Received Date : 23-Jan-2015

Revised Date : 24-Apr-2015

Accepted Date : 29-Apr-2015

Article type : Original Article

Atmospheric N Deposition Alters Connectance, but not Functional Potential Among Saprotrophic Bacterial Communities

Zachary B. Freedman[1] and Donald R. Zak[1,2]

[1]School of Natural Resources & Environment and [2]Department of Ecology and Evolution, University of Michigan, Ann Arbor, Michigan, USA 48109

#Corresponding author

Name: Zachary Freedman

Address: 440 Church Street

Ann Arbor, MI 48109

e-mail: zacf@umich.edu

tel: (734)763-8003

fax: (734)763-8395

Running title: N deposition alters bacterial co-occurrence

Abstract

The use of co-occurrence patterns to investigate interactions between microorganisms has provided novel insight into organismal interactions within microbial communities. However, anthropogenic impacts on microbial co-occurrence patterns and ecosystem function remain an important gap in our ecological knowledge. In a northern hardwood forest ecosystem located in Michigan, USA, twenty years of experimentally increased atmospheric N deposition has reduced forest floor decay and increased soil C storage. This ecosystem-level response occurred concomitantly with compositional changes in saprophytic fungi and bacteria. Here, we investigated the influence of experimental N deposition on biotic interactions among forest floor bacterial assemblages by employing phylogenetic and molecular ecological network analysis. When compared to the ambient treatment, the forest floor bacterial community under experimental N deposition was less rich, more phylogenetically dispersed, and exhibited a more clustered co-occurrence network topology. Together, our observations reveal the presence of increased biotic interactions among saprotrophic bacterial assemblages under future rates of N deposition. Moreover, they support the hypothesis that nearly two decades of experimental N deposition can modify the organization of microbial communities and provide further insight into why anthropogenic N deposition has reduced decomposition, increased soil C storage, and accelerated phenolic DOC production in our field experiment.

Keywords: co-occurrence network / phylogenetic dispersion / N deposition

Introduction

Network theory has been widely implemented by biologists, computer scientists, mathematicians, as well as social scientists to explore non-random interactions between entities (Proulx *et al.* 2005). Recently, this framework has been applied to microbial ecology, wherein, microbial co-

occurrence network topology (*e.g.*, clustering, density) can reveal ecologically meaningful biotic interactions that may be of functional significance (Barberan *et al.* 2012; Chaffron *et al.* 2010; Fuhrman & Steele 2008; Horner-Devine *et al.* 2007). Insights gained through microbial co-occurrence network theory includes support for the presence of microbial keystone species (Berry & Widder 2014; Steele *et al.* 2011), copiotrophic and oligotrophic life-history strategies (Barberan *et al.* 2012), and community turnover under Antarctic ice-covered lakes (Vick-Majors *et al.* 2013). Moreover, microbial co-occurrence patterns may provide insight into community stability and resilience (*i.e.*, the degree to which a community withstands change in the face of disturbance;(Allison & Martiny 2008; Shade *et al.* 2012). However, we have a nascent understanding of ecological processes that mediate microbial co-occurrence, and furthermore, how microbial co-occurrence may be affected by anthropogenic environmental change (Horner-Devine *et al.* 2007; Widder *et al.* 2014).

Anthropogenic modification of global biogeochemical cycles may arise through changes in the composition and phylogenetic structure of microbial communities (Falkowski *et al.* 2008; Zhao *et al.* 2014). For example, over the past century and a half, atmospheric deposition of biologically available nitrogen (N) has increased by an order of magnitude across much of the Northern Hemisphere (*e.g.*, from 0.5-1 to 15-20 kg N $ha^{-1}y^{-1}$); this trend is expected to continue through the next century (Galloway *et al.* 2004; Torseth *et al.* 2012). For twenty years, we have evaluated the effects of chronic experimental N deposition in replicate forest stands spanning the north-south geographic range of the sugar-maple-dominated northern-hardwood forest in the Great Lakes region of North America (Figure S1, Table 1;(Braun 1950). We have determined that experimental N deposition, at a rate expected in some locations by mid-century, increased net primary productivity (NPP) of wood, fostered soil organic matter (SOM) accumulation, and accelerated dissolved organic carbon (DOC) leaching, as well as reduced litter decay (Pregitzer *et al.* 2008; Zak *et al.* 2008). Additionally, experimental N deposition

altered the composition of bacteria and fungi in the forest floor (Entwistle *et al.* 2013; Freedman & Zak 2014), suppressed a suite of functional genes mediating soil C and N cycling processes (Edwards *et al.* 2011; Eisenlord *et al.* 2013; Freedman *et al.* 2013) and increased the abundance of the less-efficient bacterial organic-matter modifying community (Freedman & Zak 2014). However, we need more insight into the nature of these changes and why they have occurred, because they appear to be linked to dramatic biogeochemical changes in soil that have fostered greater soil C storage, a potential mechanism reducing the accumulation of anthropogenic CO_2 in the Earth's atmosphere.

Here, we sought to determine if experimental N deposition altered the biotic interactions among the saprotrophic bacterial community; it is plausible that chronic N deposition can modify interactions within the bacterial community inhabiting decaying leaf litter, which may elicit a functional response consistent with our biogeochemical observations. We refer to the whole community as "saprotrophic" because we expect the majority of the organisms living within the Oe/Oa horizons to be sustained by the metabolism of decaying organic matter. To address our objectives, we amplified bacterial 16S rRNA genes from decaying forest floor and used Pacific Biosciences high-throughput DNA sequencing technology to determine if the taxonomic, phylogenetic, and co-occurrence network structure of saprotrophic bacteria were altered by experimental N deposition. Moreover, we employed predictive functional profiling to assess whether chronic N deposition altered the functional potential of the bacterial community. If twenty years of experimental N deposition affected biotic interactions among saprotrophic bacteria, we expect that the microbial community would exhibit an altered phylogenetically structure and a more clustered network topology. Moreover, if experimental N deposition did, in fact, alter the bacterial phylogenetic and network structure and also affected its functional potential, it would suggest that the saprotrophic microbial community is not functionally resilient to anthropogenic N deposition. However, if topological changes in the microbial community do

not occur concomitantly with change in microbial functional potential, it suggests the microbial community is resilient to this pervasive agent of environmental change.

