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Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems

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Abstract. Ecosystems worldwide are receiving increasing amounts of reactive nitrogen (N) through anthropogenic activities. Although the effects of increased N inputs on plant communities have been reasonably well studied, few comparable studies have examined impacts on whole soil bacterial communities, though they play critical roles in ecosystem functioning. We sampled soils from two long-term ecological research (LTER) experimental N gradients, both of which have been amended with NH_4NO_3; a grassland at Cedar Creek (27 years of N additions) and an agricultural field at Kellogg Biological Station (8 years of N additions). By examining shifts in bacterial communities across these contrasting ecosystem types, we could test competing hypotheses about the direct and indirect factors that might drive bacterial responses to elevated N inputs. Bacterial community structure was highly responsive to N additions. We observed predictable and consistent changes in the structure of the bacterial communities across both ecosystem types. Our results suggest that bacterial communities across these gradients are more structured by N and/or soil carbon availability than by shifts in the plant community or soil pH associated with the elevated nitrogen inputs. In contrast to the pronounced shifts in bacterial community composition and in direct contrast to the patterns often observed in plant communities, increases in N availability did not have consistent effects on the richness and diversity of soil bacterial communities.

Key words: 16S rRNA genes; microbial ecology; N fertilization; nitrogen; pyrosequencing; soil bacteria; soil bacterial diversity; soil microbiology.

Introduction

Currently, nitrogen (N) inputs from anthropogenic sources are 30% greater than those from natural terrestrial sources, and 10-fold greater than anthropogenic inputs from 100 years ago (Galloway et al. 2004). This dramatic and relatively recent increase in N inputs has stimulated interest in understanding the environmental consequences of anthropogenic N additions. It is well established that elevated N additions to ecosystems can have wide-ranging impacts on biogeochemical cycles, the emissions of greenhouse gases, and plant biodiversity (Tilman 1987, Gough et al. 2000, Vitousek et al. 2002, Gilliam 2006). Less well understood are the impacts of increased N inputs on soil bacterial communities, even though bacteria represent a major portion of living biomass in terrestrial ecosystems (Fierer et al. 2009) and their activities are intimately tied to belowground processes.

Many studies have used experimental N gradients to examine the effects of increased N availability on plant communities. Across most ecosystem types, a significant increase in aboveground plant biomass is commonly observed (Tilman 1987, Bowman et al. 1993, Gough et al. 2000) followed, in nearly all cases, by changes in plant community composition (Gough et al. 2000, Stevens and Carson 2002, Suding et al. 2005, Gilliam 2006). As available N increases in a system, plant community diversity often decreases, favoring nitrophilous species, and eliminating slower growing and N sensitive species (Tilman 1987, Vitousek and Howarth 1991). In contrast to the relatively large number of studies examining N impacts on plant communities, we lack a comprehensive understanding of N effects on soil bacterial communities.

A number of studies have examined N fertilization effects on microbial biomass (e.g., Waldrop et al. 2004, Zeglin et al. 2007) and specific microbial activities such as extracellular enzyme production and organic carbon mineralization rates (e.g., Sinsabaugh et al. 2005, Treseder 2008). Yet the response patterns are frequently mixed and lack consistency (Treseder 2008). Other studies have focused on the responses of specific bacterial taxa directly involved in soil N cycling, such as nitrifying bacteria (e.g., Prosser and Nicol 2008). A critical gap in our knowledge is how the overall structure of bacterial communities may respond to anthropogenic

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N inputs, even though similar research has been conducted on plant communities for decades. N addition effects on whole bacterial communities have been determined via fingerprinting-based techniques (Hallin et al. 2009), but these approaches do not provide sufficient information to comprehensively resolve the phylogenetic and taxonomic responses of bacterial communities to N. With the exception of the work by Nemergut et al. (2008), few, if any, published studies have used high-resolution, sequence-based approaches to analyze bacterial community shifts across N gradients and/or to directly compare responses across ecosystem types.

To understand better how N additions impact soil bacterial community composition and diversity, we selected two experimental N fertilization studies established in contrasting ecosystems, a successional grassland and a cultivated agriculture field. In the grassland study, plant composition was permitted to respond to the treatment, while in the agricultural study, plant composition was held constant. Admittedly, our chosen gradients apply N at rates far higher than most soils are likely to receive (up to 800 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ at Cedar Creek and 270 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ at Kellogg). However, using an N gradient that spans a wide range of N amendment rates (including rates that few, if any, soils are likely to experience) is advantageous because it allows us to determine how the possible elimination of N limitation may alter microbial communities, the threshold of this response, and possible mechanisms driving this response. Additionally, since both of these gradients are well studied, having been used to understand plant responses to N additions, we can compare microbial community and plant community responses.

