leaf litter and root types. As streams are conduits of nutrients being supplied by adjacent riparian habitats, these effects will also be seen in stream sediments, but to a lesser degree.

METHODS

Study area and experimental manipulation

The study area is located at Konza Prairie Biological Station in northeastern Kansas, and three watersheds draining the Kings Creek stream network (AL, N2B, N4D, Fig. 1) were sampled. Watersheds N2B and N4D are both grazed by American bison (*Bos bison*), but are burned every 2 or 4 years, respectively. AL is not grazed and is burned every year. The last prescribed burn of N2B and N4D prior to our sampling occurred in April 2013, whereas AL was burned in March 2014.

At AL and N4D, woody, riparian vegetation was mechanically removed within a ~30 m area parallel to the stream during December 2007. Trees were girdled and left standing, whereas shrubs were removed via brushcutting. All cut vegetation was then moved outside of the removal area (see Riley and Dodds 2012). Maintenance of the removal area through removal of any additional woody growth and relocating cut wood outside the removal area occurred between 2007 and 2009. During February 2014, any regrowth of woody, riparian vegetation was removed again at these watersheds. AL and N4D removal reaches were 36 and 33 m in stream length (Riley and Dodds 2012). N2B had the entire western fork of the watershed cleared of riparian, woody vegetation (4.8 km of stream length) during December 2010 similarly to AL and N4D removals. The removal areas within N2B have been maintained annually since 2010. Wooded areas are dominated by *Quercus macrocarpa* (bur oak), *Q. muehlenbergii* (chinquapin oak), *Ulmus americana* (American elm), *Gleditsia triacanthos* (honey locust), *Cercis canadensis* (Eastern redbud), *C. occidentalis* (Western redbud) and *Cornus drummondii* (Roughleaf dogwood) (Briggs *et al.* 2005, D. Carter, unpublished data).

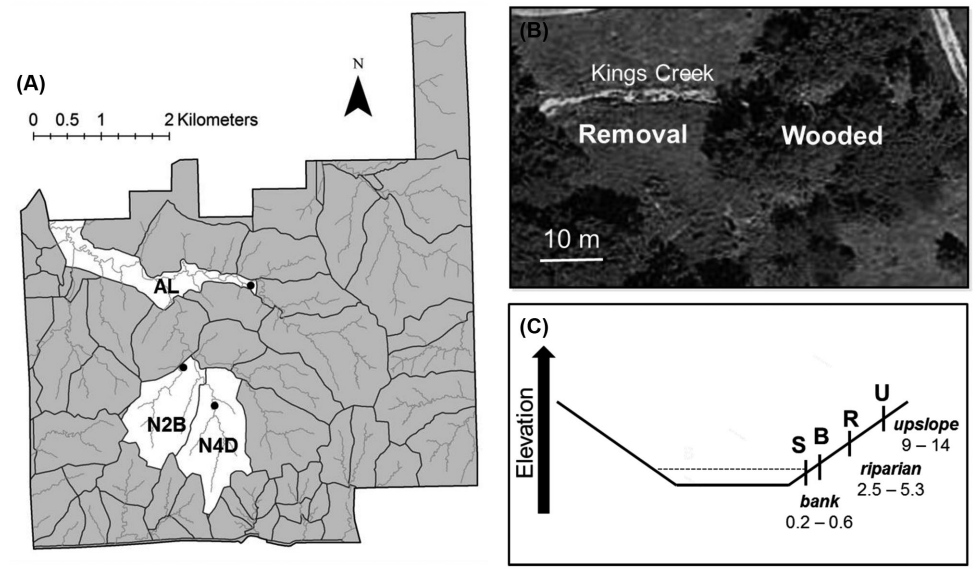

Figure 1. Location of the three watersheds (**A**) located at Konza Prairie Biological Station near Manhattan, KS. At each watershed, ~30 m area had woody vegetation removed parallel to the stream channel (AL is shown as an example in panel **B**), except at N2B with removal in entire western fork (reach upstream of sampling point shown). In both removal and wooded treatments, samples were collected from stream margin sediments to upslope soils (see panel **C** for stream channel cross-section schematic). In panel C, dashed line denotes stream water surface and ranges of distance from stream (S, distance = 0) for each position (B = bank, R = riparian, U = upslope) are given.

Soil sampling

Soils were sampled on 6–7 July 2014. Within each of the three watersheds, two 'treatments' were sampled: an area that had undergone riparian, woody vegetation removal (termed 'removal' throughout remaining text) and an area adjacent to this with riparian, woody vegetation intact (termed 'wooded'). Within each of these two treatments at each of three watersheds, soil cores were collected along four transects beginning at stream margins and ending in terrestrial upslope areas (Fig. 1). Specifically, for each transect, soil cores were taken at (1) the stream margin, (2) stream bank (average 0.32 m from stream margin), (3) nearby riparian soils (average 4.1 m from stream margin) and (4) more distant upslope soil (average 11.0 m from stream margin, Fig. 1). These categories, termed 'landscape position' throughout the remaining text, were chosen as they represent a gradual continuum of habitats (e.g. water availability, soil particle size and vegetation) spanning aquatic to terrestrial environments. Stream margin samples were taken at the edge of streams in little to no flow areas and were water saturated, whereas stream banks were within the channel. Unlike stream margin samples, bank sediments were not under water, and had some vegetation present. Terrestrial riparian and upslope soils were outside of the stream channel and had much denser vegetation present (grasses in removal areas, grasses, trees and shrubs in the wooded area), but only differed from each other based on their distance from stream margins. At each sampling point (streams, banks, riparian and upslope) along each of four replicate transects, within each treatment in a watershed, we collected three soil cores (top 5 cm) using a steel pipe with a 3.81 cm diameter and pooled into one for a total of 96 samples across the experiment. The samples were stored on ice until arrival to the laboratory where they were frozen at −20°C. Soil processing was completed within 2 weeks of collection. Soils were placed at 4°C until thawed (~48 h), thoroughly homogenized and sieved (2 mm mesh size).