Materials and Methods

Site description and sample collection

The influence of experimental N deposition on saprotrophic bacterial community composition, structure, and functional potential was investigated in four floristically and edaphically similar sugar-maple (*Acer saccarum* Marsh.)-dominated northern-hardwood forest stands in Lower and Upper Michigan, USA (Figure S1). The sites span the north-south geographic range of northern hardwood forests in the Great Lakes region (Braun 1950) and lie along a climatic and atmospheric N deposition gradient (Table 1). The thin Oi horizon is primarily comprised of sugar maple leaf litter, whereas the Oe/Oa horizons are interpenetrated by a dense root mat. The soils are characterized as sandy (85-90%), well-drained, isotic, frigid Typic Haplothods of the Kalkaska series. At each stand, six 30-m by 30-m plots were established in 1994; three of which receive ambient N deposition and three receive experimental N deposition. Experimental N deposition consists of six equal applications of $NaNO_3$ pellets broadcast over the forest floor during the growing season (30 kg N $ha^{-1}y^{-1}$); NO_3^- comprises ~60% of atmospheric N deposition (wet plus dry) in our study sites (Zak *et al.* 2008).

Forest floor was sampled in May 2012, a time in which ample moisture favors high rates of microbial activity. Previously, it has been documented that litter decay has been reduced in the Oe and Oa horizons (Zak *et al.* 2008), which we collected for this study. From each 30-m x 30-m plot, 10 random 0.1-m x 0.1-m forest floor samples (Oe/Oa horizons) were collected after removal of the freshly fallen Oi horizon. All samples were composited by plot, hand-homogenized in the field, and transported on ice to the University of Michigan where they were held at −80 °C prior to DNA extraction.

DNA extraction, PCR amplification, and high-throughput sequencing.

Genomic DNA was extracted from 2.5 g (fresh weight) of forest floor using the PowerMax Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) and purified using the PowerClean DNA Clean-up kit (MoBio) following manufacturer's instructions. Purified DNA quality was determined using an ND8000 Nanodrop (Thermo Scientific, Waltham, MA, USA) and quantified by Quant-iT PicoGreen (Invitrogen, Carlsbad, CA, USA) on a Synergy HT fluorometer. All DNA was stored at -80°C.

PCR amplifications were performed in triplicate using the Expand High Fidelity PCR System (Roche, Indianapolis, IN, USA) on a Mastercycler ProS thermocycler (Eppendorf, Hauppauge, NY, USA). The V1-V3 region of the 16S rRNA gene was amplified from each plot (n = 24) using barcoded universal bacterial primers 27F and 519R (Lane 1991). PCR conditions included an initial denaturation stage of 95 °C for 10 minutes, then 25 cycles of 95 °C for 30 sec followed by 1 min each at 55 °C and 72 °C. The resulting amplicons were purified using the MinElute PCR Purification Kit (Qiagen, Valencia, CA, USA). Purified PCR products were pooled in equi-molar concentrations within each experimental plot and were sequenced on the PacBio-RS II system (Pacific Biosciences, Menlo Park, CA, USA) using C2 chemistry and standard protocols (Eid *et al.* 2009). Barcoded PCR amplicons from two experimental plots were pooled in a SMRT cell and sequenced using PacBio circular consensus technology, which can generate at least 99.5% sequence accuracy for DNA fragments ranging up to 500 bp (Fichot & Norman 2013; Travers *et al.* 2010).

DNA sequence processing and bacterial community composition

Fastq files of the dataset used in this analysis have been deposited to NCBI under project accession number SRR1950983. Sequences were initially processed using the pbh5tools package (Pacific Biosciences), downstream processing was performed in mothur (Version 1.31.1;(Schloss *et al.* 2009).

Initial quality control removed any sequence with a consensus fold-coverage < 5, average quality score < 25 (50 bp rolling window), anomalous length (< 450 or > 550 bp), an ambiguous base, > 8 homopolymers, or a > 1 bp mismatch to either the barcode or primer (Freedman & Zak 2014; Marshall *et al.* 2012). High-quality sequences were de-replicated and aligned with the Greengenes database (Version 13.5;(DeSantis *et al.* 2006) using k-mer searching (octamers) with Needleman-Wunsch global, pairwise alignment methods (Needleman & Wunsch 1970), and checked for chimeras using UCHIME (Edgar *et al.* 2011). Sequences were taxonomically assigned using a Bayesian classifier (Wang *et al.* 2007) with a bootstrap cutoff of 80% against the Greengenes database.

To investigate how experimental N deposition has altered the structure of the saprotrophic bacterial community at the 'species' level, operational taxonomic units (OTUs) were generated using the average neighbor algorithm at 97% sequence similarity. Given the ecosystem-level focus of this work and the inherent variability associated with a study of this scale (Figure S1, Table 1), we chose to include only those OTUs that were present in at least 50% of our experimental plots (n = 6 plots per treatment) to determine the biotic associations among the "core" bacterial community (*sensu*(Brown & Jumpponen 2014; Unterseher *et al.* 2011). Sequences were first rarefied to 5,557 sequences per plot, and those representing the core community were rarified to 2,000 sequences per plot, and were used in all subsequent analyses.

Taxonomic composition of saprotrophic Bacteria under ambient and experimental N deposition

To determine if chronic N deposition altered biotic interactions within the saprotrophic bacterial community, we considered three complimentary metrics of community structure: i) taxonomic composition, ii) phylogenetic dispersion, and iii) co-occurrence network topology. Significance was accepted at $\alpha = 0.05$. Calculation of dissimilarity coefficients and all downstream analysis were

performed in mothur and Primer (version 6, Primer-E Ltd., Plymouth, UK). The effects of location, experimental N deposition, and their combined interaction on 16S rRNA assemblage diversity (Shannon Index (H';(Shannon & Weaver 1963), and Chao1 Richness;(Chao 1984) were initially determined by two-way analysis of variance (ANOVA); means were compared with a protected Fisher's LSD (SPSS Statistics, Version 20, IBM Corp., Armonk, NY, USA).

