Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe

Jonathan W. Leff[a,b], Stuart Jones[c], Suzanne M. Prober[d], Albert Barberán[a], Elizabeth T. Borer[e], Jennifer Firn[f], W. Stanley Harpole[g,h,i], Sarah E. Hobbie[e], Kirsten S. Hofmockel[j], Johannes M. H. Knops[k], Rebecca L. McCulley[l], Kimberly J La Pierre[m], Anita C. Risch[n], Eric W. Seabloom[o], Martin Schütz[n], Christopher Steenbock[b], Carly J. Stevens[p], and Noah Fierer[a,b,1]

[a] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA [b] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA [c] Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA [d] CSIRO Land and Water Flagship, Private Bag 5, Wembley, WA 6913, Australia [e] Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA [f] School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, Australia [g] Department of Physiological Diversity, Helmholtz Center for Environmental Research UFZ, Permoserstr. 15, 04318 Leipzig, Germany [h] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany [i] Institute of Biology, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, 06108 Halle (Saale), Germany [j] Ecology, Evolution and Organismal Biology Department, Iowa State University, Ames, IA 50011, USA [k] School of Biological Sciences, 348 Manter Hall, University of Nebraska, Lincoln, NE 68588, USA [l] Department of Plant & Soil Sciences, N-222D Ag. Science North, University of Kentucky, Lexington, KY 40546, USA [m] Department of Integrative Biology, University of California, Berkeley, 3001 Valley Life Sciences Building, Berkeley CA 94720, USA [n] Swiss Federal Institute for Forest, Snow and Landscape Research, Community Ecology, Zuercherstrasse 111, 8903 Birmensdorf, Switzerland [o] Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN 55108, USA [p] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

Submitted to Proceedings of the National Academy of Sciences of the United States of America

Soil microorganisms are critical to ecosystem functioning and the maintenance of soil fertility. However, despite global increases in the inputs of nitrogen (N) and phosphorus (P) to ecosystems due to human activities, we lack a predictive understanding of how microbial communities respond to elevated nutrient inputs across environmental gradients. Here we used high-throughput sequencing of marker genes to elucidate the responses of soil fungal, archaeal, and bacterial communities using an N and P addition experiment replicated at 25 globally distributed grassland sites. We also sequenced metagenomes from a subset of the sites to determine how the functional attributes of bacterial communities change in response to elevated nutrients. Despite strong compositional differences across sites, microbial communities shifted in a consistent manner with N or P additions, and the magnitude of these shifts was related to the magnitude of plant community responses to nutrient inputs. Mycorrhizal fungi and methanogenic archaea decreased in relative abundance with nutrient additions, as did the relative abundances of oligotrophic bacterial taxa. The metagenomic data provided additional evidence for this shift in bacterial life history strategies since nutrient additions decreased the average genome sizes of the bacterial community members and elicited changes in the relative abundances of representative functional genes. Our results suggest that elevated N and P inputs lead to predictable shifts in the taxonomic and functional traits of soil microbial communities, including increases in the relative abundances of faster growing, copiotrophic bacterial taxa, with these shifts likely to impact belowground ecosystems worldwide.

soil bacteria | soil fungi | shotgun metagenomics | soil ecology | fertilization

Introduction

Human activities associated with fossil fuel combustion, agricultural fertilization, and dust or ash production have greatly increased nitrogen (N) and phosphorus (P) inputs to ecosystems around the globe relative to their pre-industrial levels (1, 2). The impacts of elevated N and P inputs on grassland ecosystems, which cover 26% of the global land surface (3), are expected to occur on relatively short time scales, with potentially important effects on plant biodiversity and terrestrial carbon (C) dynamics (4–7). A large body of research focusing on plant community responses has demonstrated consistent loss of grassland plant diversity with nutrient additions (7, 8). In many cases, nutrient additions also shift the composition of plant communities with faster-growing plants that are good competitors for light being favored under conditions where nutrients are less limiting to growth (9, 10). The associated belowground microbial responses to nutrient additions, including general taxonomic and trait shifts, remain poorly understood, even though soil microbes represent a large fraction of the living biomass in grassland systems (11) and can have important effects on terrestrial C dynamics, soil fertility, and plant diversity (12). In particular, integrated, cross-site, experimental investigations of both plant and soil microbial responses to nutrient additions are needed to inform understanding of how the structure and functional attributes of soil microbial communities shift in response to anthropogenic inputs of N and P and whether these shifts are consistent across sites.

Soil microbial communities are often sensitive to nutrient inputs. For instance, N fertilization typically reduces microbial biomass and respiration rates (13–15), with specific functional groups of microbes, including ammonia oxidizers and mycorrhizal fungi, often being very sensitive to N additions (16–18). A few

Significance

Human activities have resulted in large increases in the availability of nutrients in terrestrial ecosystems worldwide. While plant community responses to elevated nutrients have been well-studied, soil microbial community responses remain poorly understood despite their critical importance to ecosystem functioning. Using DNA sequencing approaches, we assessed the response of soil microbial communities to experimentally added nitrogen and phosphorus at 25 grassland sites across the globe. Our results demonstrate that the composition of these communities shifts in consistent ways with elevated nutrient inputs, and that there are corresponding shifts in the ecological attributes of the community members. This study represents an important step forward for understanding the connection between elevated nutrient inputs, shifts in soil microbial communities, and altered ecosystem functioning.