Edaphic variables and analyses

Once sieved, the samples were analyzed for extractable NO_3^--N, NH_4^+-N, soil water content, total nitrogen (TN), total carbon (TC), C:N and soil pH. Extractable NO_3^--N and NH_4^+-N were extracted overnight (~12 h) in a 2 M KCl solution (5:1 KCl v: soil v) and the extract filtered (Whatman Nucleopore, 0.2 μm size, GE Healthcare Companies). Extractable NO_3^--N was analyzed by cadmium reduction and colorimetric reaction, whereas extractable NH_4^+-N was measured by an indophenol colorimetric reaction and both measured using a Rapid Flow Analyzer (Model RFA-300, Alpkem Corporation, Clackamas, Oregon, USA). Soil pH was measured in a 1:1 soil/deionized water solution. Additional soil was weighed and dried at 60°C for at least 48 h to calculate soil water content and prepare soils for TN and TC analysis. TN and TC were determined by grinding dried soil into a fine powder using a ball mill and then analyzed by a Carlo Erba NA 1500 Analyzer. Due to high concentrations of calcium carbonate in Konza Prairie stream sediments, stream and bank sediments were treated with 3% HCl to volatilize calcium carbonate prior to TN and TC analyses.

DNA extractions, PCR and Illumina MiSeq sequencing

Total genomic DNA was extracted from 0.25 to 0.3 g of soil from each point along each transect (32 samples per watershed, 95 total) using a MoBio PowerSoil Extraction Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). One riparian sample was potentially contaminated during thawing and therefore not prepared for microbial community analysis. DNA yield was determined using a Nanodrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and the DNA templates adjusted to a 2 ng/μL concentration.

We analyzed DNA for both bacterial (16S) and fungal (Internal Transcribed Spacer 2, ITS2) communities using a two-step PCR approach to avoid a 3′-end amplification bias resulting from the

sample-specific DNA tags (Berry *et al.* 2011). For bacterial communities, we first amplified the V4 region within the 16S ribosomal RNA (rRNA) gene using 515F and 806R primers (Caporaso *et al.* 2012). Each sample was amplified in three independent 25 μL reactions, which consisted of 1 μM of forward and reverse primers, 10 ng of template DNA, 12.5 μL proofreading Phusion High-Fidelity Master Mix (New England Biolabs, Inc., Ipswich, MA, USA) and 5 μL of molecular grade water. For fungal communities, we first amplified the entire ITS region flanked by the 18S and 28S rRNA genes using the ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990) primers. Each sample was amplified in three 25 μL reactions consisting of 1 μM of forward and reverse primers, 10 ng of template DNA, 200 μM of each deoxynucleotide phosphate, 1 μM of $MgCl_2$, 0.5 units of proofreading Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, Wilmington, Delaware, USA) and 5 μL of 5X Green HF PCR buffer (Thermo Scientific, Wilmington, Delaware, USA). Thermal cycler parameters (Eppendorf, Hamburg, Germany) for bacterial communities consisted of an initial denaturation at 98°C for 5 min, followed by 25 cycles with denaturation at 94°C for 1 min, annealing for 30 s at 50°C, extension for 1 min at 72°C, with final extension for 10 min. Fungal community PCRs were the same except for 30 cycles. Negative controls were included in both bacterial and fungal PCRs to detect contamination and all remained contaminant free.

The PCR amplicons were cleaned using Agencourt AmPure SPRI system (1:1 ratio of bead solution to PCR volume) to reduce carryover of primary PCR primers. The three technical replicates of the cleaned amplicons were pooled and diluted (bacteria 1:5; fungi 1:2) for secondary PCRs. The different dilutions were necessary as the initial 1:5 mixtures yielded poor amplification of fungal templates for several samples. In the secondary PCRs, 10 μL of cleaned and diluted primary PCR products were amplified as above except only using five cycles. In addition, the reverse primer included a 12 bp unique Multiplexing Identifier tag (MID-806R). Secondary PCRs for fungal communities were similar to those for bacteria and had 12 bp MIDs in the reverse primer (MID-ITS4), but we used a nested PCR with the fITS7 primer (Ihrmark *et al.* 2012) instead of ITS1F to generate optimally sized amplicons for Illumina MiSeq. This nested PCR approach also minimizes non-target plant amplicons that often result from environmental samples using this primer combination. All primary and secondary PCRs were visualized on a 1.5% agarose (w/v) gel to ensure successful amplification. Secondary PCRs were cleaned using Agencourt AmPure similarly to primary PCRs. Amplicon DNA concentration for each experimental unit was measured and pooled at equal amounts (150 ng for bacteria, 120 ng for fungi). Both amplicon libraries were paired-end sequenced using the Illumina MiSeq. Illumina-specific primers and adapters were ligated using a NEBNext® DNA MasterMix for Illumina kit (Protocol E6040, New England Biolabs Inc., Ipswich, MA, USA) and sequenced using a MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA) with 500 cycles.

Bioinformatics

Sequences (.fastq) were processed using mothur (version 1.33.3, Schloss *et al.* 2009). Both bacterial and fungal .fastq files were contiged and any sequences with any ambiguous bases, with more than two mismatches to the primers, any mismatches to the MID, homopolymers longer than 8 bp and any without a minimum overlap of 50 bp were removed. This yielded 4 268 545 and 710 633 sequences for bacteria and fungi, respectively. Bacterial sequences were aligned against a SILVA reference, then preclustered to remove any erroneous reads (Huse *et al.* 2010), screened for chimeras (125 723 chimeric sequences) with the UCHIME algorithm (Edgar *et al.* 2011) and non-chimeric sequences were assigned to taxa using the Naïve Bayesian Classifier (Wang *et al.* 2007) against the RDP training set (version 10) with 51% bootstrap threshold. Originally, sequences were assigned to taxa using an 80% threshold, but many were only assigned to Domain Bacteria. Non-target sequences (mitochondria, chloroplast, Archaea) were removed. We subsampled 1.5 million sequences (out of 3.03 million) from the entire dataset and calculated a pairwise distance matrix. Sequences were clustered to OTUs at a 97% similarity threshold using nearest neighbor (single linkage) joining that conservatively assigns sequences to OTUs.

After pre-processing and chimera removal (31 458 chimeric sequences) as described for bacteria, the fungal sequences were assigned to taxa using the Naïve Bayesian Classifier (Wang *et al.* 2007) and the UNITE-curated International Nucleotide Sequence Database reference database (Abarenkov *et al.* 2010). Any sequences not assigned to the Kingdom Fungi were removed and remaining sequences pairwise aligned to calculate a pairwise distance matrix. This distance matrix was used to cluster fungal sequences into OTUs at a 97% threshold using nearest neighbor joining as described for bacteria. All bacterial and fungal sequence data were accessioned into the Sequence Read Archive (Sequence Read Archive Accession SRX1129668–1129669).

