environmental microbiology

Environmental Microbiology (2018) **20**(3), 1112–1119 doi:10.1111/1462-2920.14051

Taxonomic patterns in the nitrogen assimilation of soil prokaryotes

Ember M. Morrissey,[1,2]* Rebecca L. Mau,[1] Egbert Schwartz,[1,3] Benjamin J. Koch,[1] Michaela Hayer[1] and Bruce A. Hungate[1,3]

[1]*Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ, USA.*

[2]*Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA.*

[3]*Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA.*

Summary

Nitrogen (N) is frequently a limiting nutrient in soil; its availability can govern ecosystem functions such as primary production and decomposition. Assimilation of N by microorganisms impacts the availability of N in soil. Despite its established ecological significance, the contributions of microbial taxa to N assimilation are unknown. Here we measure N uptake and use by microbial phylotypes and taxonomic groups within a diverse assemblage of soil microbes through quantitative stable isotope probing (qSIP) with ^{15}N. Following incubation with $^{15}NH_4^+$, distinct patterns of ^{15}N assimilation among taxonomic groups were observed. For instance, glucose addition stimulated ^{15}N assimilation in most members of Actinobacteria and Proteobacteria but generally decreased ^{15}N use by Firmicutes and Bacteriodetes. While NH_4^+ is considered a preferred and universal source of N to prokaryotes, the majority ($>$ 80%) of N assimilation in our soils could be attributed to a handful of active orders. Characterizing N assimilation of taxonomic groups with ^{15}N qSIP may provide a basis for understanding how microbial community composition influences N availability in the environment.

Introduction

Soil microorganisms assimilate and release N as they live, grow and die. The balance between N immobilization and mineralization by microorganisms regulates N availability to plants and thus ecosystem productivity (Reich *et al.*, 1997; Lebauer and Treseder, 2008). Predicting N uptake and release by microorganisms under varying environmental conditions is crucial to understanding how N availability can influence ecosystem response to global change. For instance, while elevated CO_2 can stimulate plant biomass production and terrestrial carbon storage, this feedback is limited by N availability (Hungate *et al.*, 2003). Indeed, additional terrestrial C storage in response to elevated CO_2 may slow over time as N becomes increasingly immobilized in plant and microbial biomass and accessible N availability declines (Zak *et al.*, 2000; Gill *et al.*, 2002; Finzi *et al.*, 2006; Norby *et al.*, 2010).

While microbial N uptake and release are recognized as the key drivers of ecosystem function, we remain unable to predict this process. A meta-analysis of 84 sites across the USA found that precipitation, soil texture, soil carbon and N contents could only explain approximately one-third of the variation in net N mineralization rates (Colman and Schimel, 2013). The authors posit that microbial community composition may be important in explaining patterns of N immobilization and release and thus is a research priority. This sentiment is further supported by correlations of microbial community composition with N immobilization (Zhu *et al.*, 2013; Heijboer *et al.*, 2016) and mineralization (Balser and Firestone, 2005; Smithwick *et al.*, 2005) rates.

Microbial community composition has been hypothesized to be a driver of processes that are performed by a relatively select group of microorganisms but not processes that are performed by many, seemingly redundant, types of organisms (Schimel, 1995; McGuire and Treseder, 2010). As all microorganisms require N for growth, N immobilization is practiced by many microbial taxa and, therefore, community composition is predicted to be unimportant (Schimel, 1995). However, if taxa vary in their rates of N assimilation, the membership of communities could influence N immobilization rates. Quantitative variation among species in the type and rate of N uptake has been documented in plant communities (Miller and Bowman, 2003), and such differences presumably contribute to the effects of plant community composition on soil N availability, nitrate leaching and nitrification (e.g., Wedin and Tilman, 1990; Hooper and Vitousek, 1998; Scherer-Lorenzen *et al.*, 2003; Byrnes *et al.*, 2014).

Received 10 October, 2017; revised 27 December, 2017; accepted 14 January, 2018. *For correspondence. E-mail ember.morrissey@mail.wvu.edu.

Very little is known about how soil microbial taxa differ in their N assimilation rates, likely due to the difficulties associated with measuring the activities of microorganisms within natural assemblages.

Assimilation of N by microbial taxa can be assessed by stable isotope probing (SIP) to track the movement of ^{15}N into the DNA of microorganisms (Buckley *et al.*, 2007a; Bell *et al.*, 2011). However, SIP with ^{15}N is challenging and rarely utilized because N comprises only ~1% of DNA by mass and high levels of enrichment are required to separate natural abundance from isotopically enriched DNA (Cadisch *et al.*, 2005). This challenge can be overcome by disentangling the effects of genomic guanine + cytosine (GC) content and isotope enrichment on DNA buoyant density (Buckley *et al.*, 2007b; Cupples *et al.*, 2007).