Reserved for Publication Footnotes

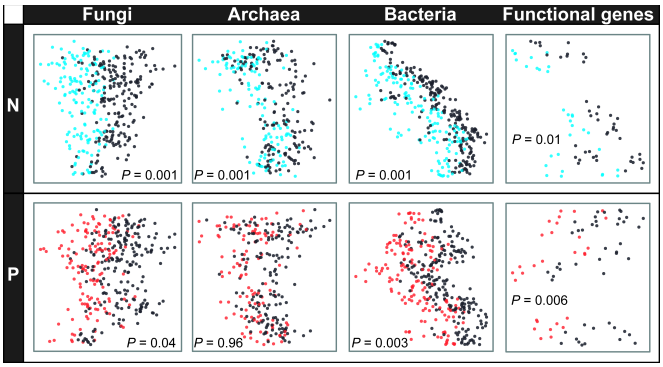

Fig. 1. Constrained ordinations showing differences between microbial communities from plots that did not receive the indicated nutrient (gray points) and from plots receiving N (blue) or P (red) additions (colored points). Colored points include samples receiving both nutrients. P-values refer to PERMANOVA results.

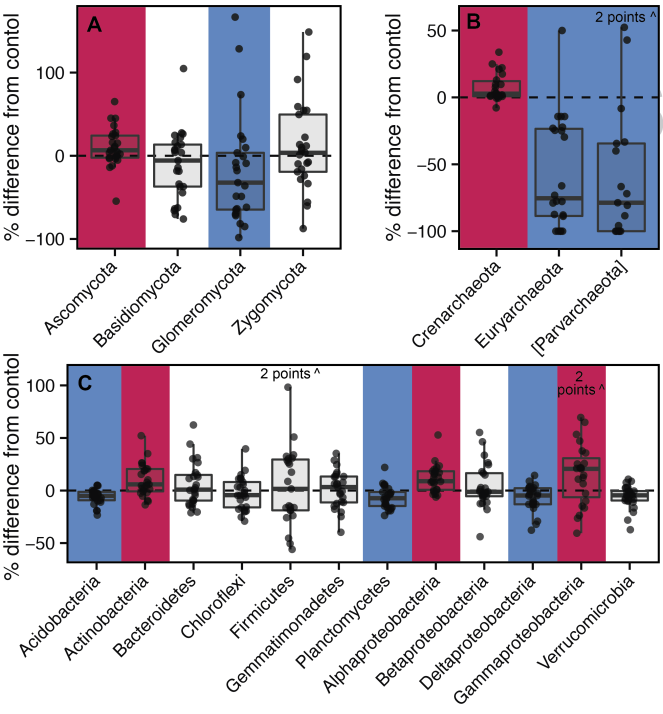

Fig. 2. Differences in the relative abundance of higher-level taxa between control and nutrient addition plots. Fungal (A) and bacterial (C) taxa differences are comparisons to +N,+P plots, and archaeal taxa differences (B) are comparisons to +N differences since P additions did not significantly affect the relative abundance of archaeal taxa, nor was there an interaction between N and P additions. Points represent site means, and boxplots show quartile values for each taxon. Red and blue backgrounds show significant increases and decreases in the relative abundances of specific taxa, respectively (FDR-corrected $P < 0.05$). Only taxa with relative abundances >1% in any of the treatments are shown. Points with values greater than the plot axis maximum are indicated.

studies conducted at individual sites also have shown that elevated N inputs can alter the overall composition of bacterial or fungal communities (17, 19–22). Understanding of soil microbial community responses to elevated P inputs remains more limited even though many regions experience elevated inputs of both N and P (2), and anthropogenic activities can alter N:P ratios in soil (1, 23). We are not aware of any studies that have used standardized nutrient treatments to evaluate the generality and local context dependence of soil bacterial, archaeal, and fungal communities to

