Soil Biology & Biochemistry 66 (2013) 130–138

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Towards a molecular understanding of N cycling in northern hardwood forests under future rates of N deposition

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ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form
3 June 2013
Accepted 18 July 2013
Available online 31 July 2013

Keywords:
N deposition
Bacteria
Archaea
Climate change
Dispersion
N cycle

ABSTRACT

The combustion of fossil fuels and fertilizer use has increased the amount of biologically available N over the last 150 years. Future rates of atmospheric N deposition may slow organic matter decay and alter microbial community composition and function. However, our understanding of how anthropogenic N enrichment may alter the physiological mechanisms by which soil microorganisms assimilate and cycle N in soil are largely unknown. Since 1994, we have experimentally increased NO_3 deposition to replicate ($n = 4$) northern hardwood forest stands across a 500-km climatic gradient in the Great Lakes region of North America. Our goal was to examine how functional genes mediating N-cycle processes in soil microbial communities have responded to experimental N deposition using the functional gene microarray, GeoChip 4.0. Experimental N deposition decreased the abundance and richness of key protein-coding genes in Archaea and Bacteria responsible for N fixation, ammonification, denitrification and assimilatory nitrate reduction; the same was true for bacterial genes mediating nitrification and dissimilatory nitrate reduction. However, the extent to which experimental N deposition decreased abundance and richness was site-specific, which was revealed by a significant site by treatment interaction. Experimental N deposition also caused a community composition shift via dispersion (increased β-diversity) in archaeal and bacterial gene assemblages. In combination, our observations suggest future rates of atmospheric N deposition could fundamentally alter the physiological potential of soil microbial communities.

1. Introduction

Atmospheric deposition of biologically available nitrogen (N) has increased ten-fold across much of the Northern Hemisphere over the last 150 years (e.g., from 0.5 to 1 to 15–20 kg N ha^{-1} y^{-1}) and is projected to more than double in the next century (Galloway et al., 2004). At present, anthropogenic creation of reactive N surpasses that of all natural terrestrial sources combined (Vitousek et al., 1997). Increased N availability in terrestrial ecosystems, such as temperate forests in which plant growth is often N limited (Vitousek et al., 2002), may lead to a phenomenon called N saturation (Aber et al., 1998, 1989) which models a series of plant and soil responses as N limitation is alleviated. Sugar maple (*Acer saccharum* Marsh.) dominated forests in the upper Great Lakes region, U. S. A., are especially prone to N saturation (Zak et al., 2006) due to their high rates of net N mineralization (80–120 kg N ha^{-1} y^{-1}; Zak and Pregitzer, 1990), and moderate rates of atmospheric deposition (7–12 kg N ha^{-1} y^{-1}; MacDonald et al., 1991).

Since 1994, replicate forest stands have experimentally received experimental NO_3^- deposition across a 500-km climatic gradient spanning the north-south geographic range of the sugar maple dominated northern hardwood forest in the Great Lakes region of North America (Braun, 1950). We have demonstrated that experimental N deposition at a rate expected by 2050 across North America and other regions (Galloway et al., 2004) has increased woody biomass production, as well as organic matter stored in the forest floor and surface mineral soil (Pregitzer et al., 2008). Surprisingly, the observed increase in soil C sequestration has occurred despite no change in above- or belowground litter production (Burton et al., 2004), indicating organic matter has accumulated due to a reduction in plant litter decay (Zak et al., 2008).

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http://dx.doi.org/10.1016/j.soilbio.2013.07.010

It is well established that litter decay rates are correlated with the initial ratios of C:N, lignin:N (Aerts, 1997; Hobbie, 2008; Prescott, 2010), and interactions among N, lignin, and cellulose (Talbot and Treseder, 2012). Thus, our understanding of how microbial communities directly involved in N cycling respond to anthropogenic N enrichment is key to understanding the fate of C and N under environmental change. Nitrogen enrichment can decrease microbial respiration (Burton et al., 2004; Janssens et al., 2010) and biomass (Wallenstein et al., 2006) and alter archaeal (Nemergut et al., 2008), bacterial (Eisenlord and Zak, 2010; Fierer et al., 2011; Nemergut et al., 2008), and fungal (Allison et al., 2007; Edwards et al., 2011) community composition. Our knowledge regarding the function of archaeal communities in the microbial N-cycle is both emerging and essential, as Archaea exhibit a common presence in soils (Bates et al., 2011) and the ability to mediate both assimilatory and dissimilatory processes of the N cycle (Cabello et al., 2004). For example, Archaea are dominant among ammonia-oxidizing prokaryotes across a variety of soil types (Leininger et al., 2006). Although most studies focus on how anthropogenic N enrichment may affect microbial populations, impacts on specific microbial communities responsible for soil N cycling processes is poorly understood.

We hypothesized that the experimental N deposition has altered the functional potential of forest floor microbial assemblages. From this hypothesis, we predict a decline in abundance and diversity of archaeal and bacterial functional genes related to N-cycling, as well a shift in community composition of functional gene assemblages mediating N cycling processes in response to chronic N deposition. To test our hypothesis, we used GeoChip 4.0, a PCR-independent microarray encompassing 73 archaeal and 2803 bacterial genes mediating N cycling processes in soil.

2. Materials and methods

2.1. Site description

The influence of simulated N deposition on soil microbial communities was investigated in four sugar maple (*A. saccharum* Marsh.) dominated northern hardwood forest stands in lower and upper Michigan, USA (Fig. 1). These locations span the north-south geographic range of the northern hardwood forests in the Great Lakes region of North America (Braun, 1950). The sites are floristically and edaphically similar, but span a climatic and atmospheric N deposition gradient (Table 1; MacDonald et al., 1991; Zak et al., 2008). The forest floor is composed of a thin Oi horizon dominated by sugar maple leaf litter, and a thicker Oe horizon interpenetrated by a dense root mat. At each study site, six 30-m by 30-m plots were established in 1994. Three plots in each stand receive ambient N deposition, and three receive experimental N deposition which consists of six equal applications of $NaNO_3$ pellets delivered to the forest floor over the growing season (30 kg N ha^{-1} y^{-1}). In our study sites, NO_3^- comprises ~60% of wet plus dry atmospheric N deposition (MacDonald et al., 1992).

Fig. 1. The geographic distribution of the study sites A–D in Lower and Upper Michigan. In each stand beginning in 1994, three plots received ambient atmospheric N deposition and three plots received ambient plus 30 kg NO_3–N ha^{-1} yr^{-1}.

