

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

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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

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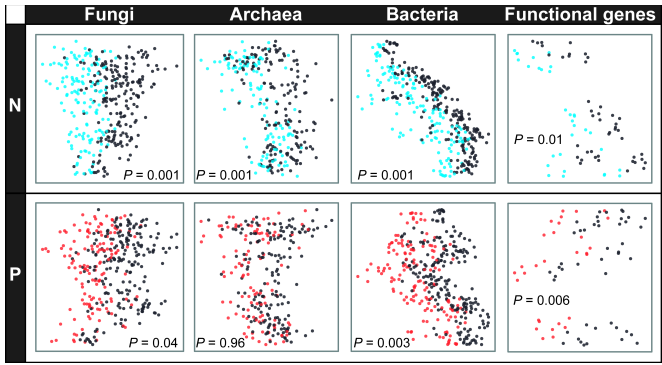


Fig. 1. Constrained ordinations showing differences between microbial communities from plots that did not receive the indicated nutrient (gray points) and from plots receiving N (blue) or P (red) additions (colored points). Colored points include samples receiving both nutrients. P-values refer to PERMANOVA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

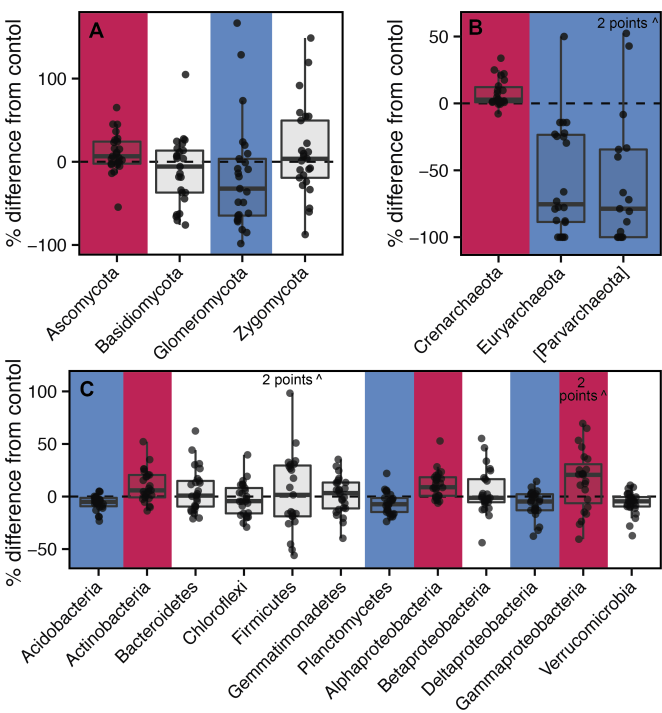


Fig. 2. Differences in the relative abundance of higher-level taxa between control and nutrient addition plots. Fungal (A) and bacterial (C) taxa differences are comparisons to +N,+P plots, and archaeal taxa differences (B) are comparisons to +N differences since P additions did not significantly affect 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

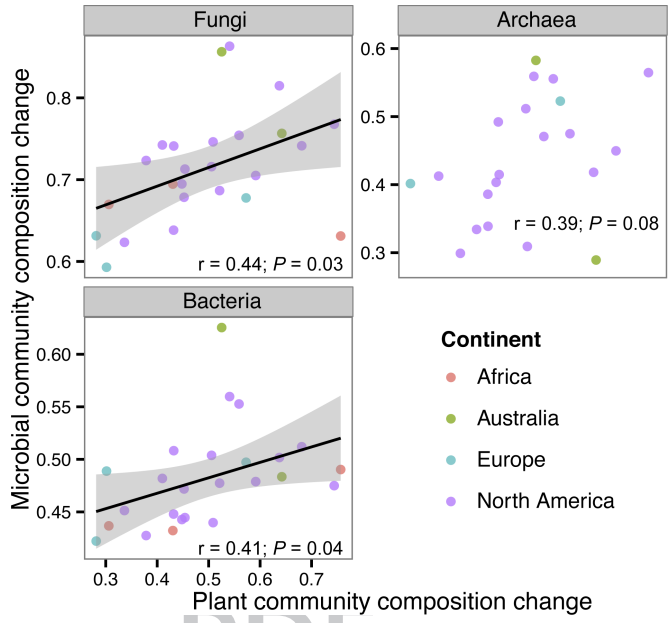


Fig. 3. Correlations between changes in microbial and plant community composition with N and P additions across the sites for fungal, archaeal, and bacterial communities. Change in community composition was calculated as the mean Bray-Curtis dissimilarity between control plots and those plots amended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