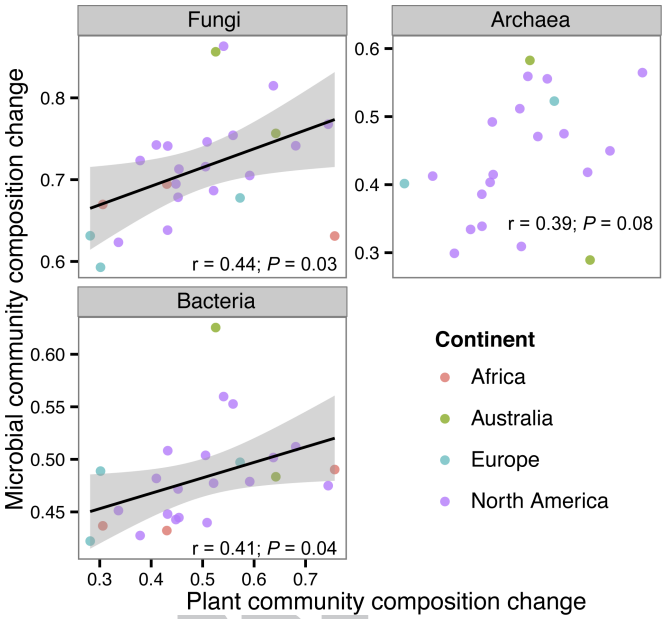

Fig. 3. Correlations between changes in microbial and plant community composition with N and P additions across the sites for fungal, archaeal, and bacterial communities. Change in community composition was calculated as the mean Bray-Curtis dissimilarity between control plots and those plots amended with nutrients. Relationships were assessed using Pearson correlations.

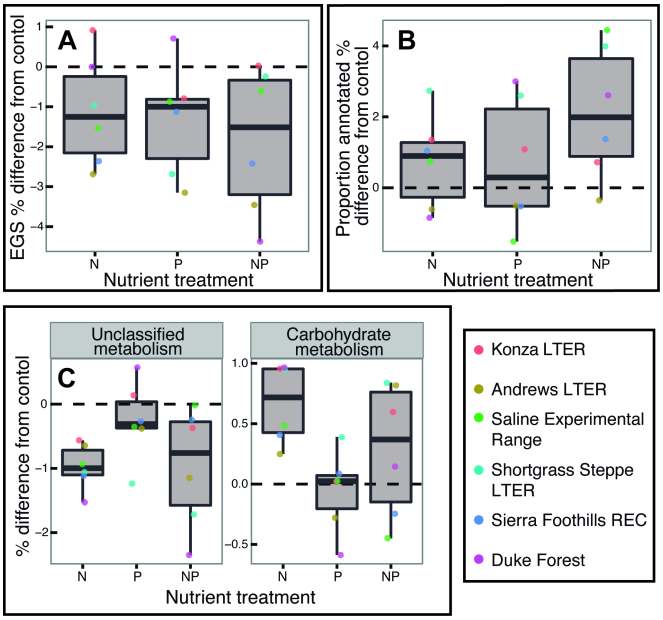

Fig. 4. Shifts in metagenomic characteristics with the addition of nutrients. Differences in the proportion of annotated genes (A), effective genome size (B), and the relative abundance of metabolic genes (C) are shown with boxplots and mean responses for each site (points). Gene categories in (C) were chosen by selecting those that most greatly differed between control and treatment plots ($P < 0.02$ for each; Table S5).

N and P amendments across a wide range of soil types. Individual studies conducted at specific sites are useful, but inconsistencies in methods and site characteristics limit the ability to make robust generalizations of how belowground microbial communities will respond to elevated nutrient inputs across sites.

While previous studies have shown that soil microbial communities can shift in response to nutrient additions at individual grassland sites (18, 20, 22, 24), relating these taxonomic or phylogenetic shifts to changes in the functional attributes of these communities is not trivial. Simply documenting how communities shift in composition might not tell us how the aggregated traits of these communities change in response to nutrient additions because soil microorganisms are incredibly diverse and most soil microbial taxa remain uncharacterized (25). Such trait-level information is arguably more important for linking changes in soil microbial communities to changes in belowground processes than simply documenting how nutrients increase or decrease the relative abundances of community members (26). Just as the aggregated traits of plant communities can shift in predictable directions with nutrient additions (9, 10), we expect that the aggregated traits of soil microbial communities will also shift in a predictable manner with fertilization. Here, we focus on the aggregated traits of bacterial communities, and specifically, we expect that increases in nutrient availability will tend to favor copiotrophic (i.e. fast growing, low C use efficiency) bacterial taxa and reduce the abundances of more oligotrophic (i.e. slow growing, high C use efficiency) taxa (20, 27). Although there is some evidence that we can use taxonomic information to place soil bacteria along this continuum in life history strategies (28), we can use shotgun metagenomic information to more accurately infer the aggregated traits of soil bacterial communities and determine whether copiotrophic traits are actually favored under conditions of elevated nutrient availability.

For this study we sought to build a predictive understanding of the responses of diverse soil microbes to elevated nutrient inputs that is generalizable across grasslands. We collected soils from an N and P addition experiment replicated at 25 grassland sites spanning four continents and quantified shifts in bacterial, archaeal, and fungal community structure in response to experimentally increased soil nutrients using high-throughput sequencing of marker genes. In addition, we investigated potential shifts in bacterial community-level traits by analyzing functional gene metagenomic sequences from a subset of those sites. We hypothesized that N and P additions would: induce shifts in fungal communities with mycorrhizal fungi decreasing in relative abundance, alter archaeal community composition by increasing the abundances of those taxa presumed to be capable of ammonia oxidation (29), and shift bacterial communities to favor copiotrophic over more oligotrophic taxa. Further, we hypothesized that the degree to which microbial communities shifted in response to nutrient additions would be positively correlated with the magnitude of the shifts in plant community composition. Those sites where nutrient additions have the largest effects on plant communities are also those sites where we would expect to see the largest responses in belowground microbial communities due to the direct associations between plants and microbes or their shared responses to fertilization.