Here, we used quantitative stable isotope probing (qSIP; Hungate *et al.*, 2015) with $^{15}NH_4^+$ to assess the N assimilation patterns of soil microbial taxa. This new approach involves sequencing the DNA in multiple fractions along a CsCl density gradient for both natural abundance and isotopically enriched samples. This allows a determination of each taxon's GC content and isotope enrichment (as described in Hungate *et al.*, 2015). Because qSIP disentangles the effects of GC content and isotopic enrichment, relatively subtle shifts in density of individual taxa, such as those produced by ^{15}N, should be detectable. Beyond allowing greater sensitivity, qSIP is a marked improvement over traditional ^{15}N SIP because it provides quantitative information regarding the proportion of N in DNA that is isotopically enriched. Presuming the excess atom fraction (EAF) ^{15}N in microbial DNA is approximately equal to that of biomass, qSIP can provide an estimate of biomass-specific N assimilation by microbial taxa. In this study, soil from a mixed conifer forest in northern Arizona was incubated with $^{14}NH_4^+$ or $^{15}NH_4^+$ in the presence or absence of glucose for one week. The glucose addition was intended to simulate a pulse of labile C from root exudates and promote N uptake by microorganisms. We hypothesized that taxonomic groups would exhibit distinct patterns of N assimilation in the presence and absence of added glucose.

Results and discussion

The average density of prokaryotic DNA increased following exposure to $^{15}NH_4^+$ isotope, but this increase was not statistically significant [analysis of variance (ANOVA), $p = 0.26$]. DNA from soils that received only N had a density (mean ± SE) of 1.6878 ± 0.0018 mg l^{-1} when incubated with $^{14}NH_4^+$ and 1.6902 ± 0.0013 mg l^{-1} with $^{15}NH_4^+$. Similarly, in the presence of glucose, $^{15}NH_4^+$ increased the density of DNA from 1.6895 ± 0.0020 to 1.6914 ± 0.0017 mg l^{-1}. To verify isotopic labeling, we used isotope ratio mass spectroscopy to measure the ^{15}N content of the total DNA extracted from the soil. DNA extracted from soil incubated with $^{15}NH_4^+$ was significantly enriched in ^{15}N ($p < 0.01$, Fig. 1), with an excess atom fraction (EAF) of 0.057 ± 0.005 in the control and, slightly higher (0.069 ± 0.004), with added glucose ($p = 0.13$).

Biomass-specific nitrogen assimilation, as determined by the ^{15}N EAF in each taxon's DNA, ranged from 0 to 0.34 EAF in the control (i.e., $^+NH_4$) and 0 to 0.38 EAF in the glucose-amended (i.e., $^+$glucose and $^+NH_4$) soils (Supporting Information Fig. S1). To determine the sensitivity of qSIP with ^{15}N, we calculated the 90% confidence interval for each taxon. The median confidence interval across all taxa was ±0.060 EAF ^{15}N when considering both the control and glucose-amended samples, suggesting that on average, qSIP with ^{15}N is sensitive enough to detect an enrichment as low as 0.06 EAF with 90% confidence. This represents a considerable improvement over traditional SIP with ^{15}N, which required enrichment in excess of 0.40 EAF to fully distinguish unlabeled from labeled DNA (Cadisch *et al.*, 2005).

To estimate community-level enrichment using qSIP, we integrated taxon-specific measurements by calculating the average ^{15}N EAF (mean ± SE) weighted by relative abundance of all taxa. The average ^{15}N EAF (mean ± SE) was 0.067 ± 0.017 EAF ^{15}N in the control and 0.109 ± 0.034 EAF ^{15}N in the glucose amended soils. These values are comparable in magnitude to the ^{15}N EAF measured in bulk DNA (Fig. 1) and also show greater enrichment in soils that received glucose. Some disparity between the community-level average enrichment estimated with qSIP

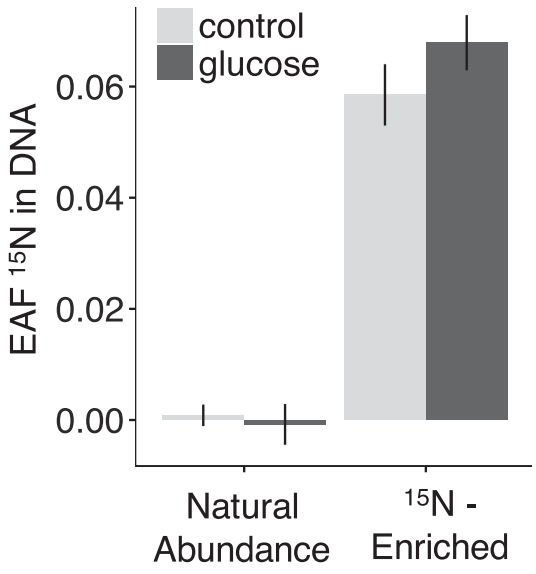

Fig. 1. Excess atom fraction (EAF) ^{15}N in total DNA (mean ± standard error) extracted from soil 1 week following the addition of either natural abundance or 0.97 EAF ^{15}N-enriched ammonium and with or without glucose.

and the measure of ^{15}N in bulk DNA was expected because some microbial groups present in the DNA pool (fungi and other eukaryotes) were not included in the qSIP analysis. Furthermore, the relative abundance of a taxon in the 16S rRNA gene library may not reflect its fraction of the total DNA pool because organisms vary in rRNA copy number per genome and genome size (Fogel *et al*., 1999; Větrovský and Baldrian, 2013). However, the general correspondence between these two independent measurements lends confidence that ^{15}N EAF values measured with the qSIP technique are representative of N assimilation into microbial DNA. The trend of enhanced N assimilation following glucose addition observed here is in agreement with the augmented rates of N immobilization after labile C addition commonly reported in the literature (e.g., Hamner and Kirchmann, 2005; Sawada *et al*., 2015; Buchkowski *et al*., 2015; Romero *et al*., 2015).