From these experimental gradients we can test competing hypotheses, recognizing that N additions can have both direct and indirect effects on soil biota (Treseder 2008). If pH is an important control on bacterial community composition and diversity, as shown by Fierer and Jackson (2006) and Lauber et al. (2009), then we should observe shifts in specific bacterial taxa that correspond with the fertilizer-induced changes in soil pH (H_1). If plant community composition or diversity is an important control, then bacterial community responses should only be observed at the grassland site, where plant community shifts are realized across the N treatments (H_2). If carbon availability is an important control, then both sites should experience similar changes in bacterial community composition (H_3). We pose H_3 in the context of C availability, as we know of no previous studies demonstrating which specific taxa (other than taxa associated with N cycling) would be expected to respond specifically and solely to increases in N availability. Thus, we used two contrasting N experiments to assess the influence of N additions on bacterial community composition and diversity using high-throughput pyrosequencing coupled with detailed soil measurements.

Methods

Site descriptions

We collected soil from two long-term experimental N gradients, one established at Cedar Creek Ecosystem Science Reserve (CC; Minnesota, USA) and the other at Kellogg Biological Station (KBS; Michigan, USA; see Plate 1), both part of the Long-Term Ecological Research (LTER) network. The CC N gradient was established in 1982 on successional grasslands with soils of a sandy texture. N is added to the plots twice per year as NH_4NO_3 at eight levels (0, 30, 60, 100, 160, 280, 500, and 800 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$). Plots also received a mixture of micronutrients (P, K, Ca, Mg, and trace metals) and approximately 6 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of atmospheric N deposition. We collected samples in July 2008 from two replicate fields (experiment E001: fields A and B), randomly choosing three of the six replicates of each treatment, yielding 48 samples in total, 24 from each field. Additional information on this experiment can be found in Tilman (1987) and at the CC web site (*available online*).[7]

The KBS N gradient was established in 2001 in an agricultural field that has a yearly rotating cropping system, with crops harvested and the plots tilled at the end of each growing season. Fields were corn (*Zea mays*) during 2008. Soils are mainly fine-loamy and coarse-loamy in texture (Crum and Collins 1995). This experimental design includes nine levels of N additions (0, 34, 67, 101, 134, 168, 202, 246, and 267 kg/ha), added yearly as NH_4NO_3 and an estimated 6 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of atmospheric N deposition. The N gradient is replicated over eight "replicate" blocks, four of which are rain-fed (R) only (1–4) and four of which also receive irrigation (I) water (5–8). Soil samples were collected in July 2008 from all blocks (eight) and plots (nine per block), yielding a total of 72 samples from this site. Further site description and information can be found on the KBS web site (*available online*).[8]

Sample collection and soil measurements

Fields A and B of CC, and rain-fed and irrigated blocks of KBS are distinct fields/blocks and were treated as such in the subsequent analyses. Soil cores (diameter 2 cm), 0–5 cm deep, were collected from six randomly selected locations within each plot, combined and sieved to 2 mm on site. Samples were frozen and shipped to the University of Colorado for analysis. A select number of samples (0, 100, and 800 $kg\ NH_4NO_3{\cdot}ha^{-1}{\cdot}yr^{-1}$ of field A from CC and the 0, 101, and 267 $kg\ NH_4NO_3{\cdot}ha^{-1}{\cdot}yr^{-1}$ samples of rain-fed blocks 2, 3, and 4 from KBS) were also shipped on ice for microbial biomass and C availability determination at the University of Georgia.

[7] ⟨http://www.cedarcreek.umn.edu/research/experiments/e001.php⟩

[8] ⟨http://lter.kbs.msu.edu/research/long_term_experiments/fertility_gradient.php⟩

PLATE 1. Just as the vegetation clearly responds to the nitrogen fertility gradient at Kellogg Biological Station, Michigan, USA, high-throughput sequencing demonstrates that the soil bacterial community concurrently experiences consistent, directional shifts in community composition. Photo credit: K. S. Ramirez.