Bacterial and fungal communities were first subsampled at 10 000 and 2000 sequences, for community composition analyses. Bacterial and fungal communities were subsampled at this depth to retain as many samples as possible without sacrificing sequencing depth. After subsampling at these depths, 3 and 8 experimental units were still removed from the bacterial and fungal community dataset, respectively. After subsampling, low abundance OTUs were removed ($\leq$10 sequences across all experimental units) as they may be PCR or sequencing artifacts (Tedersoo *et al.* 2010; Brown *et al.* 2015). The final rarefied datasets had 2754 and 1597 OTUs, and 1070 480 and 226 542 sequences for bacteria and fungi, respectively. We estimated richness and diversity metrics for both bacterial and fungal communities in mothur (Schloss *et al.* 2009). Observed OTU richness (S_{obs}), the complement of Simpson's diversity (1D: $1-\sum p_i^2$), and Simpson's evenness (E_D: $1/\sum p_i^2/S$), with p_i representing frequency of each OTU within a sample, were iteratively calculated and subsampled at 8000 sequences for bacteria and 1500 for fungi.

Statistical analyses

A two-way ANOVA model was used to determine the influence of landscape position (streams, banks, riparian, upslope) and treatment (removal, wooded) on edaphic conditions, microbial (bacteria and fungi) diversity and dominant microbial phyla ($\geq$1.0% of total sequence counts) relative abundance. We also assessed these influences on dominant fungal families ($\geq$1.0% total sequence counts) since there is limited ecological information at the Phylum level for fungi. We also assessed the interaction between landscape position and treatment for these response variables. Multiple linear regressions were also used to determine if microbial diversity and phyla and family relative abundance were correlated with edaphic variables. All edaphic variables (except soil pH and C:N), relative abundance of fungal families and relative abundance of Cyanobacteria, Firmicutes, Deltaproteobacteria, Chytridiomycota and Zygomycota were $\log_{10}$ transformed prior to analyses.

Bray–Curtis distance matrices were constructed for both bacterial and fungal communities and were implemented in

Table 1. Summary statistics for edaphic variables measured across landscape position (streams, banks, riparian, upslope habitats) and treatments (W = wooded, R = removal areas). Means (standard deviation) are reported. Letters denote Tukey's HSD post-hoc comparisons for edaphic variables across landscape position only. †denotes edaphic variables that differed across treatment. No edaphic variables had a significant landscape position and treatment interaction.

Landscape Position	Treatment	NH_4^+-N ($\mu g\ g^{-1}$ soil)†	NO_3^--N ($\mu g\ g^{-1}$ soil)	TN (mg g^{-1} soil)	TC (mg g^{-1} soil)	C:N	Soil Water Content (%)†	Soil pH
Stream	W	7.60 (3.94)	0.31 (0.15)^A	1.57 (0.66)^A	17.74 (11.99)^A	10.44 (2.45)^A	0.51 (0.11)^A	7.82 (0.09)^A
	R	5.97 (2.39)	0.32 (0.15)	1.60 (0.40)	16.71 (6.36)	10.13 (1.63)	0.53 (0.16)	7.80 (0.13)
Bank	W	8.82 (5.38)	0.48 (0.52)^A	1.39 (0.39)^A	13.74 (6.14)^A	9.33 (2.76)^A	0.27 (0.06)^B	7.85 (0.23)^A
	R	6.13 (3.05)	0.29 (0.30)	1.27 (0.24)	11.17 (4.72)	9.19 (1.57)	0.26 (0.04)	7.87 (0.11)
Riparian	W	8.82 (3.63)	3.0 (1.55)^B	9.41 (2.06)^B	150.11 (36.58)^B	16.30 (3.54)^B	0.14 (0.04)^C	7.55 (0.32)^B
	R	6.42 (2.69)	2.21 (2.15)	8.19 (1.84)	123.22 (23.98)	15.29 (2.80)	0.12 (0.03)	7.51 (0.42)
Upslope	W	12.05 (3.42)	3.70 (2.65)^B	11.64 (3.74)^B	154.60 (55.66)^B	13.18 (1.06)^C	0.18 (0.03)^C	7.33 (0.26)^B
	R	7.73 (4.73)	3.19 (2.43)	9.15 (3.85)	121.44 (40.68)	13.95 (3.16)	0.13 (0.04)	7.48 (0.43)

non-metric dimensional scaling (NMDS) ordinations to visualize bacterial and fungal community composition. Permutational multivariate ANOVA tests calculate F-ratios from among-group sum of square distances (Bray–Curtis) and within-group sum of square distances (Anderson 2001), and then randomly shuffle observations among groups iteratively and compare this F-ratio frequency distribution to the observed F-ratio. We chose to use PERMANOVAs with 1000 permutations to determine if landscape position, treatment and their interaction influenced bacterial and fungal community composition. Lastly, we performed an indicator species analysis to determine which OTUs occurred more frequently between treatments and across landscape position. We only included the 100 most abundant OTUs in both bacterial and fungal indicator species analysis. These OTUs comprised 78% and 58% of all sequences across the experiment for bacteria and fungi, respectively. FDR corrections were used for post-hoc multiple comparisons of statistical significance for indicator species analysis. A representative sequence was submitted to BLAST using nucleotide (nr/nt) as a search database to determine additional taxonomic information for any indicator OTU that was unclassified beyond Kingdom Fungi. Only top significant alignments with e-values < 0.01 and identity percentage above 97% were considered.

All statistical analyses were implemented in R (version 3.1.1, R Development Core Team 2014). ANOVAs were carried out in the *stats* package, NMDS (function *metaMDS*) and PERMANOVAs (function *adonis*) in the *vegan* package (Oksanen *et al.* 2015), and indicator species analysis in the *indicspecies* package (De Caceres and Jansen 2015).

RESULTS

Edaphic factors

Soil water content differed across landscape position ($F_{4,90}$ = 111.44, $P < 0.01$) and between treatments ($F_{1,93} = 5.93$, $P = 0.02$). Soil water content was greater in wooded compared to removal treatments and was greatest in stream sediments compared to terrestrial soils (Tukey's HSD, $P < 0.01$; Table 1). Soil pH differed across landscape position ($F_{4,83} = 13.62$, $P < 0.01$) and was greater in stream and bank sediments compared to terrestrial soils (Tukey's HSD, $P < 0.01$; Table 1), but did not differ between treatments ($P > 0.1$). Extractable soil NH_4^+-N was greater in wooded areas compared to removal treatments ($F_{1,90} = 10.74$, $P < 0.01$; Table 1), but was not influenced by landscape position ($P > 0.1$). Extractable soil NO_3^--N ($F_{4,87} = 34.02$), TN ($F_{4,83} = 225.54$) and TC ($F_{4,84} = 148.75$) differed across landscape position ($P < 0.01$) and were greater in terrestrial soils (riparian and upslope habitats) than stream and bank sediments (Tukey's HSD, $P < 0.01$; Table 1). C:N differed across landscape position ($F_{4,83} = 22.89$, $P < 0.01$) and was greatest in riparian soils compared to other landscape positions, whereas stream and bank sediments had the lowest C:N (Tukey's HSD, $P < 0.05$; Table 1). Extractable soil NO_3^--N, TN, TC and C:N did not differ between treatments ($P > 0.1$). There was no significant landscape position by treatment interactions for any edaphic variable.