The soil prokaryotic community composition did not significantly change in response to glucose addition over the 1-week incubation period (PerMANOVA, $F = 0.98$, $p = 0.35$). Communities were dominated by Proteobacteria, accounting for ~35% of 16S rRNA genes, followed by Actinobacteria (~20%), Acidobacteria (~13%), Bacteroidetes (~13%) and Verrucomicrobia (~10%; Supporting Information Fig. S2). These phyla are commonly reported to dominate temperate forest and grassland soil (Prober *et al*., 2015; Šťursová *et al*., 2016; Kaiser *et al*., 2016), suggesting that findings from our study system may apply to understanding temperate soil communities more broadly.

Phylogenetic patterns in N assimilation were explored using Blomberg's *K* and Pagel's λ phylogenetic signal analyses. Assimilation of $^{15}NH_4^+$ exhibited a phylogenetic signal when added alone or in combination with glucose (Fig. 2). Similarly, the change in ^{15}N EAF following glucose addition was nonrandom with respect to phylogeny. This phylogenetic signal indicates that closely related organisms assimilated similar amounts of N. The quantity of ^{15}N in each taxon's DNA is influenced by that taxon's growth and accompanying synthesis of DNA. Growth rate has previously been shown to exhibit phylogenetic organization with distinct patterns of ^{18}O assimilation under different resource conditions (Morrissey *et al*., 2016) and may underpin some of the patterns in biomass specific ^{15}N assimilation observed here. For instance, relatively high amounts of ^{15}N enrichment were observed following glucose addition in Micrococcaceae (mean ± SD, 0.329 ± 0.048 EAF ^{15}N) and Comamonadaceae (0.164 ± 0.030 EAF ^{15}N), groups that have been previously shown to grow rapidly in the presence of glucose (Morrissey *et al*., 2016). Additional variation among taxa could be caused by differences in their preference for NH_4^+ over other N sources. Microbes vary in their uptake and assimilation of inorganic and organic N sources (Stolte and Riegman, 1995; Reay *et al*., 1999; Zubkov *et al*., 2003). While ammonium is considered as the preferred