A Bray-Curtis dissimilarity matrix (Legendre & Legendre 1998) based on OTU abundance was generated, from which, the significance of compositional differences between ambient and experimental N deposition assemblages were confirmed by permutational multivariate analysis of variance (PerMANOVA;(Anderson 2001). PerMANOVA allows multivariate information to be partitioned according to the experimental design (with interaction terms), and determines significance by random permutation, but it cannot determine if observed shifts result from differences in assemblage location or heterogeneity in multivariate space. For this reason, a distance-based test for homogeneity of multivariate dispersions (PERMDISP;(Anderson 2004) was used to discern the nature of observed assemblage shifts.

Phylogenetic Dispersion of Saprotrophic bacteria Under Future Rates of N Deposition

To determine if experimental N deposition altered the phylogenetic dispersion of the saprotrophic bacterial community, phylogenetic structure was reconstructed using the FastTree approximate maximum-likelihood algorithm (version 2;(Price *et al.* 2010). Differences in the phylogenetic composition of bacterial communities under ambient and experimental N deposition were initially determined using the abundance-weighted UniFrac distance, which measures the sum of unique branch length attributable to one treatment or the other, but not both (*i.e.,* ambient vs. experimental N deposition;(Lozupone & Knight 2005); significance was assessed by PerMANOVA. Phylocom (version

4.2;(Webb *et al.* 2008) was used to calculate metrics of phylogenetic structure (*i.e.*, Rao's quadratic entropy (Dp;(Rao 1982), the net relatedness index (NRI), and the nearest taxon index (NTI;(Webb 2000)). Dp is a measure of abundance-weighted pairwise distances of unique branch lengths attributable to a given assemblage in the phylogram (*i.e.*, phylogenetic diversity). NRI is a tree-wide measure of phylogenetic clustering of sequences from a given assemblage, whereas the NTI quantifies the phylogenetic distance between closest relatives in an assemblage. Increasingly positive NRI and NTI scores indicate that communities are more phylogenetically related than expected by chance (phylogenetic clustering), whereas, increasingly negative scores indicate that communities are less phylogenetically related than expected by chance (phylogenetic overdispersion); significance was determined by comparative analysis to the *phylogeny shuffle* null model (999 randomizations;(Webb *et al.* 2008).

Molecular Ecological Network Analysis

Molecular Ecological Network (MEN) analysis was implemented to determine the influence of experimental N deposition on biotic associations within the saprotrophic bacterial community. Initially, co-occurrence patterns were assessed using the checkerboard score (C-score;(Stone & Roberts 1990) in mothur, wherein non-random patterns were tested against a randomly assembled community preserving site frequencies (Barberan *et al.* 2012). Networks were then constructed based on OTU co-occurrence (*i.e.*, significant positive correlations between OTUs). Spearman correlations between all OTUs were calculated in mothur, and OTU pairs that exhibited a significant correlation ($\rho \geq 0.6$; $P < 0.05$) were extracted and imported to Cytoscape (Shannon *et al.* 2003). Edges weights were defined as the strength of correlation between nodes. The resulting networks were analyzed using the NetworkAnalyzer plugin within Cytoscape (Assenov *et al.* 2008), and network modularity was calculated using the ClusterMaker plugin (Morris *et al.* 2011). To determine whether our data comprised a non-

random network, we compared each network (*i.e.*, ambient and experimental N deposition) to 100 randomly generated undirected networks of similar size using the Erdös-Rényi model (Erdös & Rényi 1959) with the Random Network tool in Cytoscape. The significance of site, treatment, and their interaction on bacterial co-occurrence network structure was determined by PerMANOVA *sensu* Williams et al. (2014). A detailed description of network metrics can be found in Table 2.

Prediction of Functional Traits

To determine whether experimental N deposition altered the functional potential of the saprotrophic bacterial community, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PiCRUSt (version 1);(Langille *et al.* 2013) was implemented within Galaxy (Goecks *et al.* 2010). PiCRUSt infers metagenomic functional content from 16S rRNA gene data in a two-step process, wherein: i) metagenome prediction is computed by homology with genomes according to a 16S rRNA gene phylogeny and ii) predicted functional gene content is corrected to the expected 16S rRNA gene copy number inferred by phylogeny. The OTU abundance table was imported into PiCRUSt and functional predictions (Kyoto Encyclopedia of Genes and Genomes (KEGG) classifications;(Kanehisa & Goto 2000) were made according to the metagenome inference workflow within Galaxy. The effect of site, experimental N deposition, and their interaction on predicted KEGG pathway abundance and composition was determined by two-way ANOVA combined with a Fisher's LSD (SPSS), and PerMANOVA, respectively.

Results and Discussion

Composition of saprotrophic bacteria under ambient and experimental N deposition

Prior to quality control (QC), we obtained 456,221 16S rRNA gene sequences; post-QC, 214,424 sequences remained (53% loss). After quality filtering and OTU clustering (97% similarity), 133,368 DNA

sequences (5,557 sequences per plot; n = 24) were distributed among 48,746 OTUs. The "core" community (*i.e.*, OTUs present in half of experimental plots for each treatment) consisted of 2,385, and 2,198 OTUs in ambient and experimental N deposition treatments, respectively, which were included in all subsequent analyses.

Experimental N deposition did not change the relative abundances of core bacterial phyla in the forest floor (Figure S2; $P > 0.05$), but it did reduce the diversity and altered the composition of the core bacterial community. Bacterial assemblages in the forest floor were dominated by OTUs classified as *Proteobacteria* (~45%), *Actinobacteria* (~18%), *Bacteroidetes* (~18%), and *Acidobacteria* (~12%). The bacterial community was less rich (Chao1; -13%; $F_{1, 23} = 13.4$; $P < 0.01$; Table S1), and slightly less diverse (Shannon Index; -0.5%; $F_{1, 23} = 6.8$; $P = 0.02$) under experimental N deposition. Bacterial assemblages were similarly affected by the experimental N deposition across sites, evidenced by a non-significant site by treatment ($P > 0.30$). Bacterial diversity was similar across sites (site effect; $P > 0.10$).

Bacterial community composition was altered by experimental N deposition (Figure S3; PerMANOVA; Pseudo-F = 1.64; $P = 0.02$); it also differed across the four forest stands (Pseudo-F = 2.48; $P < 0.01$). The bacterial community exhibited greater heterogeneity under experimental N deposition, relative to the ambient treatment (PERMDISP; ambient dispersion = 39.5 ± 0.75; experimental N deposition dispersion = 42.2 ± 0.74; $P = 0.02$) and responded similarly to the experimental N deposition across the four sites (site × treatment; Pseudo-F = 1.22; $P = 0.09$).