Biomass was determined using the substrate-induced respiration (SIR) procedure described previously (Fierer et al. 2003). To estimate microbial soil C availability, an identical procedure was used but only H_2O was added (no yeast extract); we assumed that in the absence of moisture limitation and at a constant temperature, the rate of CO_2 production in the unamended samples corresponds to the amount of C readily available for microbial mineralization (Fierer et al. 2003).

Soil moisture, pH, extractable N, and soil carbon and nitrogen concentrations, were determined for each soil sample. Soil pH was measured on supernatants of a 1:1 (mass : volume), soil to water slurry, after being shaken for 1 h. Total extractable inorganic N (NH_4^+ and $NO_3^- + NO_2^-$) was determined from 4 g (dry mass) of soil using a KCl extraction procedure, and analyzed in the Kiowa Chemistry Laboratory at the Institute of Arctic and Alpine Research (INSTAAR) on an OI Analytical Flow Solution IV Analyzer (OI Analytical, College Station, Texas, USA). Total soil C and N concentrations were determined using a CHN 4010 Elemental Combustion System (Costech Analytical Technologies, Valencia, California, USA). Total aboveground plant biomass (AGB) measurements were obtained for 2008 from the CC plots, but comparable data were not available for KBS.

Community level sequence analysis

To determine the soil bacterial composition for each sample, we extracted DNA, and PCR amplified the 27-338 region (*E. coli* numbering) of the 16S rRNA gene using error-correcting bar-coded primers (Hamady et al. 2008). Samples were pooled and then pyrosequenced on a Roche FLX 454 pyrosequencing machine (454 Life Sciences, Bradford, Connecticut, USA). All procedures, including the sequence analyses, were carried out following protocols described previously (Costello et al. 2009, Lauber et al. 2009). Although the communities were surveyed to a high level of resolution (on average $>$1900 sequence reads per sample) bacterial taxa that are rare may not have been resolved. Yet, the approach provides a robust and quantitative determination of the overall shifts in bacterial community structure to unprecedented detail across multiple (120 in total) samples. Relatedness of bacterial communities between each pair of samples was determined using the unweighted UniFrac algorithm (Lozupone and Knight 2005, 2008). UniFrac is a phylogenetic metric that uses branch length overlap to calculate the amount of phylogenetic distance (or degree of relatedness) between pairs of communities (Hamady and Knight 2009). It has been shown to be well-suited for quantifying shifts in highly diverse microbial communities (Reeder and Knight 2009). To identify changes in bacterial diversity, we calculated three indices: the number of unique phylotypes, the Shannon-Wiener index of phylotype diversity (Hill et al. 2003), and Faith's index of phylogenetic diversity (PD) (Faith 1992). For all diversity indices, we used a randomly selected subset of 1500 sequences from each sample to correct for differences in sequencing effort between samples, with phylotype richness and Shannon-Wiener diversity de-

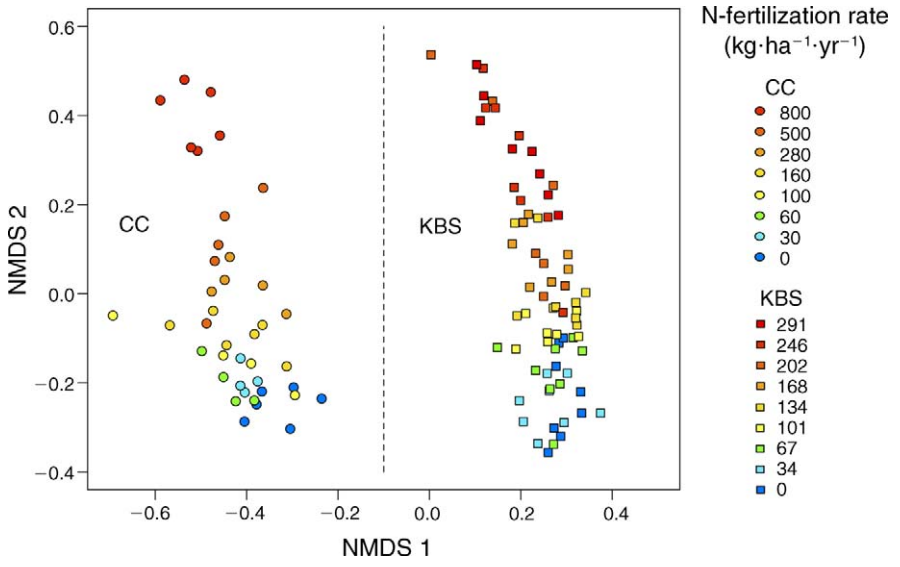

FIG. 1. Nonmetric multidimensional (NMDS) ordination plot of unweighted UniFrac pairwise distances. Symbol shapes represent experimental sites and fill color N-addition rates. Increasing distance between symbols indicates increasing dissimilarity in phylogenetic composition; a result of both experimental site and differences in N-fertilization rates. Locations are KBS, Kellogg Biological Station (Michigan, USA); CC, Cedar Creek Ecosystem Science Reserve (Minnesota, USA).