Microbial richness and diversity

Bacterial OTU richness and diversity differed across landscape position ($F_{3,89} = 60.03$, $P < 0.01$). Both were greatest in stream and bank sediments (Tukey's HSD, $P < 0.01$), and lowest in riparian and upslope soils ($P < 0.01$, Fig. 2, panels A and B). Bacterial evenness marginally differed across landscape position ($F_{3,89} = 2.27$, $P = 0.09$) and between treatments ($F_{1,91} = 3.27$, $P = 0.07$). Evenness was marginally lower in upslope soils than stream sediments (Tukey's HSD, $P = 0.08$), whereas wooded treatments had marginally greater evenness than removal soils ($P = 0.07$). Multiple edaphic variables were correlated with bacterial richness, diversity and evenness that primarily differed across landscape position (NO_3^--N, TN, C:N, soil water content and pH; Table S1, Supporting Information).

Fungal OTU richness differed across landscape position ($F_{3,78}$ = 8.07, $P < 0.01$), treatment ($F_{1,80} = 5.26$, $P = 0.02$) and had a significant landscape position by treatment interaction ($F_{3,78} = 3.71$, P = 0.02, Fig. 2, panel D). Stream sediments had richer fungal communities than riparian or upslope soils (Tukey's HSD; $P < 0.01$) in wooded treatments, but removal treatments minimized these differences and resulted in similar fungal richness across landscape positions (Fig. 2, panel D). Fungal diversity and evenness did not differ across landscape position or treatment ($P > 0.1$). TN and pH were correlated with fungal richness (Table S1, Supporting Information).

Bacterial community compositional shifts

A total of 29 bacterial phyla were found across the experiment, with 12 phyla (or class for Proteobacteria) dominating all samples (≥ 1% relative abundance across all samples) collected (Table S2, Supporting Information). A small proportion of sequences (7.5%) were unclassified beyond Domain Bacteria.

Nine dominant bacterial phyla differed in relative abundance over landscape position ($P \leq 0.01$), and two differed between treatments ($P < 0.01$; Table 2). Due to large differences in NO_3^--N

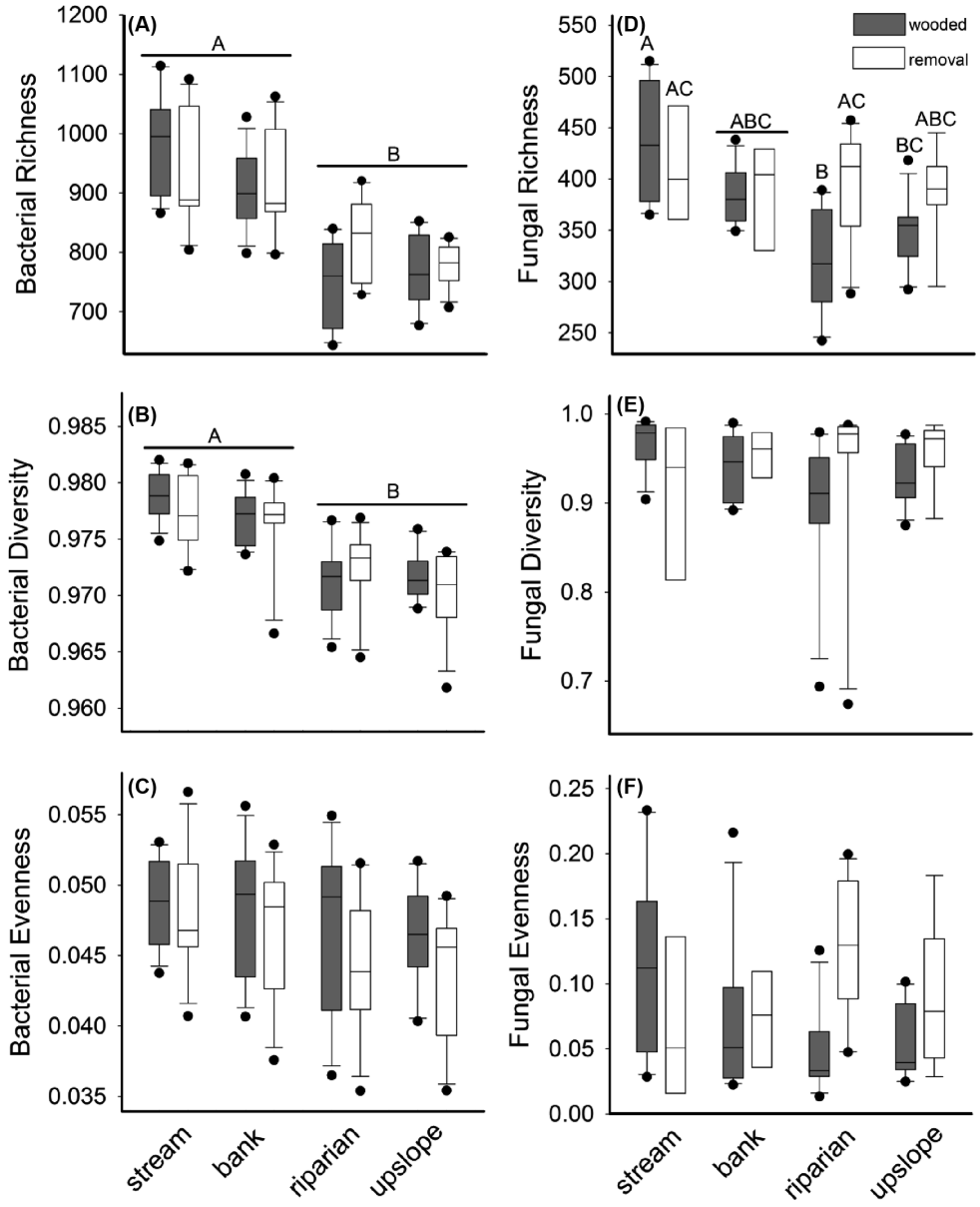

Figure 2. The distribution of bacterial (**A**, **B**, **C**) and fungal (**D**, **E**, **F**) richness, diversity and evenness across landscape positions within removal and wooded treatments. Letters denote Tukey's HSD post-hoc comparisons. All letters denote comparisons between landscape positions, except for fungal richness which denotes differences between landscape positions and treatments (significant landscape position × treatment interaction). Gray boxes = wooded treatment, white boxes = removal treatment.