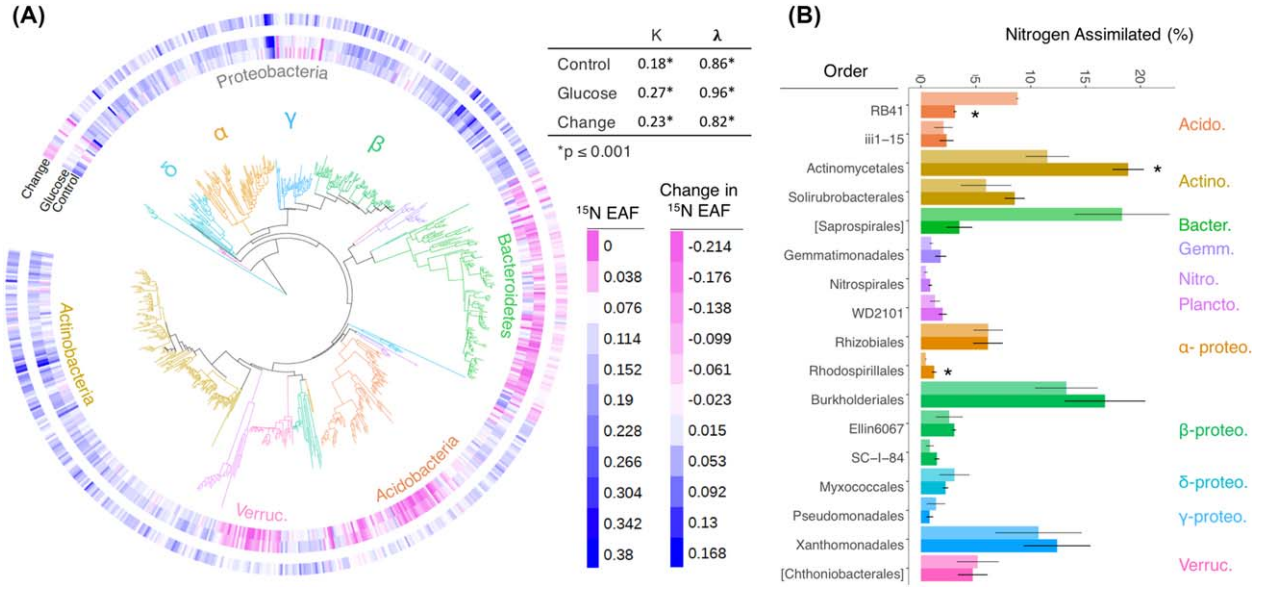

Fig. 2. Nitrogen assimilation by prokaryotic taxa.
A. Phylogenetic tree and heat map of ^{15}N isotope incorporation of prokaryotic taxa. Inner circles correspond to the excess atom fraction (EAF) ^{15}N in each taxon's DNA after incubation with $^{15}NH_4^+$ (control) or glucose and $^{15}NH_4^+$ (glucose). The outer circle represents the change in ^{15}N EAF between the control and glucose added soils.
B. Percentage of ^{15}N (mean ± standard error, $n = 3$) assimilated by orders accounting for at least 1% of the total ^{15}N assimilated. Significant differences (*) between control (lighter shade) and glucose amended (darker shade) soils were determined using paired *t*-tests ($\alpha = 0.05$).

source of N for bacteria (Geisseler *et al*., 2010 and references therein), some bacterial taxa can utilize NO_3^- as rapidly as ammonium (Reay *et al*., 1999), while others have a high affinity for organic N (Zubkov *et al*., 2003). Inter-taxon variation in the use of ammonium versus other N sources likely arises from differences in N transport and metabolism pathways (Gonzalez *et al*., 2006; Geisseler *et al*., 2010) that may correlate with phylogeny, as suggested by metagenomic analyses (Nelson *et al*., 2016).

A phylogenetic signal in N assimilation suggests that taxonomic groups exhibit distinct patterns in N assimilation. To test this possibility, we examined between-group variation of orders. The assimilation of ^{15}N varied significantly among prokaryotic orders (Supporting Information Fig. S3). Order membership explained much of the variation in ^{15}N EAF, accounting for the majority of the variation in the glucose amended (Adj $R^2 = 0.65$) and just under half of the variation in the control (Adj $R^2 = 0.47$) soils. Members of Burkholderiales exhibited consistently high ^{15}N assimilation (mean $\pm$ SD: control $= 0.140 \pm 0.039$, glucose $= 0.182 \pm 0.056$ ^{15}N EAF), while assimilation was much lower by taxa within Acidobacteriales (control $= 0.032 \pm 0.037$, glucose $= 0.039 \pm 0.017$ ^{15}N EAF). Taxonomic patterns were observed even at the coarse level of phylum. For instance, most phylotypes within Acidobacteria (74%), Actinobacteria (93%), Verrucomicrobia (83%) and Proteobacteria (82%) assimilated more N in the presence of glucose. Conversely, all taxa within Firmicutes and Crenarchaeota, as well as the majority of Bacteriodetes (84%), had greater N assimilation in the absence of added carbon. In order to be functionally redundant, organisms or groups of organisms must be quantitatively equivalent in their functioning (Allison and Martiny, 2008). Consequently, the differences we observed suggest that prokaryotic phylogenetic groups (e.g., orders) are not functionally redundant with respect to biomass-specific ammonium assimilation in soil.

To understand which taxonomic groups were most important for N assimilation in our system, we estimated the proportion of added $^{15}NH_4$ that was assimilated by microbial orders based on their relative abundance (shown in Supporting Information Fig. S2) and ^{15}N EAF. Of the total amount of ^{15}N assimilated by the prokaryotic assemblage in both the control and glucose-amended soils, much of the N assimilation (82%–88%) was concentrated in a handful of dominant orders (Fig. 2B). These orders varied in their response to glucose, with some proportionally increasing (e.g., Actinomycetales and Rhodopirillales) and others decreasing (e.g., RB41 and Saprosirales) nitrogen assimilation. The phylogenetic organization in combination with this concentration of activity into a few groups suggests that it may be feasible to characterize the ecology of influential phylogenetic groups and use this information to more accurately model soil nitrogen immobilization. Particularly, Burkholderiales, Xanthomonadales, Sapropirales and Actinomycetales accounted for large fractions of the nitrogen assimilated. Others have found that Actinomycetes tends to be more abundant in soils where nitrogen is being immobilized (Balser and Firestone, 2005; Smithwick *et al*., 2005; Heijboer *et al*., 2016).

Here, we used a new method to study N assimilation by microorganisms, qSIP with ^{15}N. This approach can identify microbial taxa that have incorporated ^{15}N into their DNA with greater sensitivity than traditional SIP and provides a quantitative measure of isotopic enrichment (i.e., EAF ^{15}N in DNA). We used this information in combination with a few important assumptions to gain ecological insight into the relevance of microbial taxa and groups for N assimilation. To estimate the biomass-specific N assimilation, we assumed the EAF ^{15}N in a taxon's DNA to be approximately equal to that of its biomass. Furthermore, we estimated the proportion of added $^{15}NH_4$ that was assimilated by microbial groups based on their enrichment (^{15}N EAF) and relative abundance. This estimate relies on the assumption that the relative abundance of an organism or group in the 16S rRNA gene library is proportional to its representation in the microbial biomass pool. Given these caveats, our approach is intended to represent a step toward a quantitative connection between microbial community composition and soil N assimilation. Testing the above assumptions and developing approaches to more accurately scale from DNA-based information to community-level processes represents a priority for future research.

