Nutrients cause consolidation of soil carbon flux to small proportion of bacterial community

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Nutrient amendment diminished bacterial functional diversity, consolidating carbon flow through fewer bacterial taxa. Here, we show strong differences in the bacterial taxa responsible for respiration from four ecosystems, indicating the potential for taxon-specific control over soil carbon cycling. Trends in functional diversity, defined as the richness of bacteria contributing to carbon flux and their equitability of carbon use, paralleled trends in taxonomic diversity although functional diversity was lower overall. Among genera common to all ecosystems, *Bradyrhizobium*, the Acidobacteria genus *RB41*, and *Streptomyces* together composed 45–57% of carbon flow through bacterial productivity and respiration. Bacteria that utilized the most carbon amendment (glucose) were also those that utilized the most native soil carbon, suggesting that the behavior of key soil taxa may influence carbon balance. Mapping carbon flow through different microbial taxa as demonstrated here is crucial in developing taxon-sensitive soil carbon models that may reduce the uncertainty in climate change projections.
Global climate projections depend on estimates of soil carbon accumulation and decomposition, processes driven by microorganisms. Given the vast diversity of soil microorganisms, different microbial taxa may have individualistic effects on C fluxes in soil, yet testing this idea has been challenging. Soils hold over twice as much organic carbon as terrestrial vegetation, and soil C turns over much more slowly. Soil microbial communities contain thousands of different heterotrophic microbial taxa that, together, influence soil C content, but the quantitative contributions of individual microbial taxa to the processes governing soil C accumulation and loss are not known. While some soil biogeochemical processes are physiologically specialized and dominated by a few phylogenetically specific groups, processes involved in heterotrophic decomposition are broadly distributed across the bacterial tree of life. With many taxa contributing to the same process, the functional evenness of heterotrophic decomposition might be expected to be approximately equivalent to the evenness in abundance of heterotrophic decomposers, with each taxon contributing to decomposition in proportion to its abundance. As bacterial abundances are logarithmically distributed, we might expect that the contributions to soil C may be similarly distributed despite differences in ecosystem or bacterial community composition.

We used a combination of measurements and models to evaluate the contributions of individual bacterial taxa to heterotrophic growth and respiration in four soils along a climate gradient in northern Arizona. Taxon-specific growth rates were measured using quantitative stable isotope probing with 18O-water (qSIP, see “Methods” section) for soils collected from desert grassland (GL), Pinyon-Juniper scrubland (PJ), Ponderosa Pine forest (PF), and mixed conifer forest (MC) sites, as described previously. Mean annual temperature for all respective sites: 8.5, 7, 5.5, and 4 °C and mean annual precipitation: 230, 380, 660, and 790 mm. To determine how taxon-specific contributions to growth and respiration varied with resource availability, measurements were conducted in the laboratory using unamended soil, soil with supplemental glucose, and soil with glucose plus a nitrogen source accessible to microbes, [NH4]2SO4 (carbon + nitrogen). Isotopic signatures of specific 16S sequences were combined with 16S abundances from quantitative PCR to yield quantitative estimates of taxon-specific population size and growth.

### Results and discussion

**Bacterial efficiency and respiration.** Taxon-specific productivity (µg C g soil⁻¹ week⁻¹) was modeled as a function of per-capita growth rate, taking into account relative abundance, 16S content per unit soil, as well as 16S copy number and genome size (as per Li et al.) to estimate taxon-specific cell size and carbon content (see “Methods” section). We estimated taxon-specific bacterial respiration as a function of taxon-specific growth rate and taxon-specific carbon use efficiency (CUE), using several parameterizations of the growth ~ CUE relationship (Supplementary Fig. 1, see “Methods” section). The relationship between microbial growth and efficiency is complex and difficult to identify based on existing literature. Among models with different parameterizations, a unimodal relationship between growth rate and CUE was selected with the lowest AIC and further discussed in the methods (Table 1).