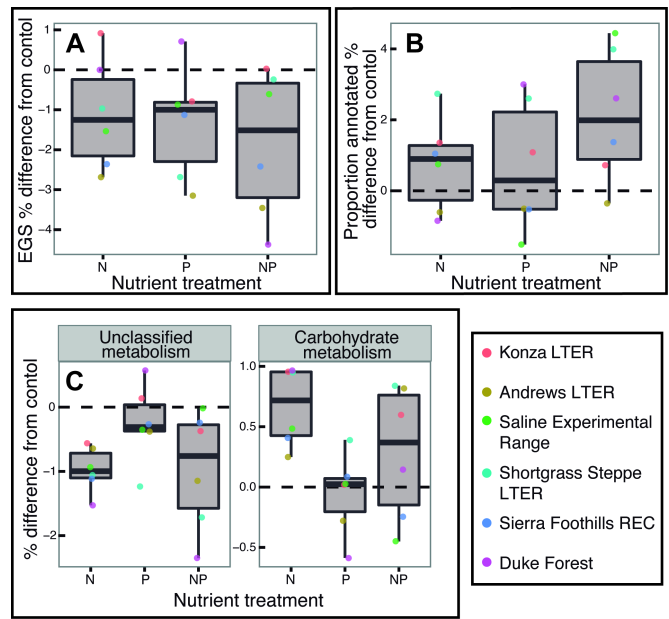


Fig. 4. Shifts in metagenomic characteristics with the addition of nutrients. Differences in the proportion of annotated genes (A), effective genome size (B), and the relative abundance of metabolic genes (C) are shown with boxplots and mean responses for each site (points). Gene categories in (C) were 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.

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

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

302 Results and Discussion

303 Effect of nutrient additions on soil fungal communities

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

341 previously been associated with differences in soil fungal community structure at these sites and others (30, 31).

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

346 In contrast to the weak effects of nutrients on fungal diversity, we observed significant effects of N ($R^2 = 0.003$; $P < 0.001$)
347 and/or P ($R^2 = 0.002$; $P = 0.04$) additions on fungal community composition, with the same taxa generally responding to nutrient
348 additions across sites despite the large cross-site variation in fungal community types (Fig. 1). With combined addition of
349 N and P, there were increases in *Ascomycota* and significant decreases in the relative abundances of *Glomeromycota* (Fig.
350 2A). The *Glomeromycota* phylum is composed almost entirely of arbuscular mycorrhizal fungi (32), and we expected these fungi
351 to decrease in relative abundance with nutrient additions since they would be less valuable to their hosts and thus provided with
352 less plant C under conditions of increased N and P availability (33–35). We further investigated nutrient effects on mycorrhizal
353 fungi by assessing the collective responses of mycorrhizal fungi, including those taxa outside the *Glomeromycota* phylum that
354 are reported in the literature as being mycorrhizal. These taxa also consistently decreased in plots receiving N and P relative
355 to the control plots ($P = 0.016$), corroborating results from a meta-analysis demonstrating declines in mycorrhizal fungi with
356 N additions (18). Interestingly, adding N and P together led to far larger decreases in the relative abundances of *Glomeromycota*
357 than when these nutrients were added individually ($P > 0.1$; Table S3), suggesting a role for both of these nutrients in shaping
358 arbuscular mycorrhizal communities.

359 The overall decrease in the proportion of mycorrhizal fungi with N and P additions, and shifts in fungal community composition
360 more broadly, could be caused by plant community shifts, changes in plant biomass, and/or the direct effects of added
361 nutrients. The magnitudes of the responses of major fungal taxonomic groups were not significantly correlated with changes in
362 key soil characteristics (Table S4). However, the magnitude of fungal community composition response (i.e. the mean community
363 dissimilarity between samples with added N and P and control samples) was significantly correlated with the magnitude of the
364 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
365 shifts in belowground communities. Those sites where nutrients had the largest impacts on plant communities were also the sites
366 that had the strongest nutrient effects on fungal communities. This suggests either that shifts in plant community composition
367 drive shifts in fungal community composition, or that both plant and fungal communities respond similarly to changes in edaphic
368 factors. Although overall fungal compositional shifts correlated with plant community composition shifts, changes in the relative
369 abundance of *Glomeromycota* were not related to changes in live plant biomass with fertilization ($P > 0.1$), nor were they related
370 to changes in surface soil nitrogen concentrations ($P > 0.1$; Table S4), suggesting that plant nutrient limitation was not a good
371 predictor of the differential responses observed across the sites.

372 Effect of nutrient additions on soil archaeal communities

373 Archaea were rare at most sites, and archaeal diversity (Fig. S1A) and community composition (Fig. S1B) were highly variable
374 across sites regardless of nutrient additions ($P < 0.001$). Archaeal phylotype richness ranged 3.7-fold across the sites, and the
375 archaeal communities were dominated by *Crenarchaeota* (92% on average) and *Euryarchaeota* (4.3% on average; Table S1). The
376 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 archaeal 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 (~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 – $94.8 \pm 2.3\%$ (mean \pm 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 background 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.

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