It is well established that soil bacterial communities are sensitive to increased N deposition (Fierer *et al.* 2012; Freedman & Zak 2014; Frey *et al.* 2004). Furthermore, certain bacterial phyla are consistently affected by N deposition across various soil types (Ramirez *et al.* 2012). In our experiment,

we determined that experimental N deposition reduced the diversity and altered the composition of the core soil bacterial community, but did not change the relative abundances of core bacterial phyla. Our results indicate that more broad measures of bacterial composition (*e.g.*, TRFLP, PLFA) may not be sufficient to capture compositional changes among the core bacterial community in our experiment, as observed changes occur at fine scales of genetic resolution.

Effects of N deposition on bacterial phylogenetic dispersion

Experimental N deposition altered the phylogenetic composition of the saprotrophic bacterial community (weighted UniFrac; PerMANOVA; Pseudo-F = 2.39; $P = 0.01$). Bacterial communities also differed in composition across sites (Pseudo-F = 2.20; $P = 0.01$). To attain a more resolved understanding of changes in phylogenetic structure due to experimental N deposition, Rao's Quadratic Entropy (Dp), net relatedness index (NRI), and nearest taxon index (NTI) were calculated. Experimental N deposition affected bacterial phylogenetic composition similarly across sites (site × treatment, $P = 0.62$); for this reason, we determined the phylogenetic structure at the treatment level (*i.e.*, ambient and experimental N deposition;(*sensu*(Cline & Zak 2014; Peay *et al.* 2010).

Bacterial assemblages under both ambient and experimental N deposition exhibited similar phylogenetic diversity (Dp = 0.63 and 0.64 for ambient and experimental N deposition assemblages, respectively), but different phylogenetic structure as indicated by the NRI and NTI. The NRI was significantly different from zero ($P < 0.05$) for bacterial assemblages under both ambient and experimental N deposition. In the ambient treatment, the significantly positive NRI (+2.25) indicated that the saprotrophic bacterial assemblages were more phylogenetically related than expected by chance (*i.e.*, phylogenetic clustering). Whereas, the bacterial community under experimental N deposition exhibited a significantly negative NRI (-2.03), suggesting that the bacterial community was

less phylogenetically related than expected by chance (*i.e.*, phylogenetic overdispersion). The NTI, which is sensitive to branch tip clustering (Webb 2000), did not differ from zero under either ambient or experimental N deposition, indicating that phylogenetic differences among bacterial communities occur at a phylogenetically deep level, and not at finer-scales of phylogenetic relatedness. Phylogenetically clustered distributions are indicative of habitat filtering, in which a group of closely related species share ecological traits, or suite of traits, that allow them to persist in a given habitat. Conversely, phylogenetic overdispersion may be due to negative (*e.g.*, competition) or positive (*e.g.*, facilitation) interactions being important drivers of microbial community assembly (Graves & Gotelli 1993; Valiente-Banuet & Verdu 2007; Webb 2000). Although we cannot definitively determine whether these ecological forces are operating *in situ*, our results suggest that bacterial communities under ambient and experimental N deposition may be responding to very different mechanisms of community assembly.

Effects of N deposition on bacterial co-occurrence network topology

Bacterial assemblages exhibited non-random co-occurrence patterns under both ambient (C-score = 0.59 ± 0.002; $P < 0.01$) and experimental N deposition (0.61 ± 0.003; $P = 0.02$). Moreover, co-occurrence patterns differed between bacterial communities in the ambient and experimental deposition treatments (PerMANOVA; Pseudo-F = 1.57, $P = 0.01$); this effect was similar across sites (site × treatment; Pseudo-F = 1.21, $P = 0.09$). Non-random co-occurrence patterns have been observed within bacterial communities in marine environments (Fuhrman & Steele 2008; Prosser *et al.* 2007), rivers (Widder *et al.* 2014), soils (Barberan *et al.* 2012; Williams *et al.* 2014), and beneath Antarctic ice-covered lakes (Vick-Majors *et al.* 2013). Moreover, non-random community assembly may be a general characteristic across all domains of life (Gotelli & McCabe 2002; Horner-Devine *et al.* 2007). Overall, soil microorganisms under both ambient and experimental N deposition tended to co-occur more than expected by chance, suggesting the dominance of deterministic processes (*e.g.*, competitive

interactions, niche differentiation) in shaping community composition (Horner-Devine *et al.* 2007). Moreover, future rates of atmospheric N deposition altered bacterial co-occurrence patterns, suggesting that this pervasive agent of global change is likely to exert a selective force on soil bacterial communities, thereby altering their composition and functional potential.

Two decades of experimental N deposition resulted in a more clustered bacterial network topology. The ambient N deposition network contained 1740 nodes (*i.e.*, OTUs) with 68,402 edges (*i.e.*, significant correlations), whereas the experimental N deposition network contained 1706 nodes with 78,840 edges (Figures S4, S5). Both networks exhibited greater connectivity than the average of 100 randomly generated networks of the same size; for example, our networks exhibited a higher clustering coefficient, average number of neighbors, and network density and a lower characteristic path length and heterogeneity (Table S2). Moreover, the OTU network under experimental N deposition exhibited greater connectivity than under ambient conditions, as indicated by a greater clustering coefficient (+12%), average number of neighbors (+18%), density (+42%), and a slightly lower characteristic path length (-1.5%) as well as number of nodes (-2.0%; Figure 1; Table S3). Networks calculated from genomic survey data in low-richness communities (i.e., < 30 OTUs) may be biased by "compositional effects", which can be overcome using corrective algorithms (*e.g.*, SparCC; Friedman & Alm 2012). Such corrections were not necessary for this dataset, because the richness of the communities from which our networks were derived (> 2,000 OTUs) are an order of magnitude greater than the low-richness communities known to produce spurious correlations (Friedman & Alm 2012). Furthermore, network topology can be less reliable between communities as the strength of habitat filtering increases (Berry & Widder 2014). However, in this study, heterogeneity between bacterial communities under ambient and experimental N deposition (~7% difference) was less than that demonstrated to adversely affect network specificity (~30%).