Results and Discussion

Effect of nutrient additions on soil fungal communities

Fungal diversity and community composition differed strongly across the 25 globally distributed grassland sites regardless of nutrient treatment ($P < 0.001$ in all cases; Fig. S1). Mean fungal phylotype (i.e. species) richness ranged 1.7-fold across the sites, and there were large variations in the relative abundances of major taxonomic groups (Table S1). The strong site effects are not surprising given the range in environmental conditions and soil characteristics found across sites spanning four continents and elevations from 50 to 2320 m (Table S2). In particular, the sites represented a broad range in soil acidity, climate, and plant community composition, factors that have previously been associated with differences in soil fungal community structure at these sites and others (30, 31).

We investigated the within-site effects of nutrient additions on fungal community structure by statistically controlling for the strong cross-site differences by including site as a random effect in our models. Fungal Shannon diversity responded weakly to nutrient additions, decreasing by only 2.7% on average when N and P were added together ($P = 0.05$), a response consistent with the weak response observed for plants (8).

In contrast to the weak effects of nutrients on fungal diversity, we observed significant effects of N ($R^2 = 0.003$; $P < 0.001$) and/or P ($R^2 = 0.002$; $P = 0.04$) additions on fungal community composition, with the same taxa generally responding to nutrient additions across sites despite the large cross-site variation in fungal community types (Fig. 1). With combined addition of N and P, there were increases in *Ascomycota* and significant decreases in the relative abundances of *Glomeromycota* (Fig. 2A). The *Glomeromycota* phylum is composed almost entirely of arbuscular mycorrhizal fungi (32), and we expected these fungi to decrease in relative abundance with nutrient additions since they would be less valuable to their hosts and thus provided with less plant C under conditions of increased N and P availability (33–35). We further investigated nutrient effects on mycorrhizal fungi by assessing the collective responses of mycorrhizal fungi, including those taxa outside the *Glomeromycota* phylum that are reported in the literature as being mycorrhizal. These taxa also consistently decreased in plots receiving N and P relative to the control plots ($P = 0.016$), corroborating results from a meta-analysis demonstrating declines in mycorrhizal fungi with N additions (18). Interestingly, adding N and P together led to far larger decreases in the relative abundances of *Glomeromycota* than when these nutrients were added individually ($P > 0.1$; Table S3), suggesting a role for both of these nutrients in shaping arbuscular mycorrhizal communities.

The overall decrease in the proportion of mycorrhizal fungi with N and P additions, and shifts in fungal community composition more broadly, could be caused by plant community shifts, changes in plant biomass, and/or the direct effects of added nutrients. The magnitudes of the responses of major fungal taxonomic groups were not significantly correlated with changes in key soil characteristics (Table S4). However, the magnitude of fungal community composition response (i.e. the mean community dissimilarity between samples with added N and P and control samples) was significantly correlated with the magnitude of the response of plant community composition to added N and P ($r = 0.44$; $P = 0.03$; Fig. 3), helping to explain site-to-site variability in shifts in belowground communities. Those sites where nutrients had the largest impacts on plant communities were also the sites that had the strongest nutrient effects on fungal communities. This suggests either that shifts in plant community composition drive shifts in fungal community composition, or that both plant and fungal communities respond similarly to changes in edaphic factors. Although overall fungal compositional shifts correlated with plant community composition shifts, changes in the relative abundance of *Glomeromycota* were not related to changes in live plant biomass with fertilization ($P > 0.1$), nor were they related to changes in surface soil nitrogen concentrations ($P > 0.1$; Table S4), suggesting that plant nutrient limitation was not a good predictor of the differential responses observed across the sites.

Effect of nutrient additions on soil archaeal communities

Archaea were rare at most sites, and archaeal diversity (Fig. S1A) and community composition (Fig. S1B) were highly variable across sites regardless of nutrient additions ($P < 0.001$). Archaeal phylotype richness ranged 3.7-fold across the sites, and the archaeal communities were dominated by *Crenarchaeota* (92% on average) and *Euryarchaeota* (4.3% on average; Table S1). The proportion of 16S rRNA reads that were of archaeal origin was

also highly variable across the sites (Fig. S2A), ranging from 0 to 0.16. This variability in archaeal communities was likely due to the large cross-site differences in environmental conditions mentioned above. For instance, previous work has shown a correlation between archaeal relative abundances and soil nutrient content (36), we know that soil N concentrations varied 33-fold across the control plots, and archaea relative abundances were inversely related to soil C:N ratios ($r = -0.67$; $P < 0.001$).