termined by defining phylotypes at the 97% sequence similarity level.

Statistical analysis

To analyze changes in phylogenetic structure, pairwise unweighted UniFrac distance matrices were compared against each of the measured edaphic variables (e.g., soil N, pH, soil C, singly or in combination) using Mantel tests (Ramette 2007). Correlations of edaphic factors, the relative abundances of each bacterial group, and estimated diversity levels with nitrogen inputs were tested for significance using Spearman correlations. Microbial biomass differences were tested using ANOVA. All statistical analyses were performed using the program R version 2.8.1 (R Development Core Team 2008).

RESULTS

Soil and plant responses

At CC, the N fertilization significantly ($P < 0.05$ in all cases) affected the edaphic and plant factors. From low to high fertilization, pH ranged from 7.4 to 6.0, soil C content from 10.4 to 28.9 mg/g soil, soil N content from 0.7 to 2.61 mg/g soil, total extractable inorganic N from 0.01 to 0.12 mg/g soil and AGB ranged from 33 to 203 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ (Appendix: Fig. A1, Table A1). From low to high fertilization microbial C mineralization, significantly increased from 0.4 to 1.2 μg $C{\cdot}CO_2$ g $soil^{-1}{\cdot}h^{-1}$ ($P < 0.01$), as determined by lab incubations, and microbial biomass ranged from 3.1 to 4.5 μg $C{\cdot}CO_2$ g $soil^{-1}{\cdot}h^{-1}$, but were only marginally significant ($P = 0.07$). At KBS, the majority of edaphic factors were also significantly correlated with the N fertilization rates ($P < 0.05$). From low to high fertilization, pH ranged from 6.9 to 5.0, soil C content from 12.8 to 17.2 mg/g soil, total N content ranged from 1.31 to 1.62 mg/g soil, and extractable N ranged from 0.01 to 0.21 mg/g soil (Appendix: Fig. A1, Table A1). Microbial C availability, ranged from 0.4 to 0.6 μg $C{\cdot}CO_2$ g $soil^{-1}{\cdot}h^{-1}$, and microbial biomass ranged from 3.5 to 5.2 μg $C{\cdot}CO_2$ g $soil^{-1}{\cdot}h^{-1}$, though increases were not significant ($P = 0.11$, $P = 0.13$, respectively).

TABLE 1. Mantel test of UniFrac distances and edaphic factors, with global R and significance reported.

Site	N addition	pH	C:N	C (mg/g)	N (mg/g)	AGB
CC field A	0.86***	0.79***	0.57***	−0.07	0.84***	0.58***
CC field B	0.81***	0.77***	0.66***	0.24*	0.59***	0.59***
CC A and B combined	0.86***	0.80***	0.67***	0.09	0.69***	0.50***
KBS rain fed (R)	0.69***	0.72***	0.40***	0.00	0.69***	NA
KBS irrigated (I)	0.70***	0.77***	0.24***	0.10	0.59***	NA
KBS R and I combined	0.65***	0.68***	0.30***	0.04	0.64***	NA

Notes: Key to abbreviations: CC, Cedar Creek Ecosystem Science Reserve; KBS, Kellogg Biological Station; AGB, aboveground plant biomass. NA, not applicable (AGB was not measured at KBS).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