concentrations across soils, we also included Nitrospirae in the analysis. Planctomycetes ($F_{1,90} = 7.58$) and Cyanobacteria ($F_{1,90} = 27.79$) relative abundance differed between treatments ($P < 0.01$). Planctomycetes had greater relative abundance in wooded treatments (Table 2), and did not differ across landscape position, whereas Cyanobacteria abundance was greater in removal treatments ($P < 0.01$). Cyanobacteria also had a significant landscape position by treatment interaction ($F_{3,88} = 3.36$, $P = 0.02$). Terrestrial soils in removal treatments had approximately 10x greater Cyanobacteria relative abundance compared to terrestrial soils in wooded treatments (Tukey's HSD $P < 0.05$; Table 2). Actinobacteria ($F_{3,88} = 9.53$) and Verrucomicrobia ($F_{3,88} = 33.06$) relative abundance was greater in terrestrial soils than stream and bank sediments (Tukey's HSD, $P < 0.01$; Table 2). Acidobacteria relative abundance was greater in banks, riparian and upslope habitats compared to streams ($F_{3,88} = 10.89$), whereas Gammaproteobacteria were greatest in bank sediments ($F_{3,88} = 3.11$, Tukey's HSD, $P \leq 0.04$). Chloroflexi ($F_{3,88} = 23.56$) relative abundance was greater in stream and bank sediments compared to all terrestrial habitats, whereas Nitrospirae ($F_{3,90} = 22.23$) had greater relative abundance in stream sediments compared to other sediment or soil habitats (Tukey's HSD, $P < 0.05$). Betaproteobacteria ($F_{3,88} = 71.53$) and Deltaproteobacteria ($F_{3,88} = 23.0$) had greater relative abundance in both stream and bank sediments compared to riparian and upslope soils (Tukey's HSD, $P < 0.01$). Most phyla's relative abundance was correlated with at least one edaphic variable (Table S3, Supporting Information).

Bacterial communities differed primarily across landscape position ($R^2 = 0.26$, $P < 0.01$), and marginally differed between treatments ($R^2 = 0.02$, $P = 0.09$). No variation in bacterial

Table 2. Bacterial phyla, fungal phyla and fungal family relative abundance across landscape position and treatment. Means (SD) are reported. Letters after means denote pairwise differences between landscape positions based on Tukey's HSD post-hoc tests. Bolded phyla labels denote a phylum or family which differed in relative abundance between treatments. Cyanobacteria and Glomeraceae had a significant landscape position by treatment interaction, therefore post-hoc comparisons are noted for landscape positions in each treatment (R = removal, W = wooded).

	Landscape position				Treatment	
Phylum	Stream	Bank	Riparian	Upslope	Removal	Wooded
Bacteria						
Alphaproteobacteria	5.6 (0.8)	5.6 (0.9)	5.7 (0.8)	5.9 (0.8)	5.7 (0.8)	5.7 (0.8)
Betaproteobacteria	9.2 (1.3)^{A}	8.7 (1.1)^{A}	5.6 (0.8)^{B}	5.4 (1.1)^{B}	7.1 (2.0)	7.4 (2.1)
Deltaproteobacteria	5.6 (1.3)^{A}	4.9 (1.0)^{A}	3.9 (0.5)^{B}	3.9 (0.5)^{B}	4.5 (1.1)	4.7 (1.3)
Gammaproteobacteria	3.8 (0.8)^{A}	4.5 (1.2)^{B}	3.9 (1.1)^{A}	3.6 (0.7)^{A}	3.9 (1.2)	4.0 (0.8)
Acidobacteria	12.5 (1.8)^{A}	14.6 (2.1)^{B}	14.9 (2.1)^{B}	15.5 (1.6)^{B}	14.4 (2.2)	14.3 (2.2)
Actinobacteria	10.9 (3.7)^{A}	11.5 (4.0)^{A}	14.7 (2.1)^{B}	14.9 (2.7)^{B}	13.2 (3.4)	12.7 (3.9)
Bacteroidetes	13.8 (2.8)	13.3 (3.1)	15.7 (2.6)	14.7 (2.0)	14.1 (2.6)	14.6 (2.9)
Chloroflexi	10.6 (3.4)^{A}	9.4 (2.3)^{A}	6.7 (2.3)^{B}	5.3 (1.8)^{B}	7.9 (2.9)	8.2 (3.6)
$Cyanobacteria_{R}$	1.3 (0.7)^{A}	1.5 (1.6)^{A}	1.4 (1.1)^{A}	1.3 (2.3)^{A,D}	1.4 (1.5)	n.a.
$Cyanobacteria_{W}$	1.1 (0.6)^{A}	0.6 (0.3)^{A,B}	0.2 (0.2)^{B,C}	0.15 (0.1)^{C,D}	n.a.	0.5 (0.5)
Firmicutes	3.1 (1.0)	2.7 (1.3)	3.1 (1.6)	3.5 (1.9)	3.3 (1.6)	2.9 (1.4)
Nitrospirae	0.7 (0.2)^{A}	0.5 (0.2)^{B}	0.4 (0.1)^{B,C}	0.3 (0.1)^{C}	0.5 (0.2)	0.5 (0.2)
Planctomycetes	3.9 (0.7)	4.0 (0.8)	4.0 (0.6)	4.2 (0.8)	3.8 (0.7)	4.2 (0.7)
Verrucomicrobia	6.0 (1.0)^{A}	5.5 (0.7)^{A}	8.5 (1.9)^{B}	9.3 (2.7)^{B}	7.6 (2.6)	7.0 (2.0)
Fungi						
Ascomycota	40.5 (15.5)	39.8 (11.5)	41.5 (13.0)	42.2 (16.3)	46.5 (13.3)	37.3 (13.2)
Herpotrichiellaceae	1.7 (1.2)^{A,B}	1.3 (0.6)^{A}	2.8 (2.3)^{B}	3.2 (2.9)^{B}	2.8 (2.8)	1.9 (1.3)
Nectriaceae	3.0 (1.8)	2.9 (1.7)	4.1 (4.1)	5.2 (5.3)	4.5 (3.6)	3.4 (3.8)
Pleosporaceae	4.3 (2.0)^{A,B}	5.9 (4.7)^{A}	3.0 (1.8)^{B}	4.8 (5.8)^{A,B}	5.8 (5.1)	3.4 (2.5)
Basidiomycota	27.2 (18.2)	29.9 (15.5)	39.2 (19.9)	38.2 (20.3)	26.6 (15.5)	39.5 (19.7)
Inocybaceae	5.0 (10.9)	3.5 (5.3)	1.7 (2.5)	2.4 (5.0)	2.3 (8.1)	3.7 (5.1)
Sebacinaceae	4.9 (6.1)	6.4 (5.4)	12.1 (17.7)	8.0 (10.8)	6.5 (13.3)	9.2 (10.2)
Thelephoraceae	3.9 (3.5)	7.4 (10.3)	7.7 (9.8)	5.8 (8.2)	3.7 (6.1)	8.3 (9.4)
Chytridiomycota	1.4 (0.8)	1.2 (1.3)	1.9 (1.8)	1.5 (1.0)	2.1 (1.7)	1.2 (0.8)
Zygomycota	3.1 (2.2)^{A}	4.9 (3.1)^{A}	7.0 (4.0)^{B}	6.5 (2.8)^{B}	6.0 (3.9)	5.2 (3.1)
Mortierellaceae	3.2 (2.0)^{A}	4.6 (2.8)^{A,B}	6.0 (3.7)^{B}	6.2 (2.7)^{B}	5.0 (3.3)	5.1 (2.9)
Glomeromycota	0.2 (0.4)	0.4 (0.4)	0.5 (0.5)	0.8 (1.6)	0.8 (1.3)	0.3 (0.3)
$Glomeraceae_{R}$	0.1 (0.2)^{A}	0.5 (0.5)^{A,B}	0.7 (0.6)^{A,B}	1.6 (2.1)^{B}	0.8 (1.2)	n.a.
$Glomeraceae_{W}$	0.2 (0.3)^{A}	0.3 (0.3)^{A}	0.3 (0.3)^{A}	0.2 (0.1)^{A}	n.a.	0.3 (0.3)