We compared this model to one without taxonomy-informed genome characteristics (16S content and genome size estimates) and without taxon-specific growth, in which individual bacterial taxa respired in direct proportion to their 16S abundance per unit soil. This comparison served to demonstrate the extent that 16S abundance data of the bacterial community alone can predict soil carbon flux. Across the four soils and nutrient amendment treatments, modeled respiration of individual bacterial taxa was summed over the bacterial assemblage and was compared with measured total soil respiration. When based on measured per-taxon growth rates, modeled bacterial respiration was positively related to total soil respiration ($R^2 = 0.83, p < 0.001$; Fig. 1a). In contrast, when estimated in proportion to a taxon’s abundance alone, modeled bacterial respiration demonstrated a comparatively poor correlation ($R^2 = 0.02, p = 0.70$; Fig. 1b). Although our methods track the incorporation of 18O-labeled water into bacterial DNA, and not carbon explicitly, these results indicate that growth of individual bacterial taxa measured through 18O assimilation can be directly associated with the movement of C through the soil. For all but two soil and treatment combinations, modeled respiration was lower than measured respiration, likely in part owing to non-bacterial contributions to measured total respiration (which were not modeled). When we amended soils with carbon (C) and carbon with nitrogen (C + N) we found elevated soil respiration, patterns which were also observed with modeled bacterial respiration (Fig. 1a). Nutrient amendments also stimulated taxon-specific bacterial respiration ($F_{2,9} = 27.2, p < 0.001$) and productivity ($F_{2,9} = 6.96, p = 0.01$) leading to higher total C use in these treatments (Fig. 2). Generally, organisms that produced more biomass also respired more (Supplementary Fig. 2).

### Distribution and consolidation of bacterial carbon use.

Soils amended with nutrients had higher productivity and respiration; however, in these soils, carbon use was less evenly distributed across the bacterial community, especially in soils provided with carbon.

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### Table 1 Comparison of per-taxon carbon use efficiency functions.

| Relation to community CUE | CUE (growth) | $\Delta AIC_{co2}$ | $\Delta AIC_{cue}$ | $\Delta AIC_{combn}$ |
|---------------------------|-------------|-------------------|-------------------|---------------------|
| Constrained               |             |                   |                   |                     |
| Unimodal0.5               | 2.98        | 5.20              | 11.17             |                     |
| Linear positive           | 6.10        | 0                 | 12.21             |                     |
| Unimodal0.05              | 0           | 14.39             | 14.39             |                     |
| Linear negative           | 2.85        | 19.87             | 25.56             |                     |
| Exponential decline       | 4.38        | 20.33             | 29.08             |                     |
| Exponential decline       | 17.43       | 46.25             | 81.10             |                     |
| Linear negative           | 21.96       | 40.89             | 84.80             |                     |
| Unimodal0.05              | 19.58       | 47.72             | 86.89             |                     |
| Linear positive           | 23.56       | 50.77             | 97.89             |                     |
| Unimodal0.5               | 23.47       | 50.99             | 97.94             |                     |

$AIC_{co2}$ indicates the fit of summed per-taxon respiration to measured respiration. $AIC_{cue}$ indicates the fit of summed relative abundance-weighted per-taxon CUE to community-level CUE. $AIC_{combn}$ indicates the sum of 2($AIC_{co2}$) and $AIC_{cue}$. Subscripts following unimodal function names indicate whether maximum per-taxon CUE was centered over a growth rate of 0.5 or the global median growth rate of 0.05 observed across all taxa.

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and nitrogen. To compare the extent that taxonomic evenness equated to functional evenness (i.e., the extent that both shared similar abundance distributions), we calculated Pielou’s evenness on the relative abundances of bacterial amplicon sequence variants (ASVs) as well as relativized growth and respiration estimates. Bacterial abundances were more evenly distributed than were estimates of bacterial productivity and respiration (Fig. 3a). Similarly, cumulative C use was strongly associated with treatment, with greater consolidation of carbon in C + N soils as shown by a lower proportion of the bacterial community responsible for a greater proportion of carbon flux (Fig. 3b).