In this study, rates of N deposition expected by mid-century altered the network topology of saprotrophic bacterial communities. The shape of the node distribution affects the stability of the complex systems; for example, macroecological network theory predicts that communities of tightly connected species should be more susceptible to disturbance (Montoya *et al.* 2006; Saavedra *et al.* 2011). Presently, empirical studies that explore the effects of anthropogenic perturbations on microbial networks are few (Faust *et al.* 2012; Widder *et al.* 2014). Here, the resulting networks are consistent with "small-world" network properties, wherein nodes exhibit greater connectivity (*i.e.*, high clustering coefficient, short characteristic path length) than a random network of similar size (Watts & Strogatz 1998). Small-world connectivity has been linked to decreased co-operative behavior in social networks and increased infectiousness in network models of disease spreading; however, the effects of "small-world" type interactions in microbial communities is not understood at this time (Watts & Strogatz 1998).

Saprotrophic bacterial assemblages under ambient and experimental N deposition exhibited a modular structure (Table S3; Q-values = 0.45 and 0.46 for ambient and N deposition, respectively; values > 0.4 suggest that the network is modular;(Newman 2006). In a network, a module is a highly interconnected group of nodes. Modularity is a characteristic of many large complex systems, including biological, social, and technological (*e.g.*, the world wide web) networks (Barabasi & Oltvai 2004; Kitano 2004; Milgram 1967; Olesen *et al.* 2007; Vick-Majors *et al.* 2013). Among biotic communities, modularity quantifies the tendency of species to be grouped into modules, in which interactions are more frequent than with the rest of the community. Communities may assemble into modules based on the functional complementarity of their traits and may offer some insight into coevolutionary dynamics between symbiotic species (Guimaraes *et al.* 2007; Thompson 2005). The modularity metrics (Q or M score) observed in our study (Q = ~0.46) are notably lower than that observed in farm food webs (M =

0.79;(Macfadyen *et al.* 2009) or pollinator networks (M = 0.52;(Olesen *et al.* 2007). It was also lower than that observed in soil microbial communities encompassing a broad range of ecosystems, climates, and soil types (Q = 0.77;(Barberan *et al.* 2012). Lower modularity in our study may be due to less pronounced niche differentiation between soil microbial communities that were sampled from the same ecosystem and soil type.

Effect of N Deposition on the Predicted Functional Potential

Changes in microbial community composition are often associated with changes in their functional capabilities (Fierer *et al.* 2012; Strickland *et al.* 2009); thus, understanding the biotic and abiotic mechanisms that affect microbial communities and their functional genes can be critical for understanding how ecosystem processes may respond to environmental change. To determine whether differences in taxonomic, phylogenetic, and network structure between ambient and experimental N deposition affected the functional potential of the saprotrophic bacterial community, we employed PiCRUSt (Langille *et al.* 2013) to predict the metagenomic structure (*i.e.*, functional potential) of each community. Comparison of the predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologies (KOs) demonstrated significant similarity between bacterial assemblages exposed to ambient and experimental N deposition (PerMANOVA; $P > 0.4$). The relative abundance of KOs were also similar between the ambient and experimental N deposition communities (Figure S6). In both communities, a majority of the predicted metagenome was attributable to the metabolism gene pathway (52%), followed by genetic information (16%), environmental information processing (14%), and unclassified (14%).

Towards a Molecular Mechanism Mediating C Storage in Northern Hardwood Forests

Experimental N deposition has altered biotic interactions among the saprotrophic microbial community, resulting in a phylogenetically over-dispersed, more tightly connected community. These community-level changes underlie a biogeochemical response, as 20 years of experimental N deposition has reduced litter decay, and increased SOM and DOC leaching in our long-term experiment (Zak *et al.* 2008), indicative of a larger phenomenon observed among terrestrial ecosystems exposed to simulated N deposition (Frey *et al.* 2004; Liu & Greaver 2010).

Evidence presented here supports the hypothesis that experimental N deposition increased biotic interactions among the saprotrophic bacterial community. However, given that the interactions between the thousands of microbial taxa (*i.e.*, nodes) are not understood, our lack of autecological knowledge limits our ability to draw conclusions regarding the effects of chronic N deposition on the nature of biotic interactions within these bacterial communities (*i.e.,* positive or negative). Taken together, evidence of phylogenetic overdispersion and a more connected (*i.e.*, "small-world") network, indicate that deterministic process and increased negative (*e.g.*, competition) or positive (*e.g.,* facilitiation) interactions together drive microbial community assembly under accelerated rates of anthropogenic N deposition (Hodkinson *et al.* 2002; Watts & Strogatz 1998). In a similar study, phylogenetic clustering decreased with increasing dissolved organic C in some soils, suggesting that as substrate availability increased, the strength of clustering and perhaps habitat filtering decreased (Horner-Devine & Bohannan 2006). The potential decrease in the strength of filtering is unlikely to be related to an increase in the role of competition for polyphenolic compounds, because competition would likely decrease with increasing organic matter availability.

Here, we observed a less rich, yet more phylogenetically dispersed, and connected saprotrophic bacterial community under experimental N deposition; however, these changes did not result in discernable differences in abundance or composition of the predicted metagenomic functional gene pathways (Figure S6). Phylogenetic conservatism of functional traits and the use of trait-based models have suggested microbial phylogeny may be useful in predicting community function (Lennon *et al.* 2012; Martiny *et al.* 2013). Moreover, metagenome prediction tools (*e.g.*, PiCRUSt) can provide inference to the functional capabilities of a community based on marker gene (*i.e.*, 16S rRNA gene) profiles and can replicate biological findings from associated shotgun metagenomes (Langille *et al.* 2013). Our evidence indicates that the saprotrophic bacterial community may exhibit functional equivalency at a broad level to future rates of anthropogenic N deposition, albeit using the tools presently available to us. Clearly, a more targeted approach is necessary to gain greater insight to whether the saprotrophic bacteria community is indeed functionally resilient to this agent of global change.