In summary, our results demonstrate that qSIP with ^{15}N can measure N assimilation into the DNA of microbial taxa and suggest ammonium assimilation may vary significantly among taxonomic groups. If taxonomic groups of microorganisms are functionally distinct with respect to N assimilation, as our data suggest, microbial community composition may be a significant driver of N immobilization rates.

Experimental procedures

Soil collection and experimental incubations

Soil was collected from a mixed conifer meadow (35.3525°N, −111.7321°W), vegetated with grass on the C. Hart Merriam elevation gradient in April 2015. This site has been previously determined to have Pachic Udic Argiboroll soil with 3.9% C and 0.35% N (Dijkstra *et al*., 2006). Three soil cores were collected (0–10 cm depth) from three distinct locations at least 5 m apart. Soils were air-dried at room temperature for 24 h and sieved through a 2 mm mesh.

To measure ^{15}N assimilation by microbial taxa, stable isotope probing incubations were conducted. Subsamples of soil from each location received either ^{15}N enriched or natural abundance NH_4^+ with and without glucose in a full factorial, randomized, block design ($n = 3$ per treatment). These 2 g of dry weight samples were weighed into 15 ml falcon tubes. Incubations received $(NH_4)_2SO_4$, either 28 μg N g^{-1} soil at

natural abundance ^{15}N or the molar equivalent (30 μg N g^{-1} soil) of 97% ^{15}N. Glucose was added to half the incubations at a concentration of 300 μg C g^{-1} soil. Immediately following nutrient additions, soils were adjusted to 60% water-holding capacity, thoroughly mixed and incubated for 7 days at 22°C. Estimates of root exudation rates range from ~200 to 3000 μg C g^{-1} root per week (e.g., Phillips *et al.*, 2011; Yin *et al.*, 2014) making our amendment a realistic approximation of C flux to root-associated soil.

Quantitative stable isotope probing

After incubation, soil samples were immediately frozen at −80°C. Total DNA was extracted using the PowerLyzer PowerSoil DNA extraction kit according to the manufacturer's instructions (MoBio Laboratories, Carlsbad, CA, USA). DNA was quantified with the Qubit double stranded DNA high-sensitivity assay kit and a Qubit 2.0 fluorometer (Invitrogen, Eugene, OR, USA). To measure ^{15}N uptake by microbial taxa, we used quantitative stable isotope probing (qSIP) as described in Hungate and colleagues (2015) with minor alterations. For density centrifugation, 1 μg of DNA was added to 2.6 ml of saturated CsCl solution after which, gradient buffer (200 mM Tris, 200 mM KCl, 2 mM EDTA) was used to completely fill the 3.3 ml OptiSeal ultracentrifuge tube (Beckman Coulter, Fullerton, CA, USA). Final density of the solution was 1.73 g cm^{-3}. Samples were centrifuged in an Optima Max bench top ultracentrifuge (Beckman Coulter) using a Beckman TLN-100 rotor at 127,000 × *g* for 72 h at 18°C. Immediately following centrifugation, the density gradient was fractionated by collecting ~125 μl fractions with a density gradient fractionation system (Brandel, Gaithersburg, MD, USA), resulting in ~30 fractions per sample. A Reichert AR200 digital refractometer (Reichert Analytical Instruments, Depew, NY, USA) was used to determine the density of each fraction.

From each density fraction, DNA was purified and 16S rRNA genes were quantified as described in Hayer *et al* (2016). Briefly, 16S rRNA genes were targeted with the 515F and 806R primer pair (Caporaso *et al.*, 2012) and quantified via real-time PCR. Thermal cycling consisted of 95°C for 1 min followed by 44 cycles of 95°C for 30 s, 64.5°C for 30 s and 72°C for 1 min on an Eppendorf Master-cycler ep Realplex system (Eppendorf, Westbury, NY, USA).

Approximately 15 fractions per sample contained ≥ 0.5% of a sample's 16S rRNA genes (determined via qPCR) and were sequenced. Sequencing was performed exactly as described in Hayer and colleagues (2016) via a two-step PCR preparation (Berry *et al.*, 2011) initially targeting the V4 region of the 16S rRNA gene with 515F and 806R primers followed by a tailing reaction. Amplicons were pooled, purified and loaded onto an Illumina MiSeq Instrument using 2 × 150 paired-end read chemistry (Hayer *et al.*, 2016). All sequence data and accompanying sample information are available on MG-RAST project name: 15N qSIP, ID: mgp82605.