We next assessed whether there were consistent shifts in archaeal relative abundance and community structure with nutrient additions by statistically controlling for the strong cross-site differences. Archaeal relative abundances generally increased with N additions ($P < 0.001$; Fig. S2B), and there was a mean 4.8% decrease in archaeal diversity with N additions when compared to control plots ($P = 0.01$). This decrease in diversity was possibly related to an N-induced growth of specific archaeal taxa. Specifically, the phylum *Crenarchaeota*, which was primarily comprised of members of the family *Nitrososphaeraceae*, consistently increased in relative abundance with N additions across the majority of sites while *Euryarchaeota*, and the candidate division *Parvarchaeota* consistently decreased (Fig. 2B). These shifts are likely related to *Archaea* being active drivers of the soil N cycle. For example, *Nitrososphaeraceae* can oxidize ammonia (29, 37), a metabolism that is expected to be advantageous with elevated ammonium supply, which should have been elevated in the N addition plots, as urea is readily hydrolyzed to ammonium. Abundances of soil *Crenarchaeota* also are positively correlated with soil N content (36). Conversely, several reports have shown the potential for members of the *Euryarchaeota*, which are predominately methanogens, to fix atmospheric N_2 (38, 39). This could place them at a competitive disadvantage under conditions of elevated N availability and explain their strong proportional decrease with N fertilization. While it has been shown that N can inhibit methanogenesis *in vitro* (40), this is, to our knowledge, the first direct evidence that N additions may also decrease methanogen populations in non-wetland soils. Still, it is important to note that these shifts in the relative abundances of archaeal phyla are not independent of one another, and decreased methanogen relative abundances could simply be the result of increased relative abundances of *Crenarchaeota*. Nonetheless, these results highlight that soil archaeal communities are sensitive to N additions, but additional research is required to determine if these community responses are associated with changes in methane fluxes or soil N cycling rates.

Effect of nutrient additions on soil bacterial communities

As with fungal and archaeal communities, bacterial diversity and community composition differed strongly across the 25 grassland sites (Fig. S1). These differences were likely due to factors such as acidity, climate, and plant community composition as has been previously observed (30, 41, 42). Mean phylotype richness ranged 1.7-fold, and the abundant phyla, including *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria*, and *Bacteroidetes*, all varied considerably in their relative abundances across the sites (Table S1).

Nutrient additions did not strongly alter bacterial diversity; P additions caused marginal (0.5%) increases in bacterial diversity ($P = 0.06$), and N had no significant effect. Our results stand in contrast to negative relationships between bacterial diversity and N additions reported from previous studies conducted at individual sites (19, 43). This points to the importance of local context and highlights the pitfalls associated with extrapolating results obtained from individual sites to other ecosystems or soil types.

Bacterial community composition was significantly affected by N ($R^2 = 0.002$; $P < 0.001$) and P additions ($R^2 = 0.002$; $P = 0.003$; Fig. 1). The community shifts corresponded to changes in the relative abundances of numerous major taxa. For example, the relative abundances of *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* consistently increased with nutrient additions across sites, while those of *Acidobacteria*, *Planctomycetes*, and *Deltaproteobacteria* consistently decreased (Fig. 2C). However, these taxonomic shifts were not always in the same direction or magnitude when N or P was added alone (Table S3). Overall, the taxonomic patterns in our cross-site study were in agreement with previous work conducted at individual grassland sites (20), and they corroborate laboratory studies which have noted similar shifts in the relative abundances of these major bacterial groups with nutrient additions (13). Our findings are generally consistent with our hypothesized shifts in general life history strategies with bacterial taxa that are faster growing and more copiotrophic (28) being favored under conditions of elevated nutrient availability (27). In particular, soil bacterial groups that are generally considered to be more copiotrophic, including *Actinobacteria* and *Alphaproteobacteria,* increased in relative abundance with nutrient additions, and the largely oligotrophic *Acidobacteria* phylum decreased in relative abundance. While original evidence for generalizations of these life history strategies across broad bacterial taxonomic groups was based on responses to labile carbon inputs (28, 44, 45), our results extend evidence for these ecological classifications to the direct or indirect bacterial responses to nutrient additions.

Genomic and metagenomic evidence for shifts in bacterial life history strategy with nutrient additions

We recognize that it is difficult to confidently assign bacterial clades into groups with copiotrophic and oligotrophic life history strategies, especially given the overwhelming amount of undescribed bacterial diversity found in soil (25). Thus, we used a combination of genomic and metagenomic approaches to provide independent assessments of how copiotroph:oligotroph ratios shifted in response to added nutrients. First, we estimated aggregate community growth rates since we expected increases in the relative abundance of copiotrophic taxa to be reflected by faster growth rates (28, 46). Thus, an increase in the estimated growth rate [i.e. a decrease in mean minimum generation time (MGT)] would suggest an increase in the relative abundance of copiotrophs. Mean MGTs were calculated for all samples from a combination of our bacterial marker gene data and published genomes; 757 of the 46,534 phylotypes could be matched to genomes. As with other attributes of community structure, estimates of MGT strongly varied across sites (Fig. S3A). Within-site differences between nutrient-amended and control samples showed that adding nutrients tended to decrease MGTs (Fig. S3B), but this trend was not significant for N additions ($P = 0.57$) or P additions ($P = 0.34$) individually. However, this analysis has important limitations in that only a small proportion ($\sim$10%) of the 16S rRNA gene sequences from our samples could be mapped to genomes for which we had MGT estimates, and this proportion differed across nutrient treatments (Fig. S3C). Thus, this analysis likely provides a conservative estimate of potential differences in MGTs associated with nutrient additions and is only weakly supportive of the hypothesis that soil bacterial MGT decreases with nutrient additions.