2.2. Forest floor collection and DNA extraction

Forest floor sampling was performed in early October 2009, a time in which ample moisture favors high rates of microbial activity. In each 30-m by 30-m plot, 10 random 0.1-m by 0.1-m forest floor samples (Oe/Oa horizons) were collected after manually removing the freshly fallen Oi horizon. Following manual removal of large organic debris, samples were composited, homogenized by hand, and were immediately frozen in the field using liquid N_2.

Genomic DNA was extracted from 2.5 g of forest floor samples using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. Extracted DNA was quantified by the Quant-iT PicoGreen (Invitrogen, Carlsbad, CA, USA) assay according to manufacturer's instructions and stored at −80 °C until further analysis.

2.3. Target preparation, GeoChip hybridization, and data preprocessing

GeoChip 4.0 was applied in this study as described previously (Lu et al., 2011). A full list of functional genes and the number of associated probes (gene variants) mediating N cycling processes are included in the supplementary information (Table S1). Briefly, 1 μg of genomic DNA from each replicate sample was purified by the Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA, USA) and labeled with fluorescent dye Cy-3 using random primers (Wu et al., 2006). The labeled gDNA was dried and rehydrated with 2.7 μl of sample tracking control to confirm sample identity, followed by incubation at 50 °C for 5 min. This DNA solution was mixed with 7.3 μL of hybridization buffer containing the universal

Table 1
Site, climactic, 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	0.76
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 and Zak (2010).

standard DNA labeled with Cy-5 dye; it was then denatured at 95 °C for 5 min and then maintained at 42 °C until it was loaded onto GeoChip arrays (NimbleGen, Madison, WI, USA). The hybridization was performed on a Hybridization Station (MAUI, Roche, CA, USA) at 42 °C for 16 h with agitation. After three washings, the arrays were scanned using a MS 200 Microarray Scanner (NimbleGen) at laser power of 100% PMT (photomultiplier tube). The signal intensities of spots on GeoChip were normalized across samples by the mean of Cy-5 labeled universal standard signal intensities and the sum of Cy-3 labeled signal intensities of hybridized spots within samples. After this two-step normalization, the unreliable or "noise" spots were removed when their original signal intensities were <2000, signal-to-noise ratio (SNR) was ≤2.0, or the coefficient of variation (CV) of the background was >0.8. Probes that appeared in only one of three replicates in each treatment group were also excluded. Natural-log transformations were then applied to all normalized signal intensities, and the final value of each spot was expressed as the ratio of finalized signal intensity to the average finalized signal intensity of all positive spots within that sample (mean ratio). The mean ratio data were subjected to statistical analyses using the pipeline developed at University of Oklahoma (http://ieg.ou.edu).

2.4. Statistical analyses

Previous studies have established that GeoChip probe signal intensities are linearly correlated to target DNA or RNA concentrations of pure cultures to environmental samples (He et al., 2007; Rhee et al., 2004; Wu et al., 2001, 2004). For this reason, resulting data matrices from microarray image analysis were considered as either: i) gene variant abundance as indicated by signal intensity of each probe or ii) binary presence/absence matrices. Additionally, relative abundance data were calculated by dividing the signal intensities of each individual probe by the sum of signal intensities for a given plot, effectively accounting for absolute differences in DNA abundance between plots.

The effects of site, simulated N deposition, and their interaction on archaeal and bacterial functional genes was initially determined by two-way analysis of variance (ANOVA), combined with a protected Fisher's LSD test of means (SPSS Statistics, Version 20, IBM Corp., Armonk, NY, USA); significance was accepted at $\alpha = 0.05$. To test the hypothesis that simulated N deposition caused a decline in abundance of functional genes mediating N cycling processes, the sum of signal intensities of gene probes were corrected, such that each GeoChip "gene category" (e.g. N fixation, denitrification) had equal influence. For example, because the number of probes designed for gene variants within each gene category were unequal, greater weight would be placed on categories with a greater number of probes on the microarray (Table S1). Presence–absence data was used to determine if experimental N deposition decreased functional richness, defined as the proportion of gene variants detected (Liu and Greaver, 2010; Van Nostrand et al., 2009), among genes mediating N cycling processes, and were similarly corrected such that each gene category had an equal weight. All proportion data were arcsine transformed prior to statistical analysis. To test the hypothesis that simulated N deposition caused a similar decline in the diversity of functional genes, the normalized signal intensity of all relevant gene variants was used to calculate the Shannon Index (H′; Shannon and Weaver, 1963). The full abundance matrix was used in the calculation of the response ratio (Luo et al., 2006) to determine if functional genes of bacterial taxa respond differently to simulated N deposition. Taxonomic information for archaeal probes lacked sufficient resolution for such analysis.

Effects of experimental N deposition on archaeal and bacterial functional gene composition, as observed in microarray presence–absence data, were further assayed based on Bray–Curtis dissimilarity coefficients (Legendre and Legendre, 1998). Calculation of dissimilarity coefficients and all downstream analysis were performed in Primer (version 6, Primer-E Ltd., Plymouth, UK). A dissimilarity matrix based on arcsine transformed proportion of gene variants detected within each functional gene category was generated for all possible pairs of samples and was used for all remaining analyses. Ordinations were obtained from principal coordinate analysis to test the hypothesis that simulated N deposition altered the composition of archaeal and bacterial gene assemblages. To determine whether specific functional genes were primarily responsible for any observed compositional changes, Spearman correlation coefficients were calculated to ascertain the degree of correlation of each gene variant to the primary principal coordinate. The significance of compositional differences between ambient and experimental N deposition assemblages were confirmed by analysis of similarity (ANOSIM; Clarke et al., 1993), and permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001). The ANOSIM test provides an *R*-statistic, ranging between −1 and 1 based on rank dissimilarities, with an *R*-statistic of zero indicating a completely random grouping. PerMANOVA allows multivariate information to be partitioned according to the experimental design (with interaction terms), and determines significance by random permutation, but cannot determine if observed shifts are due to location or dispersion. Therefore, a distance-based test for homogeneity of multivariate dispersions (PERMDISP; Anderson, 2004), a β-diversity measure, was used to discern the directional nature of observed assemblage shifts. Finally, contributions of each functional gene to the total dissimilarity between the two groups (ambient, simulated N deposition) were determined using Similarity Percentage (SIMPER; Clarke et al., 1993).