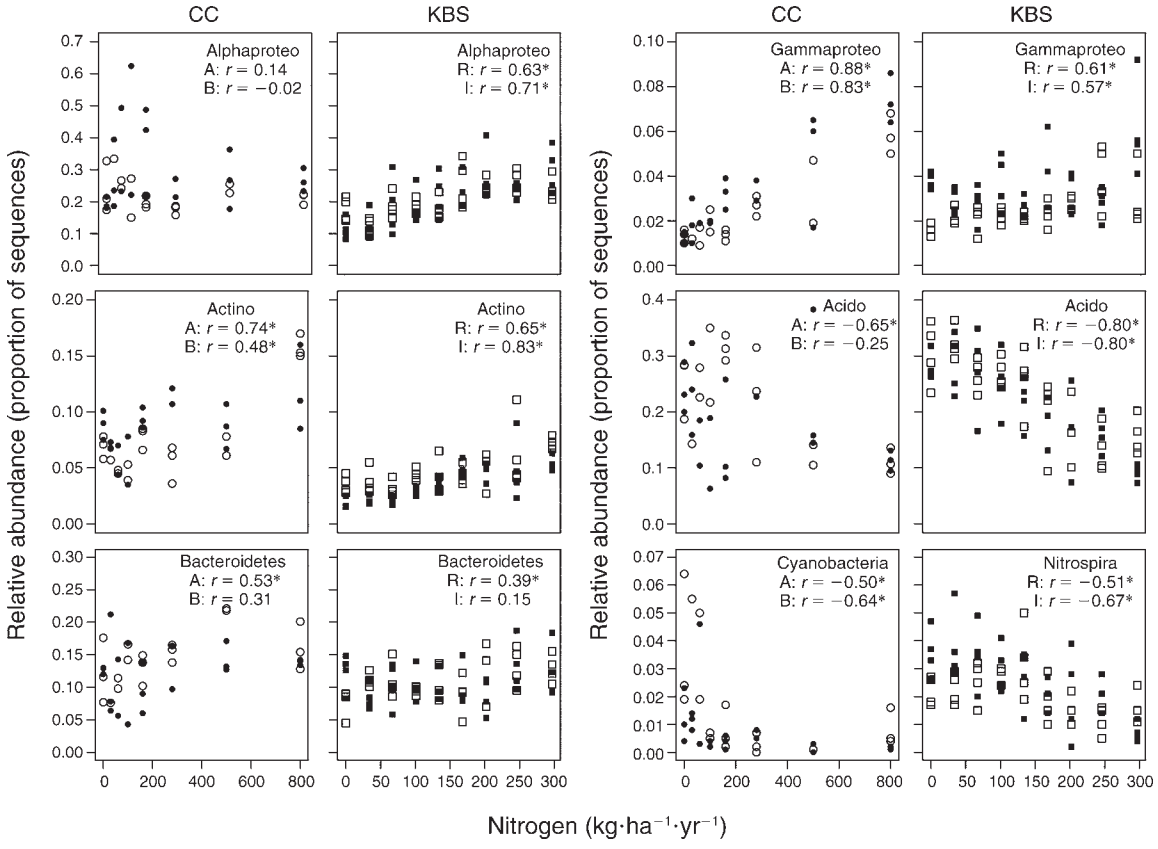

FIG. 2. Changes in the relative abundances (in proportion of sequences) of specific bacterial taxa across the N gradients, as determined by Spearman correlations. Sites are: open circles, CC field A; solid circles, CC field B; open squares, KBS rain fed (R); solid squares, KBS irrigated (I).
* $P < 0.05$.

Phylogenetic structure and diversity

CC and KBS harbor distinct soil bacterial communities, as demonstrated by phylogeny (Fig. 1) and taxonomy-based community analyses (Appendix: Table A2 and A3). The CC soils generally had higher abundances of *Proteobacteria* and lower abundances of *Acidobacteria* than the soils from KBS. However, across both N gradients, the phylogenetic structure of the bacterial communities shifted in a similar manner (Fig. 1). As N inputs increased, communities became progressively more distinct from those receiving no N fertilizer. The shifts in community composition were highly correlated with N additions at both CC (global $R = 0.86$, $P < 0.001$) and KBS (global $R = 0.65$, $P < 0.001$), as well as with pH, C:N, total N and AGB (Table 1). Changes in community composition were even observed (though less pronounced) across the lower N levels (from 0–150 μg $C{\cdot}CO_2$ g $soil^{-1}{\cdot}h^{-1}$), with significant correlations between community composition and N levels still observed at these lower levels for both CC (global $R = 0.21$, $P = 0.009$) and KBS (global $R = 0.33$, $P < 0.001$).