Microbial community structure and function are thought to be linked\textsuperscript{15,16}, but most efforts to relate them rely on aggregate community function measurements correlated against summaries of composition, diversity, or interactions (e.g., Creamer et al.\textsuperscript{17}). Interpretation of relative abundances across communities is a common exercise in contemporary studies of microbial ecology. Averaged across all ecosystems, 36 bacterial genera contributed to >50% of sequenced 16S amplicons. Of genera common to all soils, only six were necessary to obtain >50% contributions to C cycling in control and C amended soils while only three were necessary to obtain >50% C cycling in C + N amended soils. \textit{Bradyrhizobium} (Alphaproteobacteria, Family: Xanthobacteraceae), \textit{RB41} (Acidobacteria, Family: Pyrimonadaceae—Subgroup 4), and \textit{Streptomyces} (Actinobacteria, Family: Streptomycetaceae) were common to all soils and treatments in the C + N treated soils, these lineages accounted for the majority of C flux (Fig. 4a; Supplemental Table 1). These taxa also represent globally ubiquitous and abundant lineages as determined across the Earth Microbiome Project database\textsuperscript{18}.

Relative C use in the bacterial community was more consolidated within fewer lineages than the overall distribution of relative abundances might suggest. Averaged across all ecosystems and treatments, 75.7% of bacterial genera used less C than their relative abundance would otherwise predict. We assessed the relationship between relative C use and relative abundance in response to nutrient amendments using linear mixed modeling, accounting for random intercepts (and to limit pseudo-replication) across ecosystems and bacterial genera, and including an offset term to assess significant departure from the 1:1 line. In parallel with changing profiles of diversity, the relationship between taxon-specific bacterial C use and abundance was affected by treatment ($F_{2, 489.11} = 4.926, \ p = 0.008$). Specifically, we estimated that the slope of the relationship between relative C use and relative abundance was slightly but significantly higher than the 1:1 line in C + N amended soils, but not in control and C amended soils ($p = 0.02$) (Fig. 4b, c). Besides relative abundance, other potential influences on taxon-specific C

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**Fig. 1** Fit of modeled respiration scaled from taxon-specific isotopic enrichment against community soil respiration, by mass of carbon (C) per g dry soil per week (wk). a. Bacterial respiration is estimated as the sum of modeled taxon-specific respiration and plotted against measured soil respiration. B. Bacterial respiration is estimated from the community-level enrichment of all 16S copies present in a sample (per g dry soil). Points show mean respiration values ± standard error (SE) across replicates (n = 3 experimental replicates) for each ecosystem (symbol MC mixed conifer forest, PP ponderosa pine forest, PJ piñon pine-juniper scrubland, GL desert grassland) and treatment (color control = no amendment, C = glucose, C + N = glucose and \([\text{NH}_4\text{]}_2\text{SO}_4\)).

**Fig. 2** Absolute and relative carbon (C) use of bacterial families, per gram of dry soil per week (wk). Values averaged across replicates for each ecosystem (MC mixed conifer forest, PP ponderosa pine forest, PJ piñon pine-juniper scrubland, GL desert grassland) by treatment (rows: Ctrl = no amendment, C = glucose only, C + N = glucose and \([\text{NH}_4\text{]}_2\text{SO}_4\)) combination (n = 3 experimental replicates). Bar color represents bacterial family (IS shown, accounting for ≥75% of C use, remaining families designated as “Other”). a. Total C use (C-CO$_2$ respired and MBC produced) from each bacterial family. b. C use for each bacterial family, relativized by total C use.
of genome length, which was lower than Bradyrhizobium (73rd percentile), Streptomyces (88th percentile), RB41 (81st percentile) or the Burkholderia-Caballeronia-Paraburkholderia group (95th percentile), even though its growth rate was comparable.

In glucose-amended soils, the use of native soil C was closely correlated with the use of glucose across the bacterial community ($r = 0.96, p < 0.001$). In C and C + N treated soils, we performed $^{13}$C-glucose amendments in parallel to $^{12}$O-water conditions and used per-taxon $^{13}$C enrichment to estimate the amount of native ($^{12}$C) and glucose carbon utilized across the bacterial community. Our results indicate that the organisms that utilized the most glucose were also those that utilized the most native soil carbon. Thus, organisms with the capacity to grow quickly in response to easily accessible carbon substrates are important to the cycling and turnover of existing soil C. To determine the extent that the C:N stoichiometry of labile resources may change microbial C use preferences, we used Levene’s test on the variance in the relationship between $^{13}$C use and $^{12}$C use, where higher variance in response to nitrogen is indicative of shifts in the type of carbon preferred across bacterial genera. In C + N treated soils, there was significantly more variation around the trend line ($F_{(22, 770)} = 3.53, p < 0.001$, Levene’s test; Fig. 4c), indicating that labile nitrogen addition may disrupt the balance between native soil carbon use and use of a labile carbon substrate.