3. Results

3.1. Functional gene abundance

If not presented in text, all relevant means and standard errors can be found in Table S2. Two-way ANOVA of GeoChip signal intensities revealed a significant decrease in abundance of functional genes mediating N cycle processes under experimental N deposition in both archaeal ($P = 0.03$) and bacterial ($P = 0.02$) gene assemblages (Fig. 2). A significant site by treatment interaction was observed in both archaeal ($P = 0.01$) and bacterial ($P = 0.03$) assemblages; wherein, pairwise protected Fisher's LSD revealed significant effects of experimental N deposition in sites A (Archaea $P < 0.01$; Bacteria $P = 0.01$) and B (Archaea $P < 0.01$; Bacteria $P < 0.01$), with no effect in C or D ($P > 0.15$).

Archaeal gene assemblages declined in abundance evenly across each N cycle process represented on the microarray. However, when absolute differences were taken into account, the relative abundance of bacterial genes encoding assimilatory NO_3^- reduction increased ($P < 0.03$; ambient mean $= 3.7 \pm 0.05 \times 10^{-3}$; N deposition mean $= 3.9 \pm 0.1 \times 10^{-3}$), and those encoding nitrification marginally declined ($P = 0.06$; ambient mean $= 3.6 \pm 0.1 \times 10^{-3}$; N deposition mean $= 3.3 \pm 0.1 \times 10^{-3}$) disproportionately to other bacterial genes.

3.2. Diversity of functional genes

The effect of experimental N deposition on the functional richness within each gene category was investigated using the binary presence-absence data matrix. We observed that experimental N deposition caused a decline in the functional richness of archaeal ($P = 0.04$) and bacterial ($P = 0.02$) assemblages (Fig. 3), as shown by

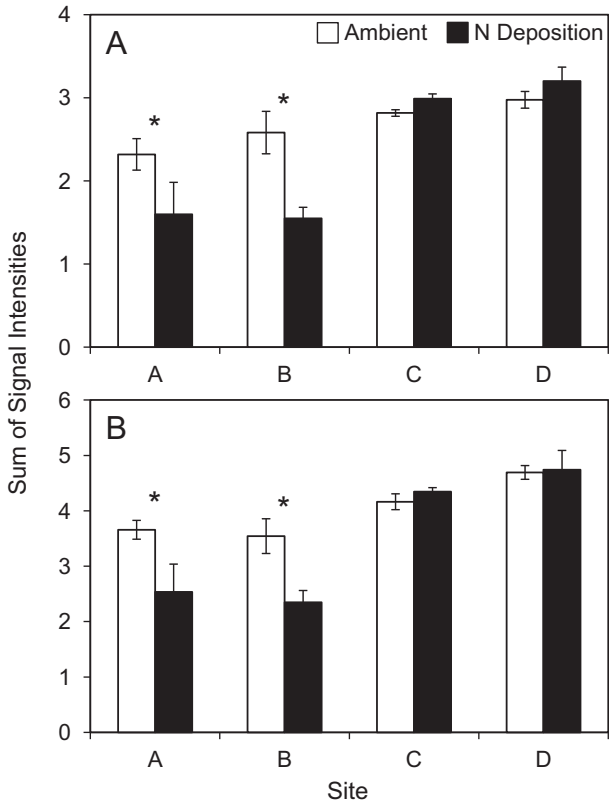

Fig. 2. Normalized signal intensities of archaeal (A) and bacterial (B) probes within the nitrogen cycle gene category across sites A–D. Error bars indicate SE, asterisk indicates significance ($P < 0.05$) by Fisher's Least Significant Difference. All means and SEs are reported in Table S2.

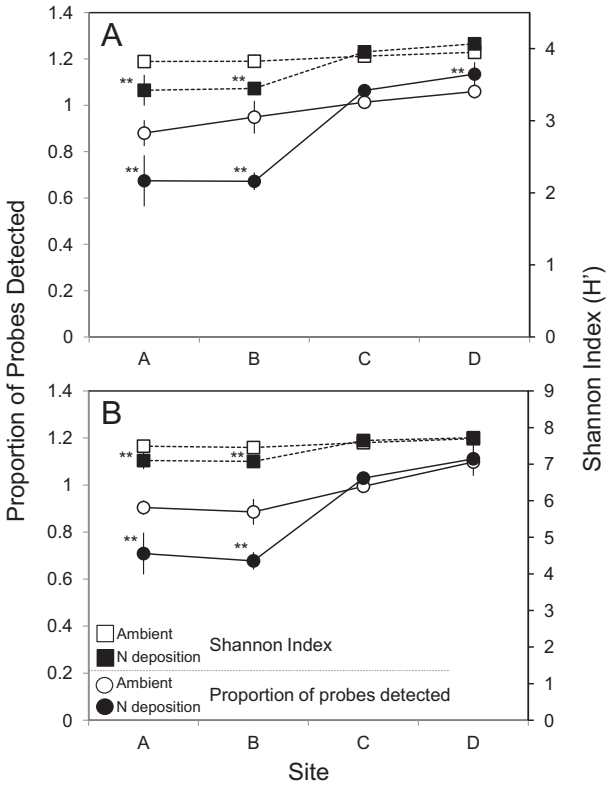

Fig. 3. Shannon index and functional richness (proportion of probes detected) of archaeal (A) and bacterial (B) probes within the nitrogen cycle gene category across sites A–D. Error bars indicate SE, asterisk indicates significance ($P < 0.05$) by Fisher's Least Significant Difference. All means and SEs are reported in table S2.

two-way ANOVA. The response was site-specific as indicated by a significant site by treatment interaction (Archaea $P = 0.04$; Bacteria $P = 0.02$), wherein experimental N deposition elicited a negative response in archaeal and bacterial genes in site A and B, and no response in sites C and D. Archaeal genes slightly increased in functional richness in site D ($P = 0.05$) in response to experimental N deposition.

The decline in functional richness occurred evenly across each gene in archaeal gene assemblages. In contrast, the relative proportion of probes detected encoding assimilatory NO_3^- reduction increased ($P = 0.03$; ambient mean $= 0.43 \pm 0.003$; N deposition mean $= 0.45 \pm 0.01$), and those encoding nitrification declined ($P = 0.05$; ambient mean $= 0.43 \pm 0.01$; N deposition mean $= 0.41 \pm 0.01$) disproportionately as compared to others.