To determine those bacterial groups responsible for the observed phylogenetic responses (Fig. 1) we examined shifts in the relative abundances of specific bacterial taxa (Appendix: Fig. A2, Tables A2 and A3). *Gammaproteobacteria* and *Actinobacteria* increased with N inputs at both sites ($P < 0.05$; Fig. 2). Conversely, *Acidobacteria*, *Cyanobacteria*, and *Nitrospira* decreased with N input rates ($P < 0.05$; Fig. 2). At CC, the relative abundances of 21 bacterial groups were significantly correlated with N amendments, representing a substantial proportion (~40–50%) of the community sequences. At KBS, the relative abundances of 30 taxa were correlated with N addition rates, representing 65–80% of the community.

In contrast to phylogenetic and taxonomic composition, responses of bacterial diversity to N fertilization were inconsistent between sites. Specifically CC displayed no significant trends, and bacterial diversity at KBS was negatively correlated with fertilization rate with nearly all of the diversity metrics employed (Fig. 3).

DISCUSSION

As has been demonstrated with plant communities (Tilman 1987, Clark et al. 2009), by studying a broad N

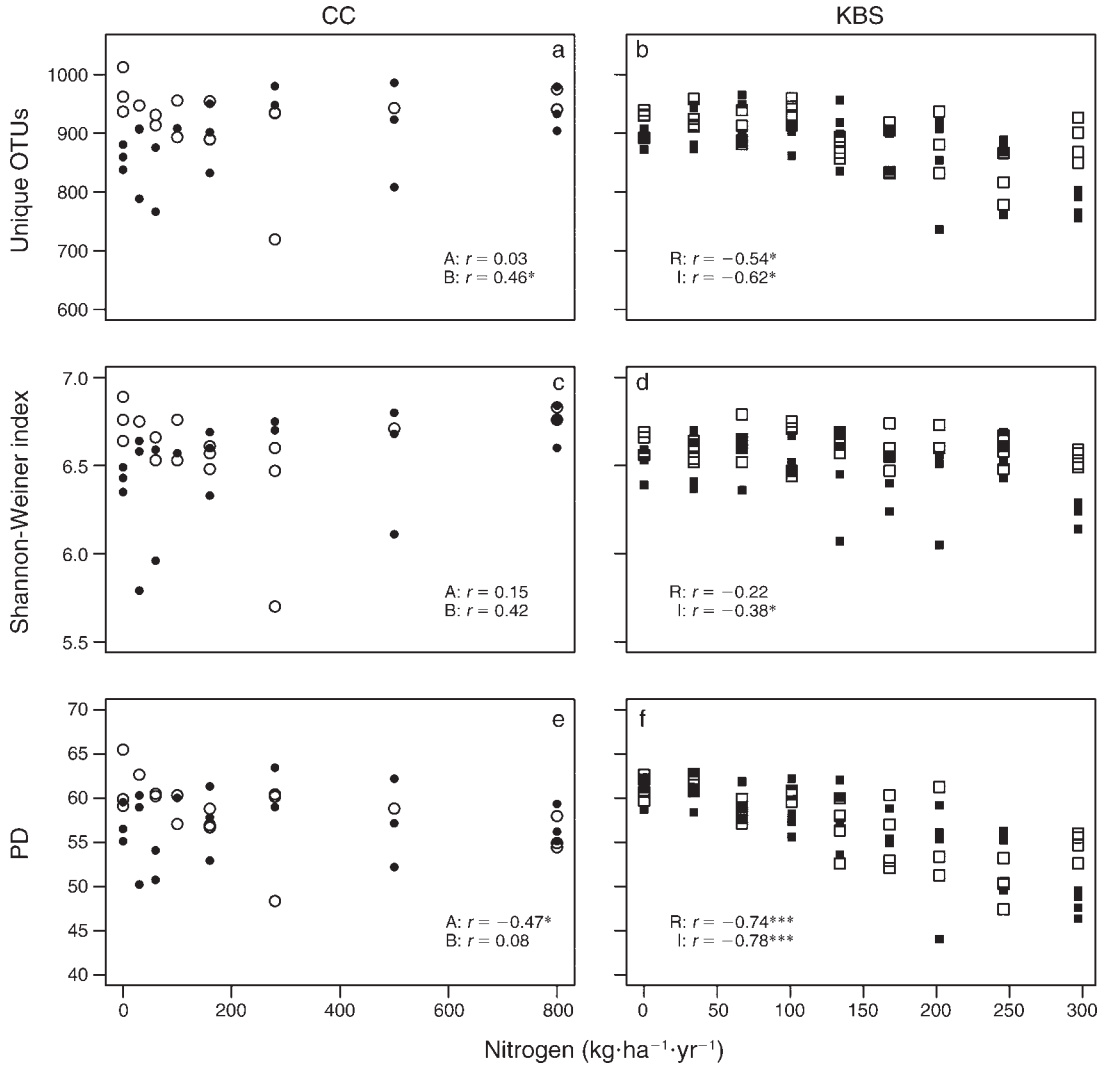

FIG. 3. Diversity measurements represented in (a, b) number of unique phylotypes (operational taxonomic units or OTUs, (c, d) the Shannon-Weiner index, and (e, f) phylogenetic diversity out of 1500 sequences per sample. Sites are: open circles, CC field A; solid circles, CC field B; open squares, KBS rain-fed (R); solid squares, KBS irrigated (I).
* $P < 0.05$; *** $P < 0.001$.

gradient we can determine which factors are structuring the microbial community, identify saturation or threshold points, and better pinpoint roles of different members of the community (e.g., which taxa are copiotrophic or nitrophilous). At both CC and KBS we observed consistent shifts in community structure (Fig. 1) and these shifts were driven largely by changes in the relative abundance of specific bacterial groups (Fig. 2). Even when testing only the lower N application rates (0–150 kg $NH_4NO_3 \cdot ha^{-1} \cdot yr^{-1}$) we find the community significantly responds, though less dramatically. We hypothesized that bacterial compositional changes might be caused by altered soil pH (H_1), plant community composition (H_2), and/or plant C inputs (H_3). Although our study design does not permit us to definitively decouple the experimental N fertilization effects on bacterial community composition from associated edaphic and plant responses, the use of the contrasting experimental studies (CC and KBS) allows us to make inferences related to those hypotheses we posed.