To further confirm the putative shifts in life history strategies in bacterial communities, we assessed functional attributes directly from functional gene (i.e. shotgun metagenomic) data collected from six of the sites used in the taxonomic analyses (Table S2). These sites were selected because they spanned a wide geographic range, encapsulated a variety of environmental conditions, and the marker gene analyses suggested the N and P effects on microbial community composition were particularly strong. The shotgun metagenomic data (hereafter referred to as "metagenomic data") were found to be almost entirely derived from bacterial genomes – 94.8 ± 2.3% (mean ± SD) of the metagenomic small subunit (SSU) rRNA gene reads were identified as

bacterial. Just as the marker gene data revealed that bacterial diversity and community composition differed strongly across sites, the metagenomic data revealed that functional gene diversity and composition also varied strongly across sites (Fig. S1). In addition, the diversity of annotated genes identified from the metagenomic data was significantly correlated with the diversity of bacterial phylotypes across the samples ($r^2 = 0.27$, $P < 0.001$; Fig. S4A), and the dissimilarity in functional gene composition was strongly related to the dissimilarity in bacterial community composition across the six sites ($\rho = 0.87$, $P < 0.001$; Fig. S4B). These findings suggest that bacterial communities that are distinct in composition tend to have distinct functional attributes, and bacterial communities that are taxonomically more diverse also have more diverse metagenomes with a broader array of annotated genes. Correspondingly, the diversity of functional genes did not change with nutrient additions ($P > 0.1$), but there were significant shifts in overall functional gene composition with N ($P = 0.01$) and P additions ($P = 0.006$; Fig. 1) as was observed for bacterial taxa. These results are supported by previous work showing a relationship between the taxonomic structure of soil bacteria and functional genes across ecosystems (41) and significant N effects on functional gene composition at two North American sites (27).

The metagenomic data yielded additional lines of evidence to support our hypothesis that nutrient additions favor copiotrophic bacterial taxa. Previous work has suggested that soil microorganisms with larger genomes should be more successful in resource-poor environments (47), and thus, we expect copiotrophic taxa to have smaller genomes. To assess this, we calculated mean effective genome size, the estimated mean size of a genome in a given sample, and found that it significantly decreased with added N or P ($P < 0.03$ in both cases; Fig. 4A). More generally, this result highlights that genome size can be considered an important ecological trait, just as bacterial genome size is correlated with range size (48) and plant genome size is an important predictor of species' ability to invade (49).

We investigated the specific gene categories that changed in proportion with nutrient additions by analyzing the quality-filtered metagenomic sequences that could be annotated. First, it is important to note that only 28.7 - 32.7% of sequences could be annotated, and soils receiving N or P had a 0.3% higher annotation rate on average ($P \leq 0.01$ in both cases; Fig. 4B), a pattern likely driven by the over-representation of copiotrophic bacteria, which are easier to culture, and are thus more commonly found in genome databases. Similarly, soils receiving N amendments tended to have a lower relative abundance of annotated, but unclassified, metabolic genes compared to control samples, likely also reflecting the better representation of copiotrophs in genome databases (Fig. 4C; Table S5). We also observed a significant increase in the relative abundances of genes associated with carbohydrate metabolism (Fig. 4C) in fertilized plots. This is consistent with the added nutrients increasing copiotroph:oligotroph ratios and potentially increasing plant carbon inputs to soil. Although <33% of the sequence reads could be annotated, a percentage that is similar to that reported in other metagenomic analyses of diverse bacterial communities e.g., (27), our results highlight that the annotated reads can be used to infer shifts in the functional capabilities of communities, shifts that are consistent with nutrient additions increasing the proportional abundance of bacteria with copiotrophic life history strategies.

Nutrients can have both direct and indirect effects on belowground bacterial communities making it difficult to unravel the mechanisms underlying the community responses described above. Potential mechanisms include direct effects of the nutrients themselves, nutrient effects on soil characteristics (e.g., pH), nutrient inputs increasing plant productivity and organic matter inputs to soils (20), and nutrient inputs mediating microbial shifts through changes in plant community composition. With N addition, soil pH decreased by an average of 0.16 units across the sites ($P < 0.001$), and pH has been shown to strongly drive shifts in soil bacterial communities (42, 50, 51). However, pH alone is not likely to have been a major driver of community shifts observed here, as the pH change was relatively small, it did not change with P additions ($P = 0.36$), and the magnitude of change in pH was unrelated to the change in the relative abundance of any of the major bacterial taxa with N and P additions across the sites (Table S4). Proportional changes in plant productivity were also unrelated to changes in the relative abundance of bacterial taxa, suggesting that elevated plant productivity in fertilized plots was not responsible for the bacterial community responses. On the other hand, the magnitude of shifts in plant community composition was directly related to the magnitude of shifts in bacterial community composition ($r = 0.41$, $P = 0.04$; Fig. 3), a pattern that mirrored that observed for fungi (Fig. 3). These findings suggest that changes in plant community composition may be more important for mediating bacterial community responses to elevated nutrient inputs than changes in edaphic characteristics or plant growth.