Data analysis

Sequence data were analyzed with MacQIIME version 1.7 (Caporaso *et al.*, 2010). First, paired end reads were joined using fastq-join (Aronesty, 2013) and quality filtered using default parameters. Using the UCLUST-based open reference protocol, reads were clustered into operational taxonomic units (97%). Taxonomy was assigned via comparison to the Greengenes 13_8 97% sequence identity reference database (Caporaso *et al.*, 2010; McDonald *et al.*, 2012). An average of 28,651 reads per sample passed quality filtering. Low abundance OTUs, accounting for less than 0.03% of sequences, were removed due to imprecision in the density measurements for rare taxa (sensu Morrissey *et al.*, 2016). This filtering retained 78.1% of the total reads and which represented 702 taxa. As the stitched sequences were too short for accurate construction of a phylogenetic tree, the representative sequence for each OTU was aligned (using parallel BLAST) against the Greengenes database version 13_8 (Edgar, 2010; McDonald *et al.*, 2012). The reference sequence with the greatest percent identity match for each OTU was used for phylogenetic assignment and downstream analyses; median percent identity was 99.6%. The Greengenes 97% OTU tree was pruned to contain only the OTUs present in our samples for phylogenetic analysis (similar to Morrissey and Franklin, 2015).

We estimated the excess atom fraction (EAF) ^{15}N enrichment for each taxon following the procedures detailed in Hungate and colleagues (2015), with the following adjustments for ^{15}N. We first calculated the maximum degree of labeling assuming all N atoms in DNA were replaced by ^{15}N. The number of N atoms per DNA nucleotide pair varies with guanine + cytosine (GC) content. There are five N atoms in guanine, three in cytosine, five in adenine and two in thymine. Thus, the number of N atoms per DNA nucleotide ($H_{NITROGEN_i}$) for taxon *i* is given as a function of that taxon's GC content (G_i):

$$H_{NITROGEN_i} = 0.5G_i + 3.5$$

where G_i is expressed as a proportion.

We assumed that prior to incubation with ^{15}N–NH_4^+, the DNA in our samples contained ^{15}N at natural abundance levels (EAF ^{15}N: 0.003663004; de Laeter *et al.*, 2003). To achieve 1.0 EAF ^{15}N for fully ^{15}N-labeled DNA, the molecular weight of DNA could increase by a maximum of 5.024851 g mol^{-1} for G and A, by 3.014911 g mol^{-1} for C and by 2.009941 g mol^{-1} for T. Therefore, for taxon *i*, the theoretical maximum molecular weight of fully ^{15}N-labeled DNA ($M_{HEAVYMAX_i}$) is

$$M_{HEAVYMAX_i} = 0.5024851G_i + 3.517396 + M_{LIGHT_i}$$

where M_{LIGHT_i} is the molecular weight of unlabeled DNA for taxon *i*.

We then calculated the proportional change in molecular weight due to isotope incorporation using equation 10 from Hungate and colleagues (2015). Excess atom fraction ^{15}N enrichment for each taxon ($A_{NITROGEN_i}$), accounting for the background fractional abundance of ^{15}N (0.003663004; de Laeter *et al.*, 2003), was then calculated as

$$A_{NITROGEN_i} = \frac{M_{LAB_i} - M_{LIGHT_i}}{M_{HEAVYMAX_i} - M_{LIGHT_i}} \cdot (1 - 0.003663004)$$

where M_{LAB_i} is the molecular weight of DNA for taxon *i* in the labeled treatment. All calculations were performed using

bootstrapping procedures to estimate variation, as described in Hungate and colleagues (2015). Variation between samples arising from inconsistency in the CsCl density gradient was corrected for as described in Morrissey and colleagues (2017).

Phylogenetic patterns in $^{15}NH_4^+$ assimilation were analyzed with Blomberg's K (Blomberg *et al.*, 2003) and Pagel's λ (Freckleton *et al.*, 2002) using the 'phylosig' function (phytools; Revell, 2012) and visualized via the Interactive Tree of Life (Letunic and Bork, 2011). To determine the functional significance of taxonomic orders for N immobilization, the proportion of N assimilated by each group (NA_i) was calculated as the product of each taxon's relative abundance (p_i, expressed as a proportion) and ^{15}N EAF ($A_{NITROGENi}$) divided by the sum of the product of relative abundance and ^{15}N EAF for the all taxa.

$$NA_i = \frac{p_i \cdot A_{NITROGENi}}{\sum_{i}^{I}(p_i \cdot A_{NITROGENi})}$$

To assess the sensitivity of ^{15}N qSIP, a 90% confidence interval was calculated for each taxon in each treatment ($n = 3$ per treatment). The effects of $^{15}NH_4^+$ and glucose additions on the density and EAF ^{15}N of DNA were analyzed with two-way ANOVA. Similarly, variation in ^{15}N EAF within and between prokaryotic orders was investigated with ANOVA for groups with a minimum of four members. The influence of glucose addition on the proportion of N assimilated by taxonomic groups was assessed with Student's *t*-tests. All analyses and calculations were performed in R (R Development Core Team, 2011).

Acknowledgements

This research was supported by grant number DE-SC0016207 from Genomic Sciences program within the Systems Biology for Energy and the Environment directorate of the Department of Energy (B.A. Hungate) and the USDA National Institute of Food and Agriculture, Hatch project accession number 1010898 (E.M. Morrissey). The authors declare no conflict of interest.

References

Aronesty, E. (2013) Comparison of sequencing utility programs. *Open Bioinformatics J* **7:** 1.

Allison, S.D., and Martiny, J.B. (2008) Resistance, resilience, and redundancy in microbial communities. *P Nal Acad Sci* **105,** 11512–11519.