Table 3. PERMANOVA statistics for bacterial and fungal communities, using Bray–Curtis distances with 1000 permutations.

Model factors	DF	*F* value	R^2	*P* value
Bacteria				
Landscape position	3	8.30	0.26	<0.01
Treatment	1	1.62	0.02	0.09
LP × T	3	0.91	0.03	0.57
Residuals	65		0.69	
Fungi				
Landscape position	3	2.05	0.09	<0.01
Treatment	1	2.18	0.03	<0.01
LP × T	3	1.20	0.06	0.04
Residuals	53		0.82	

community composition was associated with a landscape position by treatment interaction ($P > 0.1$). A large proportion of the variation in composition remained unrelated to independent variables (residuals $R^2 = 0.69$, Table 3). However, fitting environmental correlates with NMDS scores indicate that all edaphic variables were significantly correlated with bacterial community composition (Table S4, Supporting Information; Fig. 3).

Fungal community compositional shifts

Sediments and soils were dominated by Ascomycota (40.3% sequences) and Basidiomycota (36.2% sequences). However, Zygomycota (5.2% sequences), Chytridiomycota (1.3% sequences) and Glomeromycota (0.5% sequences) were also present. A total of 116 fungal families were found across the experiment, the most dominant being Sebacinaceae (8.1% sequences), Thelephoraceae (6.3% sequences), Mortierellaceae (5.1% sequences), Pleosporaceae (4.4% sequences), Nectriaceae (3.9% sequences), Inocybaceae (3.1% sequences), Herpotrichiellaceae (2.3% sequences) and Pyronemataceae (2.0% sequences). A moderate proportion of sequences (16.4%) remained unclassified beyond Kingdom Fungi.

Ascomycota, Chytridiomycota and Basidiomycota differed between treatments, but not landscape position. However, two dominant ascomycete families, Pleosporaceae ($F_{1,79} = 3.59$) and Herpotrichiellaceae ($F_{1,79} = 4.79$), did differ across landscape positions. Pleosporaceae relative abundance was greater in bank sediments compared to riparian soils (Tukey's HSD, $P = 0.02$), and Herpotrichiellaceae was greater in terrestrial soils compared to bank sediments (Tukey's HSD, $P < 0.01$). Ascomycota ($F_{1,79} = 10.17$), in addition to families Pleosporaceae ($F_{1,79} = 11.59$) and Nectriaceae ($F_{1,79} = 5.39$), and Chytridiomycota ($F_{1,79} = 8.52$) relative abundance was greater in removal areas ($P \leq 0.02$), whereas

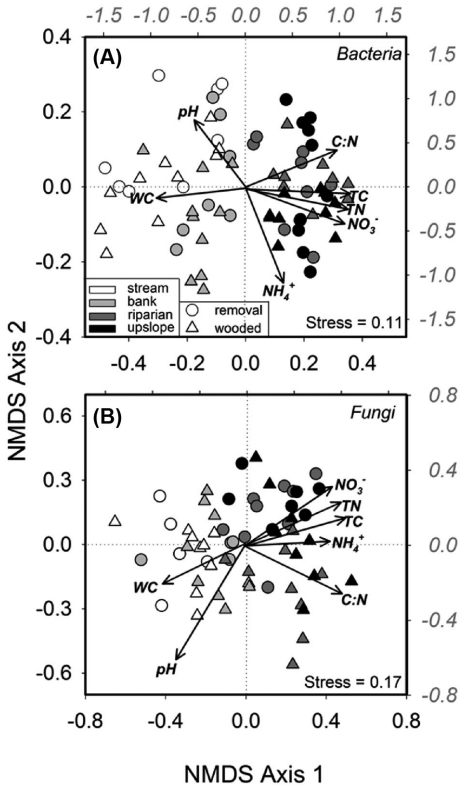

Figure 3. NMDS plots, for bacterial (**A**) and fungal (**B**) communities. Differences in colors represent landscape position: white = streams, light gray = banks, dark gray = riparian, black = upslope soils. Removal soils are represented by circles; wooded soils are represented by triangles. Stress values for each are noted, and both NMDS were created using three dimensions (k = 3). Edaphic associations with community composition are in bold with arrows. All vectors were significantly correlated with scores ($P < 0.01$). Gray axis tick labels correspond to edaphic variable vectors.