Despite some differences between 16S abundance and soil C use, across soils, differences in composition of the community significantly predicted the C use profiles ($r_s = 0.68, p < 0.001$, Mantel correlation). The four ecosystems differed in the amount of carbon used by different taxa ($R^2 = 0.69, p < 0.001$, PERMANOVA), patterns that mirrored differences in relative abundance ($R^2 = 0.69, p < 0.001$; Fig. 5). Similarly, bacterial communities changed in response to nutrient amendments, observed both with changes in relative abundance ($R^2 = 0.14, p = 0.03$) and C use ($R^2 = 0.13, p = 0.05$), though these differences were smaller than ecosystem-level separation of community composition and C use.

The strong ecosystem-specific clustering of community composition and C use (Fig. 5) is seemingly at odds with the strong treatment-specific patterns of cumulative C use (Fig. 3b), which suggests that there was a similar response to nutrient addition across all ecosystems regardless of which bacteria were responsible. However, relative abundance and C use were strongly linked (Fig. 4), and we observed that the most important contributors to bacterial C use were consistently represented by the same, abundant lineages across all ecosystems and treatments (Supplemental Table 1). Taken together, these results demonstrate that changing patterns in carbon use were driven by the consistent and abundant portion of the bacterial community which responded to the C + N amendment. Conversely, the importance of any individual lineage that occurred with low abundance towards soil C flux was difficult to determine. Rare taxa are thought to serve as a reservoir or seed bank of microbial function and diversity. Although rare lineages drove ecosystem-specific patterns in community composition and C use due to the sensitivity of multivariate dissimilarity measures to their high diversity, differences in the composition of rare lineages were negligible, contributing minimally to soil C flux. These non-dominant organisms may be best described as part of the “interchangeable” biosphere, where apart from a few consistent taxa that dominate C flux, the identities of most rare taxa are negligible towards their contributions to C flux.

Generally, dominant lineages became more dominant—in terms of abundance as well as C use—in nutrient amended soils compared to native soil conditions, especially in the Actinobacteria. Consolidation of C use increased in C + N soils for most major phyla, where a proportionally smaller number of
taxa was associated with a greater share of overall abundance and C flux in C and C + N treatments (Fig. 6). Several taxa within the Actinobacteria, mostly Streptomyces (Actinomycetaceae), Arthrobacter (Micrococcaceae), and Kribbella (Nocardioidaceae) spp., produced proportionally more 16S rRNA gene copies than other Actinobacteria during the seven-day incubation in C and C + N soils (Supplementary Fig. 3). These taxa were also dominant producers of biomass and CO₂ even after correcting for 16S rRNA gene copy number, cell mass, and growth rate (Supplementary Figs. 4–6). Across nearly all major bacterial phyla, the addition of labile nutrients tended to promote respiration of some lineages relative to others, increasing dominance, and demonstrating that the release of soil carbon as CO₂ can be concentrated in a few taxa (Figs. 3 and 6 and Supplementary Figs. 3, 4–6). These findings complement previous synthesis efforts, which have found key taxa are likely responsible for variability in carbon cycling. Generally, microbial communities are more resilient to pulses, such as our C and C + N amendments, than longer disturbances (also known as press disturbances). It is possible that nutrient addition over longer periods would elicit a different response from abundant and rare bacteria as well as changes in overall soil productivity and respiration.