Conclusions

Taken together, our results demonstrate that while microbial community composition varied considerably across the diverse grassland sites examined, nutrient availability elicits changes to the composition of microbial communities in consistent ways across sites by selecting for microbial groups that have certain functional traits. Understanding the responses of soil microbial communities to changes in nutrient availability is critical given that ecosystems across the globe are receiving increasing inputs of N and P. Our analyses represent one of the first attempts to empirically assess whether there are generalizable patterns in these responses across a wide range of climatic and edaphic environments and confirm their existence despite large cross-site differences in microbial community structure. The observed patterns correspond to broader ecological theory, and set the stage for more targeted hypothesis testing. For example, nutrient-induced shifts in copiotrophic versus oligotrophic traits could have important implications for soil C cycling (52) if their traits elicit effects rather than solely reflect responses (53). Likewise, decreases in mycorrhizae and methanogens could have important impacts on ecosystem-level processes (39, 54). This work moves us towards a more mechanistic understanding of how shifts in microbial community composition mediate and reflect the effects of anthropogenically elevated nutrient inputs on terrestrial ecosystems.

Materials and Methods

Complete documentation of the experimental design, sample collection, and analytical methods are provided in SI Materials and Methods.

Identical full factorial N and P addition experiments were established at each of the 25 sites used in this study, which included temperate-zone grasslands in Africa, Australia, Europe, and North America (Table S2). Nutrients were added annually in 10 g N or P m^{-2} yr^{-1}. Plant communities and soil characteristics were assessed as in (30). Fungal, archaeal, and bacterial community structure were characterized using barcoded Illumina sequencing of the internal transcribed spacer region of the ribosomal operon and the 16S rRNA gene for fungi and bacteria, respectively, using an approach described previously (30). These raw sequence data are available in the Sequence Read Archive at the National Center for Biotechnology Information (accession: SRP052716). The shotgun metagenomic sequences were collected and processed using an approach similar to (55) with annotation performed using the KEGG hierarchy (56). These data are available at the Integrated Microbial Genomes and Metagenomes website (http://img.jgi.doe.gov) and referenced in the Genomes Online Database (GOLD Study ID: Gs0053063). We estimated MGTs for bacterial communities by calculating MGTs in available whole bacterial genomes using the method described in (57) and mapping the 16S rRNA sequences we collected to these genomes.

Acknowledgements.

We thank Monte Lunacek of University of Colorado Research Computing for valuable computational support, and we thank Elizabeth DeLorenze

and Ryan Williams for feedback on earlier drafts of this manuscript. We are grateful to Jessica Henley and Xavier Rojas for help with the sample processing. The shotgun metagenomic analyses were made possible with support from the U.S. Department of Energy's Joint Genome Institute and their Community Sequencing Program (CSP-672). This work was supported by a grant to N.F. from the National Science Foundation (DEB0953331). The Nutrient Network (http://nutnet.org) experiment is funded at the site scale by individual researchers. Coordination and data management are supported by funding to E. Borer and E. Seabloom from the NSF Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs and the UMN Institute on the Environment (DG-0001-13). This work utilized the Janus supercomputer, which is supported by the National Science Foundation (award number CNS-0821794) and the University of Colorado Boulder.