The effect of experimental N deposition on assemblage diversity, as indicated by the Shannon index (H′), was determined based on normalized signal intensity values for all probes associated with archaeal and bacterial genes. Experimental N deposition reduced archaeal ($P = 0.04$) and bacterial ($P = 0.02$) gene diversity; a significant site by treatment interaction term indicated a site-specific response (Archaea $P = 0.02$; Bacteria $P = 0.05$). Again, experimental N deposition caused declines in diversity at sites A and B and no responses in sites C and D (Fig. 3).

3.3. Community composition of functional genes

Ordinations obtained from principal coordinate analysis (PCoA) revealed compositional similarities among ambient plots and disparate composition among experimental N deposition plots across study sites (Fig. 4). ANOSIM detected a clear divergence of gene assemblages between ambient and experimental N deposition treatments for both archaeal ($R = 0.44$, $P < 0.01$) and bacterial ($R = 0.50$, $P < 0.01$) genes mediating N cycling processes.

PerMANOVA revealed a significant treatment effect on both archaeal ($P = 0.04$) and bacterial ($P = 0.01$) functional gene composition, although site-specific responses occurred, indicated by a significant site by treatment interaction (Archaea $P = 0.01$; Bacteria $P = 0.05$). Pairwise PerMANOVA of archaeal gene assemblages indicated a significant effect of experimental N deposition in site B ($P = 0.01$), with a marginal treatment effect in sites A ($P = 0.10$) and C ($P = 0.10$); no effect occurred in site D ($P = 0.32$). Among bacterial gene assemblages, experimental N deposition caused a significant composition shift in site B ($P = 0.03$), with a slight effect in site A ($P = 0.10$), and no effect in sites C and D ($P > 0.15$). Significant PERMDISP results indicated experimental N deposition increased β-diversity of functional genes in both archaeal ($F = 6.3$, $P = 0.04$) and bacterial ($F = 14.2$, $P < 0.01$) assemblages.

Similarity Percentage (SIMPER; Clarke et al., 1993) was used to further distinguish which genes contributed to the compositional dissimilarity between ambient and experimental N deposition treatments (Table 2). Archaeal gene assemblages exhibited a mean dissimilarity of 21.2%, wherein genes mediating denitrification contributed most to assemblage dissimilarity between ambient and experimental N deposition treatments ($14.2 \pm 3.4\%$). This trend was driven by *norB* and *nosZ*, which together amount to 41.5% of the total dissimilarity. Among bacterial genes, the mean dissimilarity was 12% between ambient and experimental N deposition treatments. Contributions to assemblage dissimilarity ranged from 9 to 5%, with genes mediating nitrification (8%), N fixation (7%), and assimilatory N reduction (7%), on average, exhibiting the most difference between groups.

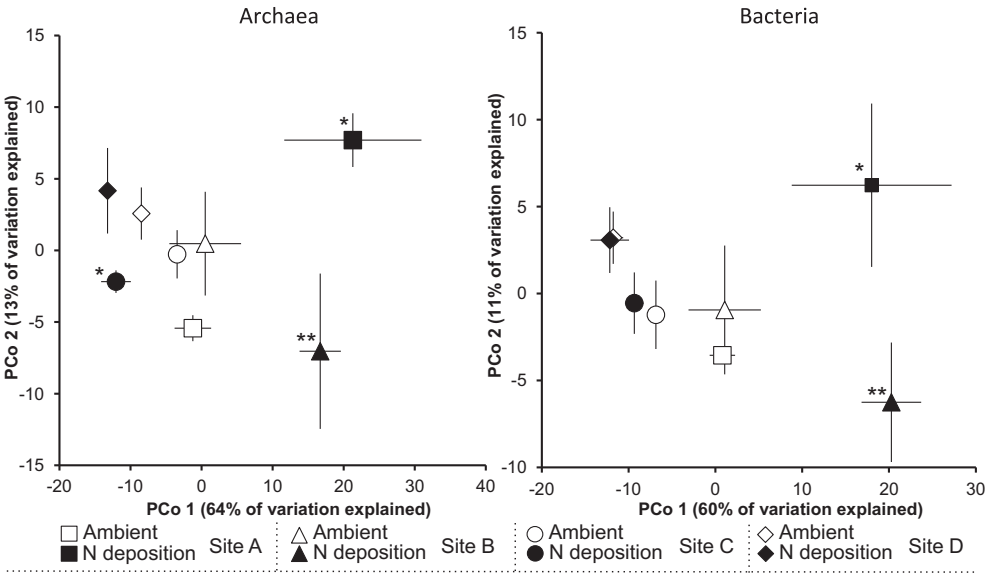

Fig. 4. Principle coordinate analysis of archaeal and bacterial N-cycle gene assemblage composition. Open symbols represent sites receiving ambient N deposition, closed symbols represent sites receiving ambient plus simulated N deposition. Data represent Bray–Curtis dissimilarity of the full presence/absence matrix. $^{**}p \leq 0.05$; $^{*}p \leq 0.10$ for each site by pairwise PerMANOVA.

Table 2
Contributions of N-cycle functional genes to compositional dissimilarity as indicated by SIMPER analysis.

	Average dissimilarity	Diss./SD	% Contribution
Archaea			
Denitrification	3.02 ± 0.73	0.98 ± 0.05	14.21 ± 3.42
norB	4.56	0.98	21.45
nosZ	4.25	0.95	20.03
nirK	1.92	1.13	9.04
narG	1.34	0.84	6.33
Ammonification	1.82 ± 0.71	1.00 ± 0.06	8.57 ± 3.34
gdh	2.53	1.06	11.91
ureC	1.11	0.94	5.23
Assimilatory N reduction	1.52 ± 0.39	0.99 ± 0.20	7.17 ± 1.84
nirA	2.39	1.25	11.27
nirB	1.29	0.54	6.08
nasA	0.88	1.18	4.17
N Fixation	0.95	1.50	4.49
nifH	0.95	1.50	4.49
Bacteria			
Nitrification	0.94	1.31	8.01
hao	0.94	1.31	8.01
N Fixation	–	–	–
nifH	0.83	1.44	7.08
Assimilatory N reduction	0.79 ± 0.06	1.45 ± 0.07	6.75 ± 0.47
nirA	0.92	1.66	7.83
nirB	0.84	1.39	7.15
nasA	0.74	1.40	6.35
NiR	0.66	1.36	5.66
Dissimilatory N reduction	0.77 ± 0.01	1.32 ± 0.04	6.58 ± 0.12
napA	0.78	1.35	6.70
nrfA	0.76	1.28	6.46
Denitrification	0.77 ± 0.06	1.26 ± 0.04	6.58 ± 0.55
nirS	1.01	1.37	8.67
nirK	0.77	1.30	6.62
nosZ	0.71	1.31	6.06
norB	0.71	1.15	6.04
narG	0.64	1.17	5.50
Ammonification	0.70 ± 0.13	1.20 ± 0.07	5.94 ± 1.04
gdh	0.82	1.13	6.98
ureC	0.57	1.27	4.90

Average dissimilarity ± SE are shown for each N-cycle process. One gene per process was present on GeoChip where no SE is included.