In conclusion, we identify the contributions of individual bacterial taxa to soil carbon flux through bacterial production and respiration in their native soil habitats, providing insight into the community dynamics that are missing in microbial carbon models. Our model identified the growth of a few highly abundant bacterial lineages in response to labile nutrient additions, whose pre-existing high abundance in the community allowed them to assimilate ~50% of carbon consumed by or available to bacteria. The well-known pattern of logarithmic bacterial frequency and abundance distributions, thus parallels the high importance of a relatively small subset of bacterial biodiversity in the carbon cycling of any given soil. Given that this pattern is universal in microbial communities, we expect that such inequality in carbon use is as well. 4 of the 20 most prolific contributors to soil respiration come from poorly understood bacterial groups, one from the Acidobacteria, a phylum often generalized as oligotrophic. However, the abundance of individual bacterial taxa, alone, was not a sufficient predictor of soil C flux. Thus, the ability to measure in situ growth rates provided by techniques like qSIP has considerable potential to resolve the ecological roles of bacterial lineages that are difficult to culture, or whose functions would otherwise require extensive physiological assays. With regard to soil respiration modeling, we propose that because the majority of bacterial carbon flux could be accounted for by 3–6 common genera from ecosystems with different temperature and precipitation regimes, and that these genera were globally abundant and ubiquitous, it is worthwhile to determine both the global ubiquity and consistency in carbon process rates, as well as their determining traits, of such highly abundant bacteria in response to climate change. Doing so may reveal a core group of the microbial community that acts as dominant carbon processors.

Methods
Sample collection and incubation. Three replicates of soil samples were collected from the top 10 cm in of plant-free patches in four ecosystems along the C. Hart Merriam elevation gradient in Northern Arizona beginning at high desert grassland (1760 m), and followed at higher elevations by pïñon-pine juniper scrubland, and desert grassland and amended with either water (Control, labeled with 18O), glucose (C), or glucose and [NH₄]₂SO₄ (C + N) (n = 3 experimental replicates). Bacterial phyla (six phyla accounted for >99% of C use) were included. Trend lines show best fit from a linear mixed model accounting for differences between ecosystems and bacterial genera. Asterisks represent significant differences of slopes from the 1:1 line (two-sided unadjusted t-tests; C:Control t₄₈₉ = −0.60 ± 0.06 (std error), p = 0.548, effect-size r = −0.0007; C + N:Control t₄₈₉ = 2.42 ± 0.06, p = 0.016, effect-size r = 0.012). C values are averages across soil replicates from four ecosystems (mixed conifer forest, ponderosa pine forest, pïñon pine-juniper scrubland, and desert grassland) and amended with either water (Control, labeled with 18O), glucose (C), or glucose and [NH₄]₂SO₄ (C + N) (n = 3 experimental replicates). A Comparison of the relative abundance and relativized C use of the top 36 most abundant genera. Colors correspond to bacterial phyla (six phyla accounted for >99% of C use). Symbols correspond to the metric being compared for each taxon (relative abundance of 16S rRNA gene amplicon sequences, relativized use of soil C, or relativized use of native soil C excluding added glucose). B Comparison of relative abundance and relativized C use across all genera. Trend lines show best fit from a linear mixed model accounting for differences between ecosystems and bacterial genera. Asterisks represent significant differences of slopes from the 1:1 line (two-sided unadjusted t-tests; C:Control t₄₈₉ = −0.60 ± 0.06 (std error), p = 0.548, effect-size r = −0.0007; C + N:Control t₄₈₉ = 2.42 ± 0.06, p = 0.016, effect-size r = 0.012). C Comparison of relativized glucose use and relativized native soil carbon use across all genera, with variance (var) around trend lines included. Asterisks represent significant differences in variance in C + N soils compared to C soils (F₂₂,₇₇₀ = 3.53, p < 0.001, Cohen’s d = 0.134).

Fig. 4 Comparison of relative abundance and relativized carbon (C) use of soil bacterial genera. Points show relative contributions from individual bacterial genera. Values are averages across soil replicates from four ecosystems (mixed conifer forest, ponderosa pine forest, pïñon pine-juniper scrubland, and desert grassland) and amended with either water (Control, labeled with 18O), glucose (C), or glucose and [NH₄]₂SO₄ (C + N) (n = 3 experimental replicates).
woodland (2020 m), ponderosa pine forest (2344 m), and mixed conifer forest (2620 m). Soils were air-dried for 24 h at room temperature, homogenized, and passed through a 2 mm sieve before being stored at 4 °C for another 24 h. Soil samples prepared for measurement of carbon flux. All soils were incubated in the dark for one week. Following incubation, soils were frozen at −80 °C for 1 week prior to DNA extraction. Soil. CO2, and microbial biomass measurements. We analyzed headspace gas of soil samples using a LI-Cor 6262 (LI-Cor Biosciences Inc, Lincoln, NE, USA) and a Picarro G2201 (Picarro Inc., Sunnyvale, CA, USA), respectively. Prior to incubation we analyzed soil MBC using the chloroform-fumigation extraction method on 10 g of soil. One sub-sample was immediately extracted with 25 ml of a 0.05 M K2SO4 solution, while a second sub-sample was first fumigated with chlorofrom (for 5 days), after which it was similarly extracted. Following K2SO4 addition, we agi- nation. Following K2SO4 addition, we agi-