In conclusion, long-term experimental N deposition consistently and significantly altered saprotrophic bacterial communities in the forest floor. Observed changes were robust, as evidenced by complimentary metrics of community composition. From this, we suggest that experimental N deposition can modify the organization of microbial communities and may be one factor underlying reduced decay, the accumulation of soil organic matter, and greater phenolic dissolved organic C production in our long-term experiment (Pregitzer *et al.* 2004). Overall, chronic experimental N deposition has fundamentally altered the composition of the saprotrophic bacterial community; however, understanding how compositional changes influence functional responses remains elusive, at least as assayed by the methods used here. Nonetheless, our observations support the idea changes in community connectedness and the nature of biotic interactions among saprotrophic soil bacteria may

be an important mechanism by which anthropogenic N deposition alters the cycling and storage of C in soil.

Tables and Figures

Table 1: Site, climatic, overstory, and ambient nitrogen deposition rates of four study sites receiving experimental NO_3^- additions.

Characteristic	Site A	Site B	Site C	Site D
Location				
Latitude, N	46°52”	45°33”	44°23”	43°40”
Longitude, W	88°53”	84°52”	85°50”	86°9”
Climate				
Mean annual temperature	4.7	6.0	6.9	7.6
Mean annual precipitation	873	871	888	812
Wet + dry NO_3^-–N deposition, g N m^{-2} yr^{-1}	0.38	0.58	0.78	.076
Wet + dry total N deposition, g N m^{-2} yr^{-1}	0.68	0.91	1.17	1.18
Vegetation				
Overstory biomass, Mg ha^{-1}	261	261	274	234
Acer saccharum biomass, Mg ha^{-1}	237	224	216	201
Soil (0-10 cm)				
Sand, %	85	89	89	87
pH (1:1 soil/H_2O)	4.8	5.0	4.5	4.7
Cation exchange capacity, $mmol_c$ kg^{-1}	3.4	3.8	2.6	3.0
Base saturation, %	71	96	73	80

Table adapted from Eisenlord et al. 2010.

Table 2: Description of network properties potentially relevant for community roles and functioning

Property	Description	Ecological Interpretation
Average Shortest Path Length	The expected distance between two connected nodes	Short path length can increase the response speed of perturbations in a biotic network[1]
Average Number of Neighbors	The average number of connections between nodes	Networks with high average number neighbors, clustering coefficients, and density, are robust and resistant to random node failure[23].
Clustering Coefficient	A cluster is a triangle of nodes. The clustering coefficient is the fraction of observed vs. possible clusters for each node.	
Density	How completely the network is populated with edges. The density is 1 when all nodes are connected and is zero with solely isolated nodes.	
Heterogeneity	The coefficient of variation of the connectivity *i.e.*, density distribution.	High heterogeneity is indicative of few highly connected nodes hub or "keystone" nodes with the majority of nodes having few connections[12].
Modularity	Tendency of a network to contain sub-clusters of nodes.	Modules have been interpreted as niches or clusters in which interactions may be more frequent than with others[14567].

[1](Barabasi & Oltvai 2004)

[2](Gutteridge *et al.* 2007)

[3](Zhao *et al.* 2006)

[4](Zhou *et al.* 2010)

[5](Vick-Majors *et al.* 2013)

[6](Dong & Horvath 2007)

[7](Barberan *et al.* 2012).

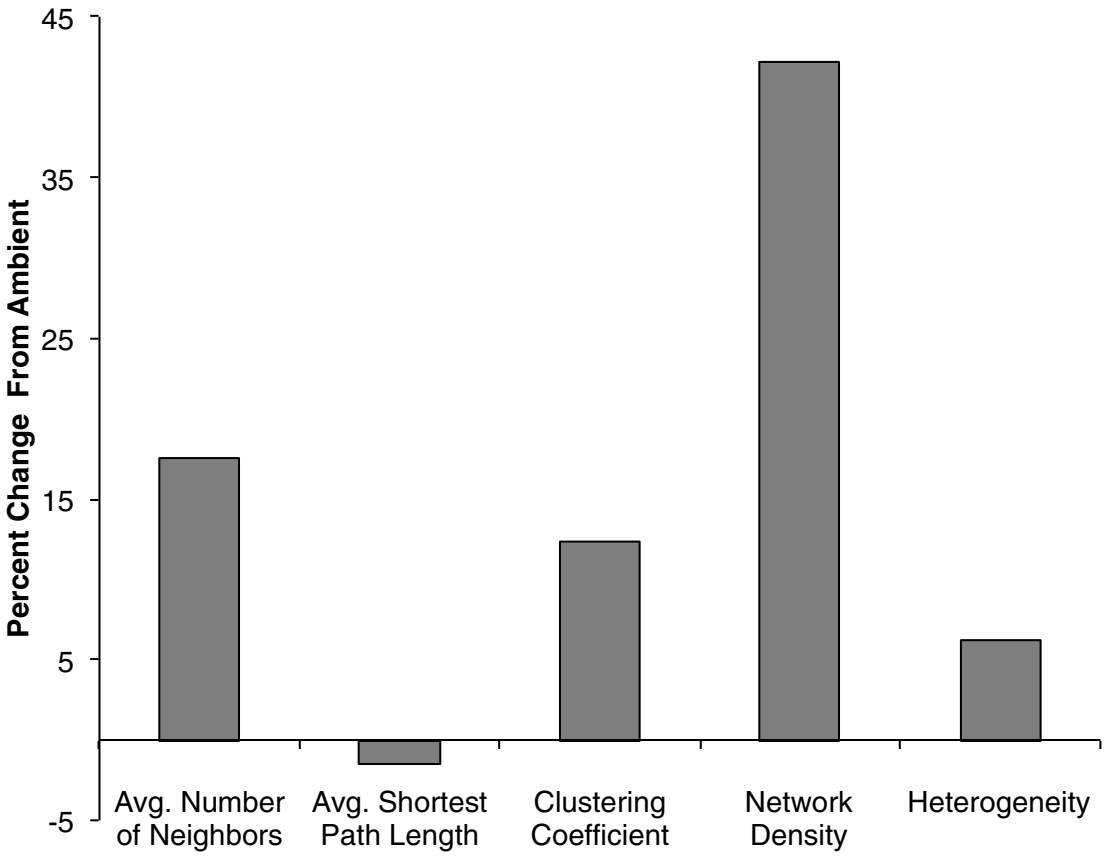

Figure 1: Network topology metrics for bacterial molecular ecological networks under ambient and experimental N deposition. Data are presented as percent change from ambient conditions. A description of network metrics can be found in Table 2, and relevant data can be found in Table S3.