In both archaeal and bacterial principal coordinate ordinations, the majority of the variation in assemblage composition was explained by principal coordinate 1 (Archaea, 64%; Bacteria, 60%). Among archaeal assemblages, genes mediating denitrification (*norB*; $\rho = -0.75$) and assimilatory N reduction (*nirA*; $\rho = -0.52 \pm 0.23$) were most highly correlated to PCo1. Among bacterial assemblages, genes mediating nitrification (*hao*, $\rho = -0.50 \pm 0.05$) and N fixation (*nifH*, $\rho = -0.49 \pm 0.01$) were more highly correlated with PCo1 than other N cycle genes.

Response ratios (Luo et al., 2006) were calculated to determine if functional genes from bacterial phyla represented on GeoChip 4.0 responded differently to experimental N deposition (Fig. S1). Functional genes representative of Bacteriodetes responded most negatively (−0.75 ± 0.51), followed by probes representing uncultured bacteria (−0.20 ± 0.04) and γ-proteobacteria (−0.17 ± 0.11), all of which are significantly less than zero based on >90% confidence intervals. Functional gene abundance of Firmicutes responded positively to experimental N deposition (1.49 ± 0.19) and was greater than zero at the 99% confidence interval. Archaeal probe taxonomic resolution was not sufficient to perform these analyses.

4. Discussion

4.1. Mixed responses of soil microbial communities under future rates of atmospheric NO_3^- deposition

In our long-term field study, experimental N deposition led to a decrease in abundance, richness (proportion of gene variants detected), and diversity (H′) of a suite of genes mediating N cycle processes, with a concomitant shift in community composition of both archaeal and bacterial functional gene assemblages. This response occurred with fidelity in the northernmost two sites, A and B, but was not evident in the southernmost sites (C and D). In addition to the response in sites A and B, chronic N deposition caused a shift in archaeal community composition in site C ($P = 0.10$), and increased the β-diversity of gene assemblages at all sites. This is not a surprising result, as a recent study demonstrated a consistent decline in microbial respiration and biomass under N enrichment across a variety of soil types (Ramirez et al., 2012);

others have demonstrated that N additions can decrease the diversity (Allison et al., 2008; Campbell et al., 2010) and richness (Shen et al., 2010) of soil microbial communities. However, unlike previous studies which focus on general microbial community composition (Campbell et al., 2010; Edwards et al., 2011; Fierer et al., 2011; Ramirez et al., 2012), this study employed a functional gene microarray, providing a unique opportunity to gain insight into the physiological potential of microbial communities in our experiment to mediate soil N cycling processes.

The significant site by treatment interactions we observed suggest that soil microbial assemblages responsible for assimilating and cycling N may be somewhat idiosyncratic in their responses to atmospheric NO_3^- deposition. Historically, microbial responses to anthropogenic N enrichment have been variable; some have reported stimulation (Neff et al., 2002; Newell et al., 1996; Tietema, 1998), or suppression (Edwards et al., 2011; Eisenlord and Zak, 2010; Gallo et al., 2004; Ramirez et al., 2012; Wallenstein et al., 2006) of microbial activities, whereas others have found no effect (Hobbie and Vitousek, 2000). Here, we observe a consistent and robust response of microbial assemblages to experimental N deposition in the northernmost two sites, A and B, with a moderate to non-significant response in sites C and D. The four experimental forest stands were chosen to span the north–south range of the northern hardwood forests in the Great Lakes region of North America; if we observed no interaction between site and treatment, it would infer a uniform response to future rates of atmospheric N deposition across an expansive and diverse ecosystem. As this was not the case, it provides an opportunity to further explore the mechanisms that underlie our observed response. Over the last decade, ambient N deposition has ranged from ~4 (site A) to ~12 (site D) kg N ha^{-1} y^{-1} across our study sites (Burton et al., 1993; National Atmospheric Deposition Program, 2005). Because our N deposition treatment (30 kg N ha^{-1} y^{-1}) is consistent across sites, site A experiences a 10-fold increase in N deposition, whereas site D experiences a 3-fold increase. It is possible that microorganisms previously exposed to relatively high concentrations of ambient N deposition may not have been affected by our treatment in a way we would be able to detect using GeoChip. It is also plausible that microbial communities in our southernmost sites (C & D), which experience relatively high levels of ambient N deposition, have already acclimated to this anthropogenic perturbation, whereas those in the northernmost sites have not.

Within the context of previous results obtained from our long-term field study, experimental N deposition more broadly suppressed microbial activities than was previously established. For example, long-term experimental N deposition resulted in significant declines in soil respiration (−15%; Burton et al., 2004), active microbial biomass (−23%; DeForest et al., 2004a), and phenol oxidase (−33%), and peroxidase (−30%) activity (DeForest et al., 2004b, 2005), which reduced decay, increased soil organic matter (SOM) content (+18%; Zak et al., 2008) as well as increased DOC (+26%) and NO_3^- (+680%) leaching (Pregitzer et al., 2004). One possible alternative explanation for an overall decline in gene abundance under experimental N deposition could be Na^+ toxicity, resulting from long-term $NaNO_3$ addition. However, Na^+ concentrations in soil water (0.45 ± 0.00 mmol; W. E. Holmes, unpublished data) are two orders of magnitude below those known to decrease microbial biomass, respiration, and enzyme activity (~40 mmol Na^+ as NaCl; Bernardet and Nakagawa, 2003; Garcia and Hernandez, 1996), indicating the observed response cannot be due to Na^+ toxicity. Refer to Zak et al. (2008) for more discussion on this matter.