Nitrogen fertilization decreased soil pH at both sites, and from previous work we would expect soil pH to have a strong influence on soil bacterial community structure and diversity (Hartman et al. 2008, Lauber et al. 2009). If the change in soil pH (H_1) across the gradients does cause the phylogenetic responses (Fig. 1), we would expect the relative abundance of *Acidobacteria* to increase at low pH, and the relative abundance of *Actinobacteria* and *Bacteriodetes* to decrease at low pH (Jones et al. 2009, Lauber et al. 2009). However, our observed trends (Fig. 2) are in direct contrast with these

predictions, suggesting that soil pH, while it may still be important in structuring the communities, was not the dominant factor responsible for the pronounced shifts in bacterial community composition across these N gradients. Alternatively, if the changes in bacterial communities were related to shifts in plant community composition and diversity (H_2), we would expect to observe bacterial community responses to N only at CC, where the plant composition was not held constant. Instead, we observed consistent bacterial community shifts between CC, a speciose grassland, and KBS, a monospecific cropland. The mechanisms proposed in H_1 and H_2 do not appear to be the most parsimonious explanations for the phylogenetic and taxonomic patterns observed across the N gradients.

Instead the similar community composition patterns observed at both CC and KBS (Figs. 1 and 2) support our hypothesis that changes in C availability (H_3) structure the bacterial communities across the N gradients. It is well established that N additions increase net primary productivity (Gough et al. 2000, LeBauer and Treseder 2008) and the inputs of labile plant C to soil (Gu et al. 2004). Our estimates of microbial available C, measured via the lab incubation, and total soil C concentrations increased with N fertilization at both CC and KBS, as we might expect from the marked increases in AGB at CC (Appendix: Table A1) and KBS (K. Ramirez, *personal observation*). Additionally, the shifts in the relative abundances of specific taxa further support H_3. Specifically, at both CC and KBS, with N fertilization we observed increases in copiotrophic (or *r*-selected) bacterial groups, such as *Bacteriodetes* and *Betaproteobacteria*, and decreases in oligotrophic (or *K*-selected) groups, such as *Acidobacteria* (as per Padmanabhan et al. 2003, Fierer et al. 2007). These taxonomic responses support our hypothesis that changes in the quantity and/or quality of C inputs might explain the observed shifts in bacterial community composition across the N gradients. Again, we are using C measurements as a proxy, and it is possibly that N alone or both N and C are structuring community composition, however at this time no study has identified how an entire bacterial community collectively responds only to N additions (while holding other variables constant).