Balser, T.C., and Firestone, M.K. (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* **73:** 395–415.

Bell, T.H., Yergeau, E., Martineau, C., Juck, D., Whyte, L.G., and Greer, C.W. (2011) Identification of nitrogen-incorporating bacteria in petroleum-contaminated arctic soils by using [15N] DNA-based stable isotope probing and pyrosequencing. *Appl Environ Microb* **77:** 4163–4171.

Berry, D., Mahfoudh, K.B., Wagner, M., and Loy, A. (2011) Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl Environ Microbiol* **77:** 7846–7849.

Blomberg, S.P., Garland, T., Jr., and Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57:** 717–745.

Buchkowski, R.W., Schmitz, O.J., and Bradford, M.A. (2015) Microbial stoichiometry overrides biomass as a regulator of soil carbon and nitrogen cycling. *Ecology* **96:** 1139–1149.

Buckley, D.H., Huangyutitham, V., Hsu, S.F., and Nelson, T.A. (2007a) Stable isotope probing with $^{15}N_2$ reveals novel noncultivated diazotrophs in soil. *Appl Environ Microb* **73:** 3196–3204.

Buckley, D.H., Huangyutitham, V., Hsu, S.F., and Nelson, T.A. (2007b) Stable isotope probing with 15N achieved by disentangling the effects of genome G+C content and isotope enrichment on DNA density. *Appl Environ Microb* **73:** 3189–3195.

Byrnes, J.E., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., *et al.* (2014) Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. *Methods Ecol Evol* **5:** 111–124.

Cadisch, G., Espana, M., Causey, R., Richter, M., Shaw, E., Morgan, J.A.W., *et al.* (2005) Technical considerations for the use of 15N-DNA stable-isotope probing for functional microbial activity in soils. *Rapid Commun Mass Spectrom* **19:** 1424–1428.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., *et al.* (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6:** 1621–1624.

Colman, B.P., and Schimel, J.P. (2013) Drivers of microbial respiration and net N mineralization at the continental scale. *Soil Biol Biochem* **60:** 65–76.

Cupples, A.M., Shaffer, E.A., Chee-Sanford, J.C., and Sims, G.K. (2007) DNA buoyant density shifts during 15 N-DNA stable isotope probing. *Microbiol Res* **162:** 328–334.

De Laeter, J.R., Böhlke, J.K., De Bièvre, P., Hidaka, H., Peiser, H.S., Rosman, K.J.R., and Taylor, P.D.P. (2003) Atomic weights of the elements. Review 2000 (IUPAC Technical Report). *Pure Appl Chem* **75:** 683–800.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V., *et al.* (2006) 13C and 15N natural abundance of the soil microbial biomass. *Soil Biol Biochem* **38:** 3257–3266.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460–2461.

Finzi, A.C., Moore, D.J.P., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., *et al.* (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. *Ecology* **87:** 15–25.

Fogel, G.B., Collins, C.R., Li, J., and Brunk, C.F. (1999) Prokaryotic genome size and SSU rDNA copy number: estimation of microbial relative abundance from a mixed population. *Microb Ecol* **38:** 93–113.

Freckleton, R.P., Harvey, P.H., and Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *Am Nat* **160:** 712–726.

Geisseler, D., Horwath, W.R., Joergensen, R.G., and Ludwig, B. (2010) Pathways of nitrogen utilization by soil microorganisms–a review. *Soil Biol Biochem* **42:** 2058–2067.

Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherali, H., and Jackson, R.B. (2002) Nonlinear grassland responses to past and future atmospheric CO2. *Nature* **417:** 279–282.

Gonzalez, P.J., Correia, C., Moura, I., Brondino, C.D., and Moura, J.J.G. (2006) Bacterial nitrate reductases: molecular and biological aspects of nitrate reduction. *J Inorg Biochem* **100:** 1015–1023.

Hamner, K., and Kirchmann, H. (2005) Net nitrogen immobilization in soil induced by small additions of energy sources. *Acta Agric Scand B* **55:** 177–185.

Hayer, M., Schwartz, E., Marks, J.C., Koch, B.J., Morrissey, E.M., Schuettenberg, A.A., and Hungate, B.A. (2016) Identification of growing bacteria during litter decomposition in freshwater through H218O quantitative stable isotope probing. *Env Microbiol Rep* **8:** 975–982.

Heijboer, A., ten Berge, H.F., de Ruiter, P.C., Jørgensen, H.B., Kowalchuk, G.A., and Bloem, J. (2016) Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15 N tracer-based approach. *Appl Soil Ecol* **107:** 251–260.

Hooper, D.U., and Vitousek, P.M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecol Monogr* **68:** 121–149.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., and Field, C.B. (2003) Nitrogen and climate change. *Science* **302:** 1512–1513.

Hungate, B.A., Mau, R.L., Schwartz, E., Caporaso, J.G., Dijkstra, P., van Gestel, N., *et al*. (2015) Quantitative microbial ecology through stable isotope probing. *Appl Environ Microb* **81:** 7570–7581.