For this study, the use of the functional gene microarray, GeoChip 4.0, provided the luxury to examine the abundance and composition of 2876 gene variants encompassing 15 genes mediating N-cycle processes while not requiring PCR amplification of each gene. However, given that probe design is limited by gene sequence availability from public databases (e.g. Genbank), the problem still remains that microarrays can only detect the limited number of sequences covered by the probes included on the array. For a more detailed discussion of advantages and disadvantages of microarrays such as GeoChip, see Van Nostrand et al. (2012). In future studies, the use shotgun metagenomics may prove helpful in elucidating more nuanced responses of microbial assemblages to experimental N deposition. Our results emphasize the assertion that, in light of new and emerging metagenomic technologies, more highly replicated experimental designs are needed to accurately assess microbial community dynamics (Knight et al., 2012).

4.2. Towards a molecular understanding of community and ecosystem-level responses to NO_3^- deposition

Taken together, our results infer communities are diverging under experimental N deposition, but these changes are not consistent among sites. Overall, experimental N deposition caused a site-dependent decrease in α-diversity, but an increase in β-diversity of functional genes, suggesting that future rates of N deposition may cause soil microbial communities to become more functionally heterogeneous. In this study, bacterial gene variants mediating nitrification were strongly affected by our N deposition treatment; these assemblages decreased in abundance and richness disproportionately relative to other gene categories and were responsible for the majority of the variation observed in PcoA (Fig. 4; PCo1 $\rho = -0.50 \pm 0.05$) and SIMPER analysis (Table 2). This observed molecular response to our N deposition treatment has clear functional implications, as Zak et al. (2006) noted a significant increase of annual net nitrification in experimental N deposition treatments (+27%), a finding consistent with other studies (Jefts et al., 2004; Lamontagne and Schiff, 2000). It is possible that composition shifts in nitrifying assemblages may favor those organisms which are more efficient in their nitrification pathways, leading to ultimately greater rates of net nitrification despite these assemblages being less abundant.

NO_3^- assimilation and denitrification may also prove to be significant transformations as N-cycling assemblages acclimate to future rates of N deposition. Previous results from our long-term field experiment report a significant microbial assimilation of $^{15}NO_3^-$ occurred within hours of addition in experimental N deposition plots (Zogg et al., 2000); similar findings have also been reported in lab incubations (Zak et al., 2006) and agricultural soils (Burger and Jackson, 2003). In this study, gene variants mediating assimilatory NO_3^- reduction disproportionately increased in abundance and richness, and contributed highly to total gene dissimilarity among treatments. The combination of field, laboratory, and molecular analyses together suggest microbial assimilation of N to be an important process by which anthropogenic N initially cycles in soil. Among archaeal assemblages, genes encoding denitrification were most affected by experimental N deposition. These assemblages were responsible for the majority of the variation in PCoA (Fig. 4; PCo1 $\rho = -0.44 \pm 0.12$) and were found to greatly contribute to assemblage dissimilarity in SIMPER analysis (Table 2). Nitrogen enrichment can increase rates of denitrification in forest soils (Buckley et al., 1998; He et al., 2007; Nicol and Schleper, 2006); however, process rates were unavailable at this time for our experiment. A robust analysis of microbial N transformations in the forest floor is needed to more clearly determine if the observed compositional shifts may elicit a functional change in response to experimental N deposition.

Several recent large-scale sequencing efforts have revealed significant and consistent phylum-level shifts in soil bacterial populations in response to N fertilization (Campbell et al., 2010;

Nemergut et al., 2008; Ramirez et al., 2012, 2010). The copiotrophic hypothesis (Fierer et al., 2007) predicts that N enrichment may induce a shift from slower growing oligotrophic taxa to more fast growing copiotrophic taxa (Fierer et al., 2007), and this has been empirically supported (Cleveland et al., 2007; Fierer et al., 2011; Nemergut et al., 2010; Ramirez et al., 2012, 2010). Our results were both consistent, as well as contrary to the copiotrophic hypothesis. Functional genes of the Firmicutes, a copiotrophic group (Cleveland et al., 2007) increased in abundance under experimental N deposition (Fig. S1); similarly, Firmicutes increased in abundance (+3.04%) under N fertilization across a variety of soil types in lab incubations (Ramirez et al., 2012). Functional genes attributable to another copiotrophic group, the Bacteroidetes, exhibited the most negative response to experimental N deposition in our study, conflicting with predictions of the copiotrophic hypothesis. Conversely, Bacteroidetes increased in abundance under N amendments in alpine tundra soils (Nemergut et al., 2008), as well as in agricultural and grassland soils (Fierer et al., 2011); however, these responses occurred at substantially greater rates of N deposition than those in our experiment (>100 kg N ha^{-1} yr^{-1} vs. 30 kg N ha^{-1} y^{-1}). Bacteroidetes are capable of utilizing macromolecules such as proteins and polysaccharides, namely cellulose (Bernardet and Nakagawa, 2003), which is often encased by lignin within plant cell walls. Experimental N deposition decreased activities of lignolytic enzymes peroxidase and phenol oxidase (DeForest et al., 2004b) in our study sites, as well as the expression of fungal laccase (*lcc*; Edwards et al., 2011). It is possible that a reduction in lignin metabolism resulted in fewer complex polysaccharides, which may adversely affect the growth, and therefore proliferation of protein-coding genes of these soil Bacteria.

4.3. The emerging appreciation of the role of Archaea in the soil N cycle

A paucity of knowledge exists regarding archaeal community responses to N enrichment. In our study, archaeal and bacterial gene abundance and composition responded similarly to experimental N deposition, namely site-dependent decreases in the abundance, richness, and diversity (H′) of functional genes mediating N cycling processes, as well as shifts in gene composition. A similar response of ecological coherence between archaeal and bacterial assemblages has been previously observed over several fertilization regimes (Wessen et al., 2010), whereas another study provided no evidence for archaeal community shifts in N-fertilized agricultural soils in which shifts in bacterial communities were observed (Taylor et al., 2012). It has been hypothesized that Archaea play an important role in soil nitrification (Bates et al., 2011; Leininger et al., 2006; Nicol and Schleper, 2006). Furthermore, possible inhibition of soil archaeal communities by high levels of available N was observed in N amended grassland soils, possibly due to competitive interactions with bacterial nitrifiers (Bates et al., 2011). Recent studies have attempted to resolve possible competitive interactions between ammonia-oxidizing Archaea (AOA) and Bacteria (AOB), with some proposing bacterial dominance in nitrification (Di et al., 2009; Long et al., 2012), whereas some suggest the opposite (Leininger et al., 2006) and others suggest a soil-dependent response (Taylor et al., 2010). Unfortunately, the probe composition of GeoChip 4.0 does not allow for such comparisons in our study. Though PCR-based molecular techniques have greatly improved existing knowledge of this domain since its inception nearly 20 years ago (Woese et al., 1990), we still have an inadequate understanding of terrestrial Archaea (Bates et al., 2011), and even less is known regarding effects of anthropogenic N deposition on these soil organisms. Genome sequencing has revealed putative NO_3^- transporters, and genes encoding enzymes involved in NO_3^- reduction and denitrification in both Crenarchaeota and Euryarchaeota, two families within the Archaea (Cabello et al., 2004). Furthermore, archaeal assimilatory NO_3^- and NO_2^- reductases have been purified from pure cultures (Martinez-Espinosa et al., 2001). Undoubtedly, information obtained through the rise in large-scale sequencing efforts will allow for more robust analyses of the archaeal response to anthropogenic N enrichment.