1. Galloway JN et al. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226.
2. Wang R et al. (2015) Significant contribution of combustion-related emissions to the atmospheric phosphorus budget. *Nat Geosci* 8:48–54.
3. Foley JA et al. (2011) Solutions for a cultivated planet. *Nature* 478:337–342.
4. Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712–5.
5. Craine JM, Morrow C, Stock WD (2008) Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytol* 179:829–36.
6. LeBauer D, Treseder K (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379.
7. Suding KN et al. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci U S A* 102:4387–92.
8. Borer ET et al. (2014) Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508:517–20.
9. Tilman D, Wedin D (1991) Plant traits and resource reduction for five grasses growing on a nitrogen gradient. *Ecology* 72:685–700.
10. Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111:1169.
11. Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. *Ecol Lett* 12:1238–49.
12. Van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296–310.
13. Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob Chang Biol* 18:1918–1927.
14. Janssens IA et al. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nat Geosci* 3:315–322.
15. Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol Lett* 11:1111–20.
16. Wessén E, Nyberg K, Jansson JK, Hallin S (2010) Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Appl Soil Ecol* 45:193–200.
17. Egerton-Warburton L, Johnson N, Allen E (2007) Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. *Ecol Monogr* 77:527–544.
18. Treseder K (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytol* 164:347–355.
19. Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environ Microbiol* 12:1842–54.
20. Ramirez KS, Lauber CL, Knight R, Bradford MA, Fierer N (2010) Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* 91:3463–3470.
21. Allison SD, Hanson CA, Treseder KK (2007) Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biol Biochem* 39:1878–1887.
22. Coolon JD, Jones KL, Todd TC, Blair JM, Herman MA (2013) Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. *PLoS One* 8.
23. Peñuelas J et al. (2013) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun* 4:2934.
24. Pan Y et al. (2014) Impact of long term N, P, K and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiol Ecol*.
25. Ramirez K et al. (2014) Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proc R Soc B* 281:20141988.
26. Fierer N, Barberán A, Laughlin DC (2014) Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. *Front Microbiol* 5:1–6.
27. Fierer N et al. (2011) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* 6:1–11.
28. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88:1354–64.
29. Leininger S et al. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442:806–9.
30. Prober SM et al. (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol Lett* 18:85–95.
31. Tedersoo L et al. (2014) Global diversity and geography of soil fungi. *Science* 346:1256688–1256688.
32. Redecker D, Raab P (2006) Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* 98:885–95.
33. Van Diepen LT a, Lilleskov E a., Pregitzer KS, Miller RM (2007) Decline of arbuscular mycorrhizal fungi in northern hardwood forests exposed to chronic nitrogen additions. *New Phytol* 176:175–183.
34. Wei C et al. (2013) Nitrogen deposition weakens plant-microbe interactions in grassland ecosystems. *Glob Chang Biol* 19:3688–97.
35. Johnson NC, Wilson GWT, Bowker M a, Wilson J a, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc Natl Acad Sci U S A* 107:2093–2098.
36. Bates ST et al. (2011) Examining the global distribution of dominant archaeal populations in soil. *ISME J* 5:908–17.
37. Gubry-Rangin C, Hai B (2011) Niche specialization of terrestrial archaeal ammonia oxidizers. *Proc Natl Acad Sci* 108:21206–21211.
38. Leigh JA (2000) Nitrogen fixation in methanogens: the archaeal perspective. *Curr Issues Mol Biol* 2:125–31.
39. Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. *Annu Rev Microbiol* 67:437–57.
40. Klüber HD, Conrad R (1998) Inhibitory effects of nitrate, nitrite, NO and N2O on methanogenesis by Methanosarcina barkeri and Methanobacterium bryantii. *FEMS Microbiol Ecol* 25:331–339.
41. Fierer N et al. (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc Natl Acad Sci* 109:21390–21395.
42. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 75:5111–20.
43. Koyama A, Wallenstein MD, Simpson RT, Moore JC (2014) Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Front Microbiol* 5:1–16.
44. Bastian F, Bouziri L, Nicolardot B, Ranjard L (2009) Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biol Biochem* 41:262–275.
45. Eilers KG, Lauber CL, Knight R, Fierer N (2010) Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol Biochem* 42:896–903.
46. Pianka E (1970) On r-and K-selection. *Am Nat* 104:592–597.
47. Konstantinidis KT, Tiedje JM (2004) Trends between gene content and genome size in prokaryotic species with larger genomes. *Proc Natl Acad Sci U S A* 101:3160–5.
48. Barberán A et al. (2014) Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecol Lett* 17:794–802.
49. Suda J, Meyerson LA, Leitch IJ, Py P (2014) The hidden side of plant invasions : the role of genome size. *New Phytol*.
50. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci U S A* 103:626–31.
51. Rousk J, Brookes PC, Glanville HC, Jones DL (2011) Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. *Appl Environ Microbiol* 77:2791–5.
52. Wieder WR, Bonan GB, Allison SD (2013) Global soil carbon projections are improved by modelling microbial processes. *Nat Clim Chang* 3:909–912.
53. Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct Ecol* 16:545–556.
54. Van der Heijden MGA et al. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72.
55. Fierer N et al. (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science* 342:621–4.
56. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res* 40:D109–14.
57. Vieira-Silva S, Rocha EPC (2010) The systemic imprint of growth and its uses in ecological (meta)genomics. *PLoS Genet* 6:e1000808.

SI References

58. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–8.
59. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461.
60. McDonald D et al. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 6:610–8.
61. Abarenkov K et al. (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytol* 186:281–285.
62. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–7.
63. Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–63.
64. Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27:863–4.
65. Markowitz VM et al. (2012) IMG: the Integrated Microbial Genomes database and comparative analysis system. *Nucleic Acids Res* 40:D115–22.
66. Kent WJ (2002) BLAT---The BLAST-Like Alignment Tool. *Genome Res* 12:656–664.
67. Fierer N et al. (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc Natl Acad Sci* 109:21390–21395.
68. Quast C et al. (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* 41:590–596.
69. Haegeman B et al. (2013) Robust estimation of microbial diversity in theory and in practice. *ISME J* 7:1092–101.
70. R Core Team (2013) R: A Language and Environment for Statistical Computing.