In contrast to phylogenetic and taxonomic composition, N fertilization did not have a consistent effect on bacterial diversity. That is, we observed no diversity response at CC with increasing N (even though plant diversity decreases; Tilman 1984), and a subtle decrease in diversity at KBS (Fig. 3). We speculate that the controls over diversity may differ from those controls on community composition. Specifically, we pose that pH, not carbon availability, may be controlling diversity. At KBS, the pH ranges from 6.9 to 5.0; Lauber et al. (2009) shows that at pH values lower than 6.5 there was a strong negative relationship with bacterial diversity across a wide range of soils. At CC, pH ranges from 7.4 to 6.0; a range over which diversity did not respond appreciably (see Lauber et al. 2009). Future work is required to ascertain why these different metrics of community structure (diversity and composition) appear to respond to different drivers.

The activities of bacterial communities directly regulate biogeochemical processes in ecosystems. By simultaneously investigating bacterial community responses to N fertilization across two contrasting ecosystems, our work suggests that C and/or N inputs consistently influence the composition of bacterial communities. Our work helps to resolve those factors, which may be important in structuring bacterial communities, and shows that their composition is sensitive to N fertilization. Still, future work is needed to ascertain how changes observed under the experimental N gradients influence ecosystem processes.

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APPENDIX

Edaphic factors and bacterial abundance (*Ecological Archives* E091-247-A1).

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ERRATA

Ramirez et al. have discovered errors in their Report ("Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems"), published in the December 2010 issue (*Ecology* 91:3463–3470). The levels of nitrogen applied at CC were reported as kg $NH_4NO_3 \cdot ha^{-2} \cdot yr^{-1}$ and the levels of nitrogen applied at KBS were reported as kg $N \cdot ha^{-2} \cdot yr^{-1}$.

In fact, nitrogen was applied as NH_4NO_3 to CC (0, 10, 20, 34, 54, 95, 170 and 272 kg $N \cdot ha^{-2} \cdot yr^{-1}$) and to KBS (0, 34, 67, 101, 134, 168, 202, 246 and 267 kg $N \cdot ha^{-2} \cdot yr^{-1}$). The microbial biomass and C availability analyses were done on the 0, 34 and 272 kg $N \cdot ha^{-2} \cdot yr^{-1}$ samples from CC. Additionally, the levels of N-fertilization rate (kg $N \cdot ha^{-2} \cdot yr^{-1}$) for CC in Figure 1 are 0, 10, 20, 34, 54, 95, 170 and 272, and the axes for CC in Figure 2 and 3 should be divided by a factor of 2.94. These changes do not affect any of the results or conclusions of this paper.

Kauffman et al. have discovered errors in their Report ("Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade"), published in the September 2010 issue (*Ecology* 91:2742–2755). A conversion error associated with the tree ring measuring device led to incorrect values being reported in the paper. The errors are found in two figures on pages 2748 and 2750 and can be corrected by simply changing the scales of the axes. In Fig. 2A, the vertical axis describing tree growth (BAI, mm^2) should have ranged from 0.0 to 10 mm^2, rather than from 0.0 to 40 mm^2. In Fig. 5A, the correct horizontal axis describing tree growth (BAI, mm^2/yr) should have ranged from 0.0 to 15 mm^2/yr, rather than from 0.0 to 60 mm^2/yr. The mistakes did not alter any analyses or conclusions described in the paper.

Fedriani and Delibes have noted a taxonomic mistake in their paper, "Dangerous liaisons disperse the Mediterranean dwarf palm: fleshy-pulp defensive role against seed predators," which appeared in the February 2011 issue (*Ecology* 92:304–315). Where the paper says "bruchid," it should instead say "curculionid" in the following places:

p. 306, right-hand column, line 13;
p. 307, right-hand column, line 11 (i.e., the subsection heading);
p. 307, right-hand column, lines 19, 21, 23, 27, and 29;
p. 308, left-hand column, line 31 (i.e., the first line of the section *Statistical analyses*);
p. 308, right-hand column, line 31 (i.e., the first subsection heading in the Results);
p. 308, right-hand column, lines 41 and 52;
p. 312, right-hand column, lines 29 and 47.

The authors noted that "bruchid" is correct in the following four instances:

p. 305, right-hand column, line 1;
p. 312, left-hand column, line 35;
p. 312, right-hand column, line 20;
p. 315, right-hand column, line 6 (i.e., in the title of the Silvius and Fragoso reference).