5. Conclusions

The results we present here support the hypothesis that chronic N deposition can decrease the abundance and diversity of functional genes mediating N cycling processes as well as altering their composition, albeit this response was site-dependent. In the northernmost two sites, the overall decline in the abundance of functional genes encoding N-cycling processes occurred as part of a broad overall suppression of microbial activities in response to anthropogenic N deposition which results in reduced decay, greater soil C storage, and increased DOC and NO_3^- leaching. Evidence from this study suggests denitrification, nitrification, and NO_3^- assimilation to be key transformations as N-cycling microbial assemblages respond to future rates of atmospheric N deposition. Our results support the assertion that ecosystem-level microbial responses to anthropogenic N deposition may fundamentally alter the physiological potential of soil microbial communities and soil N cycling dynamics within the northern hardwood forest ecosystem.

Acknowledgments

We thank Rima Upchurch and Lauren Cline (University of Michigan) for advice and assistance regarding data analysis and statistical methods, and Tong Yuan (University of Oklahoma) for her work in running the microarray. Grants from the Department of Energy's BER Program and the NSF (DEB-061442 and DEB-0814623) provided support for our work.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.07.010.

References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems – hypotheses revisited. Bioscience 48, 921–934.

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39, 378–386.

Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79, 439–449.

Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14, 1156–1168.

Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biology & Biochemistry 39, 1878–1887.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.

Anderson, M.J., 2004. PERMDISP: a Fortran Computer Program for Permutational Analysis of Multivariate Dispersions (For Any Two-factor Anova Design) Using Permutation Tests. Department of Statistics, University of Auckland, Auckland, New Zealand.

Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. The ISME Journal 5, 908–917.

Bernardet, J.F., Nakagawa, Y., 2003. An introduction to the family *Flavobacteriaceae*. In: Dworkin, M. (Ed.), The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community, third ed. Springer-Verlag, New York, N.Y.

Braun, E.L., 1950. Deciduous Forests of Eastern North America. Macmillan Publishing Co, Inc., New York, NY, p. 596.