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., *et al*. (2016) Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Sci Rep* **6.**

LeBauer, D.S., and Treseder, K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89:** 371–379.

Letunic, I., and Bork, P. (2011) Interactive tree of life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res* **39:** W475–W478. gkr201.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., *et al*. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6:** 610–618.

McGuire, K.L., and Treseder, K.K. (2010) Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biol Biochem* **42:** 529–535.

Miller, A.E., and Bowman, W.D. (2003). Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. *Plant and Soil* **250:** 283–292.

Morrissey, E.M., and Franklin, R.B. (2015) Evolutionary history influences the salinity preference of bacterial taxa in wetland soils. *Front Microbiol* **6:** 1013.

Morrissey, E.M., Mau, R.L., Schwartz, E., Caporaso, J.G., Dijkstra, P., van Gestel, N., *et al*. (2016) Phylogenetic organization of bacterial activity. *ISME J* **10:** 2336.

Morrissey, E.M., Mau, R.L., Schwartz, E., McHugh, T.A., Dijkstra, P., Koch, B.J., *et al*. (2017) Bacterial carbon use plasticity, phylogenetic diversity and the priming of soil organic matter. *ISME J* **11:** 1890–1899.

Nelson, M.B., Martiny, A.C., and Martiny, J.B. (2016) Global biogeography of microbial nitrogen-cycling traits in soil. *Proc Natl Acad Sci USA* **113:** 8033–8040.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., and McMurtrie, R.E. (2010) CO2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc Natl Acad Sci USA* **107:** 19368–19373.

Phillips, R.P., Finzi, A.C., and Bernhardt, E.S. (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. *Ecol Lett* **14:** 187–194.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., *et al*. (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol Lett* **18:** 85–95.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3–900051-07-0 [WWW document]. URL http://www.R-project.org.

Reay, D.S., Nedwell, D.B., Priddle, J., and Ellis-Evans, J.C. (1999) Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. *Appl Environ Microb* **65:** 2577–2584.

Reich, P.B., Grigal, D.F., Aber, J.D., and Gower, S.T. (1997) Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* **78:** 335–347.

Revell, L.J. (2012) Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* **3:** 217–223.

Romero, C.M., Engel, R., Chen, C., and Wallander, R. (2015) Microbial immobilization of nitrogen-15 labelled ammonium and nitrate in an agricultural soil. *Soil Sci Soc Am J* **79:** 595–602.

Sawada, K., Funakawa, S., Toyota, K., and Kosaki, T. (2015) Potential nitrogen immobilization as influenced by available carbon in Japanese arable and forest soils. *Soil Sci Plant Nutr* **61:** 917–926.

Scherer-Lorenzen, M., Palmborg, C., Prinz, A., and Schulze, E.D. (2003) The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* **84:** 1539–1552.

Schimel, J. (1995). Ecosystem consequences of microbial diversity and community structure. In *Arctic and Alpine Biodiversity: patterns, Causes and Ecosystem Consequences*. Berlin/Heidelberg: Springer, pp. 239–254.

Smithwick, E.A., Turner, M.G., Metzger, K.L., and Balser, T.C. (2005) Variation in NH 4+ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA). *Soil Biol Biochem* **37:** 1546–1559.

Stolte, W., and Riegman, R. (1995) Effect of phytoplankton cell size on transient-state nitrate and ammonium uptake kinetics. *Microbiology* **141:** 1221–1229.

Štursová, M., Bárta, J., Šantrůčková, H., and Baldrian, P. (2016) Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. *FEMS Microbiol Ecol* **92.**

Větrovský, T., and Baldrian, P. (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS One* **8:** e57923.

Wedin, D.A., and Tilman, D. (1990) Species effects on nitrogen cycling: a test with perennial grasses. *Oecologia* **84:** 433–441.

Yin, H., Wheeler, E., and Phillips, R. P. (2014) Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biol Biochem* **78:** 213–221.

Zak, D.R., Pregitzer, K.S., King, J.S., and Holmes, W.E. (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytol* **147:** 201–222.

Zhu, T., Meng, T., Zhang, J., Yin, Y., Cai, Z., Yang, W., and Zhong, W. (2013) Nitrogen mineralization, immobilization turnover, heterotrophic nitrification, and microbial groups in acid forest soils of subtropical China. *Biol Fert Soils* **49:** 323–331.

Zubkov, M.V., Fuchs, B.M., Tarran, G.A., Burkill, P.H., and Amann, R. (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl Environ Microb* **69:** 1299–1304.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Taxon-specific excess atom fraction ^{15}N shown as mean ± 90% confidence intervals following incubation with $^{15}NH_4^+$ in control and glucose amended soils.

Fig. S2. Relative abundance (mean ± standard deviation) of prokaryotic phyla (A) and orders (B) in the control (lighter shade) and glucose amended (darker shade) soils.

Fig. S3. Mean ± standard error ^{15}N EAF in the DNA of member phylotypes within each order after incubation with $^{15}NH_4^+$ (circle) or $^{15}NH_4^+$ and glucose (triangle). Orders are colored by phylum and the relative abundance of each order is proportional to the size of symbols.