Basidiomycota ($F_{1,79} = 9.78$), and families Inocybaceae ($F_{1,79} = 7.29$), Sebacinaceae ($F_{1,79} = 7.14$) and Thelephoraceae ($F_{1,79} = 9.99$), relative abundance was greater in wooded soils ($P < 0.01$). Zygomycota relative abundance differed across landscape position ($F_{3,77} = 6.66$, $P < 0.01$), but not treatment. Zygomycota and family Mortierellaceae ($F_{3,77} = 5.77$) were greatest in riparian and upslope soils (Tukey's HSD, $P < 0.01$, Table 2). Although not 'dominant' (< 1.0% relative abundance), it is notable that Glomeromycota were greater in removal soils compared to wooded ($F_{1,79} = 9.05$, $P < 0.01$). Further, Glomeraceae (0.48% relative abundance across experiment) was influenced by landscape position ($F_{3,77} = 3.12$, $P = 0.03$), treatment ($F_{1,79} = 12.42$, $P < 0.01$) and had a significant landscape position by treatment interaction ($F_{3,77} = 3.84$, $P = 0.01$). Glomeraceae relative abundance was similar across landscape positions in wooded areas, but was greatest in upslope removal soils compared to all wooded habitats and removal stream sediments (Tukey's HSD, $P < 0.02$). All fungal phyla and families, except Ascomycota and Nectriacaeae, were correlated with at least one edaphic variable (Table S5, Supporting Information).

Fungal community composition significantly differed across landscape position ($R^2 = 0.09$, $P < 0.01$), treatment ($R^2 = 0.03$, $P < 0.01$) and some variation in composition was associated with a landscape position by treatment interaction ($R^2 = 0.06$, $P = 0.04$). A large proportion of variation in community composition remained unexplained (residuals $R^2 = 0.82$, Table 3). All edaphic variables were correlated with fungal community composition (Table S4, Supporting Information; Fig. 3).

Indicator taxa

Bacteria had no indicator OTUs for removal or wooded treatment soils. For fungi, there was one indicator OTU for removal and wooded soils, respectively. The indicator OTU for removal soils was the ectomycorrhizal mushroom, OTU 72 (*Inocybe lanatodisca*, $P < 0.01$). The indicator OTU for wooded soils represented a different genus of ectomycorrhizal mushroom, OTU 46 (*Cortinarius* sp., $P < 0.01$).

Bacteria had one indicator OTU for sediments and soils across landscape position. OTU 92 (Bacteroidetes, $P < 0.01$) was an indicator OTU for stream and bank sediments. Fungal communities had several indicator OTUs across landscape positions. Stream sediments had two indicator OTUs—OTU 103 (unclassified Fungi, $P < 0.01$) and OTU 72 (*Inocybe lanatodisca*, $P < 0.01$). A BLAST search indicated that OTU 103 is most closely matched to the saprobic basidiomycete, *Coprinellus* sp. (NCBI accession EF619671.1). Further, stream and bank sediments had five indicator taxa, including OTU 55 (Ascomycota, $P = 0.02$), OTU 74, 56, 101 and 113 (unclassified Fungi, $P < 0.01$). BLAST searches indicated that OTUs 56 and 101 are most closely matched to an uncultured ascomycete (HM239694.1, AY970222.1), and OTU 113 to the basidiomycete, *Mrakiella cryoconiti* (GQ911549.1). OTU 74 was most closely matched to an uncultured fungal clone (KF297116.1). There were no indicator taxa for terrestrial soils.

DISCUSSION

Woody vegetation, and its removal, affects microbial communities

Woody encroachment in tallgrass prairie alters multiple facets of ecosystem structure and function across ecosystem compartments (Lett *et al.* 2004; Hughes *et al.* 2006; Ratajczak *et al.* 2011; Ratajczak, Nippert and Collins 2012; Riley and Dodds 2012; Reisinger *et al.* 2013). Our study design tested whether riparian restorations, in reference to woody encroached areas, affected microbial community diversity and composition across ecosystem types. Our largely observational experiment precluded explicit identification of mechanistic drivers (e.g. environmental filters) of microbial community assembly in response to restorations. Nevertheless, this study clearly indicates that riparian removals impact both bacterial and fungal communities, particularly within terrestrial soils, and these effects may be related to differences in abiotic conditions and/or organismal interactions between microbial and plant communities.

Contrary to our hypotheses, bacterial richness and diversity were similar between wooded and riparian soils, but differed across landscape positions; stream sediments harbored more bacterial OTUs and were more diverse. However, wooded stream sediments had more fungal OTUs than terrestrial soils, yet removal restorations increased fungal richness in soils resulting in similar fungal richness across stream sediments and terrestrial soils. Greater plant species richness may increase abundance of saprophytic or arbuscular mycorrhizal (AM) fungi (Chung *et al.* 2007) and woody encroachment causes declines in plant species richness (Ratajczak *et al.* 2011). Thus, removal of woody vegetation and restoration of riparian areas to their native grassland state may increase plant species richness resulting in similar changes in fungal communities. We did not test how removal restorations impacted overall plant community richness so this

statement warrants further study. In addition, bacterial and fungal richness and diversity may be driven by different processes. For example, resource quantity supplied by plants may be more influential in determining bacterial richness (De Deyn, Quirk and Bardgett 2011), which did not change across treatments (i.e. TC), whereas plant species richness or identity (Chung *et al.* 2007; De Deyn, Quirk and Bardgett 2011) may drive fungal richness.

The relative abundance of two bacterial phyla differed between wooded and removal soils, but there was only one landscape position by treatment interaction across bacterial phyla. Planctomycetes relative abundance was lower in removal soils whereas Cyanobacteria had approximately 10x greater abundance in removal terrestrial soils compared to all wooded habitats. Planctomycetes have a wide range of metabolic capability—both a diversity of C and N metabolisms including lineages that are anaerobic ammonium oxidizers (Glöckner *et al.* 2003; Strous and Jetten 2004). Planctomycetes were positively correlated with NH_4^+, so wooded soils may provide more available nutritive sources for this bacterial group as wooded areas had greater NH_4^+ than removals. Interestingly, Cyanobacteria abundance was substantially greater and more variable in removal terrestrial soils. Most cyanobacteria are photosynthetic; many are known to be desiccation-resistant (Potts 1994; Singh, Sinha and Häder 2002; Lüttge, Beck and Bartels 2011) and may thus proliferate in drier soils with greater light availability. They were negatively correlated with NH_4^+ and NO_3^- suggesting that likely due to their N-fixing activity they are outcompeted by other microbes at higher N concentrations. These data suggest that, in particular, the large increase in Cyanobacterial relative abundance may alter N cycling or primary productivity after removal restorations in soils, specifically. Surprisingly, removal restorations had no effect on stream-associated bacterial groups, indicating that although removing woody vegetation may open canopy cover and cause significant changes to stream trophic state (Riley and Dodds 2012), it has little effect on bacterial community composition.