Buckley, D.H., Graber, J.R., Schmidt, T.M., 1998. Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. Applied and Environmental Microbiology 64, 4333–4339.
Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biology & Biochemistry 35, 29–36.
Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P., Zak, D.R., 2004. Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. Global Change Biology 10, 1080–1091.
Burton, A.J., Pregitzer, K.S., MacDonald, N.W., 1993. Foliar nutrients in sugar maple forests along a regional pollution-climate gradient. Soil Science Society of America Journal 57, 1619–1628.
Cabello, P., Roldan, M.D., Moreno-Vivian, C., 2004. Nitrate reduction and the nitrogen cycle in Archaea. Microbiology 150, 3527–3546.
Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C., Schuur, E.A., 2010. The effect of nutrient deposition on bacterial communities in arctic tundra soil. Environmental Microbiology 12, 1842–1854.
Clarke, K.R., Warwick, R.M., Brown, B.E., 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92, 205–219.
Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., Townsend, A.R., 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82, 229–240.
DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004a. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology & Biochemistry 36, 965–971.
DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004b. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68, 132–138.
DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2005. Atmospheric nitrate deposition and enhanced dissolved organic carbon leaching: test of a potential mechanism. Soil Science Society of America Journal 69, 1233–1237.
Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nature Geoscience 2, 621–624.
Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., Pregitzer, K.S., 2011. Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. PLoS One 6, e20421.
Eisenlord, S.D., Zak, D.R., 2010. Simulated atmospheric nitrogen deposition alters actinobacterial community composition in forest soils. Soil Biology & Biochemistry 74, 1157–1166.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2011. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6 (5), 1007–1017.
Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R.L., Zak, D.R., 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology 48, 218–229.
Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vöosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.
Garcia, C., Hernandez, T., 1996. Influence of salinity on the biological and biochemical activity of a calciorthird soil. Plant and Soil 178, 255–263.
He, J.Z., Shen, J.P., Zhang, L.M., Zhu, Y.G., Zheng, Y.M., Xu, M.G., Di, H.J., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environmental Microbiology 9, 2364–2374.
Hobbie, S.E., Vitousek, P.M., 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81, 1867–1877.
Hobbie, S.E., 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. Ecology 89, 2633–2644.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3, 315–322.
Jefts, S., Fernandez, I.J., Rustad, L.E., Dail, D.B., 2004. Decadal responses in soil N dynamics at the bear brook watershed in Maine, USA. Forest Ecology and Management 189, 189–205.
Knight, R., Jansson, J., Field, D., Fierer, N., Desai, N., Fuhrman, J.A., Hugenholtz, P., van der Lelie, D., Meyer, F., Stevens, R., Bailey, M.J., Gordon, J.I., Kowalchuk, G.A., Gilbert, J.A., 2012. Unlocking the potential of metagenomics through replicated experimental design. Nature Biotechnology 30, 513–520.
Lamontagne, S., Schiff, S.L., 2000. Response of soil microorganisms to an elevated nitrate input in an open *Pinus banksiana – Cladina* forest. Forest Ecology and Management 137, 13–22.
Legendre, P., Legendre, L., 1998. Numerical Ecology, second ed. Elsevier, Amsterdam, The Netherlands.
Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809.
Liu, L.L., Greaver, T.L., 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecology Letters 13, 819–828.
Long, X., Chen, C.R., Xu, Z.H., Oren, R., He, J.Z., 2012. Abundance and community structure of ammonia-oxidizing bacteria and archaea in a temperate forest ecosystem under ten-years elevated CO_2. Soil Biology & Biochemistry 46, 163–171.
Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., Yang, X., Li, B., 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. New Phytologist 189, 1040–1050.
Luo, Y., Hui, D., Zhang, D., 2006. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87, 53–63.
MacDonald, N.W., Burton, A.J., Jurgensen, M.F., Mclaughlin, J.W., Mroz, G.D., 1991. Variation in forest soil properties along a great-lakes air-pollution gradient. Soil Science Society of America Journal 55, 1709–1715.
MacDonald, N.W., Burton, A.J., Liechty, H.O., Witter, J.A., Pregitzer, K.S., Mroz, G.D., Richter, D.D., 1992. Ion leaching in forest ecosystems along a great-lakes air-pollution gradient. Journal of Environmental Quality 21, 614–623.
Martinez-Espinosa, R.M., Marhuenda-Egea, F.C., Bonete, M.J., 2001. Assimilatory nitrate reductase from the haloarchaeon *Haloferax mediterranei*: purification and characterization. FEMS Microbiology Letters 204, 381–385.
National Atmospheric Deposition Program, 2005. National Atmospheric Deposition Program 2004 Annual Summary. NADP Data Report 2001–2005. Illinois State Water Survey, Champaign, IL.
Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.
Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L., Townsend, A.R., 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biology & Biochemistry 42, 2153–2160.
Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N., Schmidt, S.K., 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: Implications for carbon and nitrogen cycling. Environmental Microbiology 10, 3093–3105.
Newell, S.Y., Arsuffi, T.L., Palm, L.A., 1996. Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia 108, 495–502.
Nicol, G.W., Schleper, C., 2006. Ammonia-oxidising crenarchaeota: important players in the nitrogen cycle? Trends in Microbiology 14, 207–212.
Pregitzer, K.S., Burton, A.J., Zak, D.R., Talhelm, A.F., 2008. Simulated chronic nitrogen deposition increases carbon storage in northern temperate forests. Global Change Biology 14, 142–153.
Pregitzer, K.S., Zak, D.R., Burton, A.J., Ashby, J.A., MacDonald, N.W., 2004. Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. Biogeochemistry 68, 179–197.
Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101, 133–149.
Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18, 1918–1927.
Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463–3470 discussion 3503–3414.
Rhee, S.K., Liu, X.D., Wu, L.Y., Chong, S.C., Wan, X.F., Zhou, J.Z., 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Applied and Environmental Microbiology 70, 4303–4317.
Shannon, C.E., Weaver, W., 1963. The Mathematical Theory of Communication. University of Illinois Press, Champaign, IL.
Shen, W.S., Lin, X.G., Shi, W.M., Min, J., Gao, N., Zhang, H.Y., Yin, R., He, X.H., 2010. Higher rates of nitrogen fertilization decrease soil enzyme activities, microbial functional diversity and nitrification capacity in a Chinese polytunnel greenhouse vegetable land. Plant and Soil 337, 137–150.
Talbot, J.M., Treseder, K.K., 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry-decay relationships. Ecology 93, 345–354.
Taylor, A.E., Zeglin, L.H., Dooley, S., Myrold, D.D., Bottomley, P.J., 2010. Evidence for different contributions of archaea and bacteria to the ammonia-oxidizing potential of diverse Oregon soils. Applied and Environmental Microbiology 76, 7691–7698.
Taylor, A.E., Zeglin, L.H., Wanzek, T.A., Myrold, D.D., Bottomley, P.J., 2012. Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. The ISME Journal 6, 2024–2032.
Tietema, A., 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen deposition gradient. Forest Ecology and Management 101, 29–36.
Van Nostrand, J.D., He, Z., Zhou, J., 2012. Use of functional gene arrays for elucidating in situ biodegradation. Frontiers in Microbiology 3. http://dx.doi.org/10.3389/fmicb.2012.00339.
Van Nostrand, J.D., Wu, W.M., Wu, L., Deng, Y., Carley, J., Carroll, S., He, Z., Gu, B., Luo, J., Criddle, C.S., Watson, D.B., Jardine, P.M., Marsh, T.L., Tiedje, J.M., Hazen, T.C., Zhou, J., 2009. GeoChip-based analysis of functional microbial communities during the reoxidation of a bioreduced uranium-contaminated aquifer. Environmental Microbiology 11, 2611–2626.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7, 737–750.
Vitousek, P.M., Hattenschwiler, S., Olander, L., Allison, S., 2002. Nitrogen and nature. Ambio 31, 97–101.
Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H., 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management 222, 459–468.
Wessen, E., Hallin, S., Philippot, L., 2010. Differential responses of bacterial and archaeal groups at high taxonomical ranks to soil management. Soil Biology & Biochemistry 42, 1759–1765.
Woese, C.R., Kandler, O., Wheelis, M.L., 1990. Towards a natural system of organisms: proposal for the domains archaea, bacteria, and eucarya. Proceedings of the National Academy of Sciences of the United States of America 87, 4576–4579.
Wu, L., Liu, X., Schadt, C.W., Zhou, J., 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Applied and Environmental Microbiology 72, 4931–4941.
Wu, L.Y., Thompson, D.K., Li, G.S., Hurt, R.A., Tiedje, J.M., Zhou, J.Z., 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. Applied and Environmental Microbiology 67, 5780–5790.
Wu, L.Y., Thompson, D.K., Liu, X.D., Fields, M.W., Bagwell, C.E., Tiedje, J.M., Zhou, J.Z., 2004. Development and evaluation of microarray-based whole-genome hybridization for detection of microorganisms within the context of environmental applications. Environmental Science & Technology 38, 6775–6782.
Zak, D.R., Holmes, W.E., Burton, A.J., Pregitzer, K.S., Talhelm, A.F., 2008. Simulated atmospheric NO_3^- deposition increases soil organic matter by slowing decomposition. Ecological Applications 18, 2016–2027.
Zak, D.R., Holmes, W.E., Tomlinson, M.J., Pregitzer, K.S., Burton, A.J., 2006. Microbial cycling of C and N in northern hardwood forests receiving chronic atmospheric NO_3^- deposition. Ecosystems 9, 242–253.
Zak, D.R., Pregitzer, K.S., 1990. Spatial and temporal variability of nitrogen cycling in northern lower Michigan. Forest Science 36, 367–380.
Zogg, G.P., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81, 1858–1866.