Acknowledgements

We thank William Argiroff and Catherine Doktycz for their work in sample preparation, Sarah Eisenlord and Rima Upchurch for their guidance with molecular methods, and Christine McHenry, Joseph Washburn, and Robert Lyons for their help in DNA sequencing and analysis. We also wish to thank Rebecca Mueller (Los Alamos National Lab), other members of the Zak lab, and three anonymous reviewers for their thoughtful feedback on this manuscript. Grants from the U. S. Department of

Energy's BER program, the NSF LTREB program, and USDA McIntire-Stennis Program provided support for our work. The authors declare no conflict of interest. Supplementary information is available at *Molecular Ecology's* website.

References

Allison SD, Martiny JB (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci U S A* **105 Suppl 1**, 11512-11519.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**, 32-46.

Anderson MJ (2004) *PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests* Depertmant of Statistics, University of Auckland, Auckland, New Zealand.

Assenov Y, Ramirez F, Schelhorn SE, Lengauer T, Albrecht M (2008) Computing topological parameters of biological networks. *Bioinformatics* **24**, 282-284.

Barabasi AL, Oltvai ZN (2004) Network biology: understanding the cell's functional organization. *Nature Reviews Genetics* **5**, 101-113.

Barberan A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *The ISME Journal* **6**, 343-351.

Berry D, Widder S (2014) Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology* **5**, 219.

Braun EL (1950) *Deciduous forests of eastern North America* Macmillan Publishing Co, Inc., New York,

NY.

Brown SP, Jumpponen A (2014) Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. *Molecular Ecology* **23**, 481-497.

Chaffron S, Rehrauer H, Pernthaler J, von Mering C (2010) A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Research* **20**, 947-959.

Chao A (1984) Nonparametricestimation of the number of classes in a population. *Scandinavian Journal of Statistics* **11**, 265-270.

Cline LC, Zak DR (2014) Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. *Environmental Microbiology* **16**, 1538-1548.

DeSantis TZ, Hugenholtz P, Larsen N*, et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* **72**, 5069-5072.

Dong J, Horvath S (2007) Understanding network concepts in modules. *Bmc Systems Biology* **1**.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194-2200.

Edwards IP, Zak DR, Kellner H, Eisenlord SD, Pregitzer KS (2011) Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. *PLoS One* **6**, e20421.

Eid J, Fehr A, Gray J*, et al.* (2009) Real-time DNA sequencing from single polymerase molecules. *Science* **323**, 133-138.

Eisenlord SD, Freedman Z, Zak DR*, et al.* (2013) Microbial Mechanisms Mediating Increased Soil C Storage under Elevated Atmospheric N Deposition. *Applied and Environmental Microbiology* **79**, 1191-1199.

Entwistle EM, Zak DR, Edwards IP (2013) Long-Term Experimental Nitrogen Deposition Alters the Composition of the Active Fungal Community in the Forest Floor. *Soil Science Society of America Journal* **77**, 1648-1658.

Erdös P, Rényi A (1959) On Random Graphs. I. *Publicationes Mathematicae* **6**, 290-297.

Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**, 1034-1039.

Faust K, Sathirapongsasuti JF, Izard J*, et al.* (2012) Microbial co-occurrence relationships in the human microbiome. *PLoS Comput Biol* **8**, e1002606.

Fichot EB, Norman RS (2013) Microbial phylogenetic profiling with the Pacific Biosciences sequencing platform. *Microbiome* **1**, 10.

Fierer N, Lauber CL, Ramirez KS*, et al.* (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* **6**, 1007-1017.

Freedman Z, Eisenlord SD, Zak DR*, et al.* (2013) Towards a molecular understanding of N cycling in northern hardwood forests under future rates of N deposition. *Soil Biology and Biochemistry* **66**, 130-138.

Freedman Z, Zak DR (2014) Atmospheric N deposition increases bacterial laccase-like multicopper oxidases: implications for organic matter decay. *Applied and Environmental Microbiology* **80**,

4460-4468.

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* **196**, 159-171.

Friedman J, Alm EJ (2012) Inferring correlation networks from genomic survey data. *PLoS Computational Biology* **8**, e1002687.

Fuhrman JA, Steele JA (2008) Community structure of marine bacterioplankton: patterns, networks, and relationships to function. *Aquatic Microbial Ecology* **53**, 69-81.

Galloway JN, Dentener FJ, Capone DG*, et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**, 153-226.

Goecks J, Nekrutenko A, Taylor J, Team G (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biology* **11**.

Gotelli NJ, McCabe DJ (2002) Species co-occurrence: A meta-analysis of J. M. Diamond's assembly rules model. *Ecology* **83**, 2091-2096.

Graves GR, Gotelli NJ (1993) Assembly of Avian Mixed-Species Flocks in Amazonia. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 1388-1391.

Guimaraes PR, Jr., Rico-Gray V, Oliveira PS*, et al.* (2007) Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Currant Biology* **17**, 1797-1803.

Gutteridge A, Kanehisa M, Goto S (2007) Regulation of metabolic networks by small molecule metabolites. *Bmc Bioinformatics* **8**.

Hodkinson ID, Webb NR, Coulson SJ (2002) Primary community assembly on land - the missing stages: why are the heterotrophic organisms always there first? *Journal of Ecology* **90**, 569-577.

Horner-Devine MC, Bohannan BJ (2006) Phylogenetic clustering and overdispersion in bacterial communities. *Ecology* **87**, S100-108.

Horner-Devine MC, Silver JM, Leibold MA*, et al.* (2007) A Comparison of Taxon Co-Occurrence Patterns for Macro and Microorganisms. *Ecology* **88**, 1345-1353.

Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* **28**, 27-30.

Kitano H (2004) Biological robustness. *Nature Reviews Genetics* **5**, 826-837.

Lane DJ (1991) 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematics* (eds. Stackebrandt E, Goodfellow M), pp. 115-175. Chichester, New York, NY.

Langille MGI, Zaneveld J, Caporaso JG*, et al.* (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* **31**, 814-+.