Among fungi, Ascomycota and Chytridiomycota relative abundance was greater in removal treatments, whereas that of Basidiomycota was greater in wooded. Many basidiomycetes possess lignocellulolytic activities and their lesser abundance in removal soils may be due to a lower availability of lignocellulosic substrates (Kirk and Farrell 1987; Peláez, Martínez and Martínez 1995; Liers *et al.* 2007). However, some ascomycetes also possess similar enzyme systems enabling lignin degradation (Kirk and Farrell 1987; Rodríguez *et al.* 1997). Fungal families with common saprobic or pathogenic members (i.e. Pleosporaceae, Nectriaceae) had greater relative abundance across all removal habitats (stream sediments and terrestrial soils), whereas some potentially mycorrhizal (Sebacinaceae) or ectomycorrhizal families (Inocybaceeae, Thelephoraceae) had greater relative abundance across all wooded habitats. In addition, Glomeraceae, although not dominant, had substantially greater relative abundance in upslope removal soils compared to all wooded habitats and removal stream sediments. These data suggest that removal restorations harbor different functional groups of fungi compared to encroached areas, likely changing belowground processes. Further, by removing woody plant species, AM may benefit from the recovery of mycorrhiza-dependent grass species and facilitate greater host phosphorous uptake, biomass accumulation and overall plant health and performance (Johnson *et al.* 2010).

Neither bacterial nor fungal community composition was strongly impacted by the removal treatment. The composition of bacterial communities may be more impacted by abiotic conditions (for example, pH; Fierer and Jackson 2006), more so than by differing plant communities. In fact, composition was correlated with all edaphic variables measured with TN, TC and water content being the most influential. Fungal richness substantially differed between treatments, yet composition was only marginally different between removal and wooded treatments. Similar to bacteria, composition was correlated with all edaphic variables and TN, TC and water content were the most influential. These data suggest that although differences between removal and wooded sites impact richness of fungi OTUs and relative abundance of both bacterial and fungal phyla, there is relatively little effect on the relative abundance of microbial OTUs.

Microbial communities shift across stream and terrestrial habitats

In this ecosystem, multiple environmental gradients exist across stream and terrestrial soil habitats and are highly influential in structuring microbial communities. Bacterial and fungal richness differed across these environmental gradients, with stream sediments (greater water and pH, lower C and N concentrations) harboring more species (at least for wooded soils). In general, soils are typically considered to be the microbially richest and most diverse habitat (Torsvik, Øvreås and Thingstad 2002). There are several reasons for our contrasting results. First, during precipitation events, microbes associated with adjacent soils can be flushed into stream networks and transported downstream and eventually deposited when baseflow resumes. As a result, stream sediments likely include both stream-exclusive and soil-inhabiting microbes. Second, this ecosystem often experiences long periods of little to no precipitation. If bacteria and fungi in terrestrial soils have low drought tolerance, fewer species will exist in soils with low soil water content. However, we sampled after several rainfall events so this explanation is unlikely. Third, stream sediments may have richer microbial consortia because of multiple chemical and physical gradients that exist vertically (Lozupone and Knight 2007). This last explanation may be appropriate for bacteria, but bacteria *and* fungi were richer in streams and a majority of fungal taxa are not aquatic (excluding Ingoldian fungi and many chytridiomycetes). One fungal indicator taxon in stream sediments was *Inocybe lanatodisca*, an ectomycorrhizal mushroom, which would only have higher frequencies in streams if its spores are being deposited there. Further, we exclusively used DNA-based methods that capture the entire community (active and inactive including deposited spores). This suggests that sediments in low to no flow areas (e.g. stream margins, pools) in this ecosystem may serve as deposit reservoirs and dormant propagule banks for microbes (Lennon and Jones 2011), particularly for fungi. Future work examining changes across land–water interfaces should incorporate both RNA and DNA-based approaches (e.g. rRNA:rRNA genes) to better understand the relative proportion of active versus dormant taxa in microbial communities.

Many bacterial phyla differed in relative abundance across land–water interfaces. Some phyla were more abundant in stream sediments (i.e. Betaproteobacteria, Nitrospirae and Chloroflexi), whereas others were more abundant in terrestrial soils, such as Actinobacteria, Acidobacteria and Verrucomicrobia. As expected, phyla more abundant in streams positively correlated with soil water content, whereas those more abundant in soils were positively correlated with nutrient availability. These differences in abundance of dominant phyla and their correlation with edaphic variables across landscape position suggest that

bacteria, even when evaluated at low taxonomic resolution such as phylum, may undergo environmental filtering (Fierer, Bradford and Jackson 2007). Many other variables not measured in this study, such as sediment particle size (Jackson and Weeks 2008), or sampling near vegetation, for example, may heavily impact these conclusions. As a result, decoupling specific processes that contribute to our results is not possible. Regardless, bacteria assembled differentially across landscape position more so than fungi, whereas fungal richness, and relative abundance of phyla, was more impacted by treatment implying that different processes may control their assembly.

Our data serve as a first step towards understanding (1) if woody encroachment, and its restoration, affects bacteria and fungi across ecosystem types, and (2) what processes may affect microbial community assembly in streams and adjacent soils. Although this study focused on edaphic conditions, with emphasis on nutrients, and their relationship to microbial communities in the context of woody encroachment restorations, we did not measure ecosystem process rates. However, the removal effects (lower soil extractable NH_4^+ and soil moisture, substantially greater Cyanobacterial and Glomeraceae abundance, and higher fungal richness) that we observed suggest that restorations do affect both bacteria and fungi with consequential implications for multiple belowground processes. In addition, patterns of microbial diversity and community composition may be more driven by species-sorting (Crump *et al.* 2012) mechanisms such as species interactions (e.g. plant–fungi interactions) or by physiochemical controls (e.g. bacterial community composition associations with abiotic conditions; Leibold *et al.* 2004). Further research is needed to effectively link woody encroachment on ecosystem processes and microbial community dynamics within stream and terrestrial habitats.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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