Legendre P, Legendre L (1998) *Numerical Ecology*, 2 edn. Elsevier, Amsterdam, The Netherlands.

Lennon JT, Aanderud ZT, Lehmkuhl BK, Schoolmaster DR (2012) Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* **93**, 1867-1879.

Liu LL, Greaver TL (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters* **13**, 819-828.

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* **71**, 8228-8235.

Macfadyen S, Gibson R, Polaszek A*, et al.* (2009) Do differences in food web structure between organic and conventional farms affect the ecosystem service of pest control? *Ecology Letters* **12**, 229-238.

Marshall CW, Ross DE, Fichot EB, Norman RS, May HD (2012) Electrosynthesis of commodity chemicals by an autotrophic microbial community. *Applied and Environmental Microbiology* **78**, 8412-8420.

Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *The ISME Journal* **7**, 830-838.

Milgram S (1967) The small world problem. *Psychology Today* **2**, 60.

Montoya JM, Pimm SL, Sole RV (2006) Ecological networks and their fragility. *Nature* **442**, 259-264.

Morris JH, Apeltsin L, Newman AM*, et al.* (2011) clusterMaker: a multi-algorithm clustering plugin for Cytoscape. *Bmc Bioinformatics* **12**.

Needleman SB, Wunsch CD (1970) A general method applicable to search for similarities in amino acid sequence of 2 proteins. *Journal of Molecular Biology* **48**, 443-453.

Newman ME (2006) Modularity and community structure in networks. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 8577-8582.

Olesen JM, Bascompte J, Dupont YL, Jordano P (2007) The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19891-19896.

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD (2010) Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical

ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist* **185**, 529-542.

Pregitzer KS, Burton AJ, Zak DR, Talhelm AF (2008) Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. *Global Change Biology* **14**, 142-153.

Pregitzer KS, Zak DR, Burton AJ, Ashby JA, MacDonald NW (2004) Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry* **68**, 179-197.

Price MN, Dehal PS, Arkin AP (2010) FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One* **5**, e9490.

Prosser JI, Bohannan BJM, Curtis TP*, et al.* (2007) Essay - The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* **5**, 384-392.

Proulx SR, Promislow DE, Phillips PC (2005) Network thinking in ecology and evolution. *Trends in Ecology & Evolution* **20**, 345-353.

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* **18**, 1918-1927.

Rao R (1982) Diversity and dissimilarity coefficients: a unified approach. . *Theoretical Popualtion Biology* **21**, 24-43.

Saavedra S, Stouffer DB, Uzzi B, Bascompte J (2011) Strong contributors to network persistence are the most vulnerable to extinction. *Nature* **478**, 233-235.

Schloss PD, Westcott SL, Ryabin T*, et al.* (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**, 7537-7541.

Shade A, Hogan CS, Klimowicz AK*, et al.* (2012) Culturing captures members of the soil rare biosphere. *Environmental Microbiology* **14**, 2247-2252.

Shannon CE, Weaver W (1963) *The mathematical theory of communication* University of Illinois Press, Champaign, IL.

Shannon P, Markiel A, Ozier O*, et al.* (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research* **13**, 2498-2504.

Steele JA, Countway PD, Xia L*, et al.* (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *The ISME Journal* **5**, 1414-1425.

Stone L, Roberts A (1990) The Checkerboard Score and Species Distributions. *Oecologia* **85**, 74-79.

Strickland MS, Lauber C, Fierer N, Bradford MA (2009) Testing the functional significance of microbial community composition. *Ecology* **90**, 441-451.

Thompson JN (2005) Coevolution: the geographic mosaic of coevolutionary arms races. *Currant Biology* **15**, R992-994.

Torseth K, Aas W, Breivik K*, et al.* (2012) Introduction to the European Monitoring and Evaluation Programme (EMEP) and observed atmospheric composition change during 1972-2009. *Atmospheric Chemistry and Physics* **12**, 5447-5481.

Travers KJ, Chin CS, Rank DR, Eid JS, Turner SW (2010) A flexible and efficient template format for circular consensus sequencing and SNP detection. *Nucleic Acids Research* **38**, e159.

Unterseher M, Jumpponen A, Opik M*, et al.* (2011) Species abundance distributions and richness estimations in fungal metagenomics--lessons learned from community ecology. *Molecular Ecology* **20**, 275-285.

Valiente-Banuet A, Verdu M (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters* **10**, 1029-1036.

Vick-Majors TJ, Priscu JC, L AA-Z (2013) Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *The ISME Journal*.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**, 5261-5267.

Watts DJ, Strogatz SH (1998) Collective dynamics of 'small-world' networks. *Nature* **393**, 440-442.

Webb CO (2000) Exploring the Phylogenetic Structure of Ecological Communities: An Example for Rain Forest Trees. *American Naturalist* **156**, 145-155.

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**, 2098-2100.

Widder S, Besemer K, Singer GA*, et al.* (2014) Fluvial network organization imprints on microbial co-occurrence networks. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 12799-12804.

Williams RJ, Howe A, Hofmockel KS (2014) Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. *Frontiers in Microbiology* **5**, 358.

Zak DR, Holmes WE, Burton AJ, Pregitzer KS, Talhelm AF (2008) Simulated atmospheric NO_3^- deposition increases soil organic matter by slowing decomposition. *Ecological Applications* **18**, 2016-2027.

Zhao J, Yu H, Luo JH, Cao ZW, Li YX (2006) Hierarchical modularity of nested bow-ties in metabolic networks. *Bmc Bioinformatics* **7**.

Zhao MX, Xue K, Wang F*, et al.* (2014) Microbial mediation of biogeochemical cycles revealed by simulation of global changes with soil transplant and cropping. *The ISME Journal* **8**, 2045-2055.

Zhou JZ, Deng Y, Luo F*, et al.* (2010) Functional Molecular Ecological Networks. *MBio* **1**.

Data Accessibility

DNA Sequences: NCBI SRA: SRR1950983

Bioinformatic pipeline and OTU tables: Dryad doi: 10.5061/dryad.n20ms.

Author Contributions

D.R.Z. designed the study and sampling design. Z. B. F. prepared the samples for molecular analysis, analyzed molecular data and performed the statistical analyses. Z.B.F and D. R. Z. wrote the manuscript.