Quantitative stable isotope probing. We performed DNA extraction and 16S amplicon sequencing on 16O-incubated qSIP soils11–13. The procedures targeted the V4 region of the 16S gene as specified by the Earth Microbiome Project (EMP, http://www.earthmicrobiome.org) standard protocols.27,28 We used PowerSoil DNA extraction kits following manufacturer instructions to isolate DNA from soil (Molbio laboratories, Carlsbad, CA, USA). We quantified extracted DNA using the Qubit dsDNA High-Sensitivity assay kit and a Qubit 2.0 Fluorometer (Invitrogen, Eugene, OR, USA). To quantify the degree of 16O-isotope incorporation into bacterial DNA, we performed density fractionation and sequenced 15–18 fractions separately following methods modified from the canonical publication.2 We added 1 µg of DNA to 2.6 mL of saturated CaCl2 solution in combination with a gradient buffer (200 mM Tris, 200 mM KCL, 2 mM EDTA) in a 3.3 mL OptiSeal ultra-

centrifuge tube (Beckman Coulter, Fullerton, CA, USA). The solution was cen-

Sequence processing and qSIP analysis. The raw sequence data of forward and reverse reads (FASTQ) were processed within the QIIME 2 environment (release 2018.6.29,30, denoising sequences with the available DADA2 pipeline.31 We clustered the remaining sequences into amplicon sequence variants or ASVs (at 100% sequence identity) against the SILVA 132 database32 using an open-reference Naïve Bayesian classifier33. We removed singletons and doubletons from ASVs, non-bacterial lineages, and samples with less than 4000 sequence reads. Removal of global singles and doubletons resulted in the removal of 2241 unique ASVs from the feature table yielding 115,647 out of 117,888 (a retention of 98% of all ASVs) as well as the loss of 4018 sequences leaving 37,765,678 (a retention >99% of all sequences). We combined taxonomic information and ASV sequence counts with per-fraction qPCR and density measurements using the phyloseq package (version 1.24.2), in R (version 3.5.1).34 Because high-throughput sequencing produces relativized measures of abundance, we converted ASV sequencing abund-

Calculation of 16S rRNA gene copy numbers and cell mass. In parallel to taxonomic assignment, we compared quality-filtered 16S sequences against a database of 12,415 complete prokaryote genomes obtained from GenBank. From the list of 16S rRNA gene counts, we estimated the production of new 16S rRNA gene copies of each ASV per g dry soil per week using the following equation:

\[
\frac{dN_i}{dt} = N_i - N_i e^{-c_i}
\]

Where \(N_i\) is the number of 16S rRNA gene copies of taxon i at time t (here after 7 days) and \(g\) represents the per-capita growth rate (calculated as a daily rate). See Supplementary Fig. 3 for results on the production of 16S rRNA copies.
gene copy number and total genome size. For ASVs that did not find an exact match, we assigned 16S rRNA gene copy number values and genome sizes based on the median values observed in the most specific possible taxonomic rank. We estimated the mass of individual cells for each population using published allometric scaling relationships between genome length and cellular mass from West and Brown. We estimated that 20% of cellular mass is composed of carbon. For ASVs that did not find an exact match, we assigned 16S rRNA gene copy number values and genome sizes based on the median values observed in the most specific possible taxonomic rank. We estimated the mass of individual cells for each population using published allometric scaling relationships between genome length and cellular mass from West and Brown. We estimated that 20% of cellular mass is composed of carbon.

Efficiency and respiration modeling. We estimated rates of respiration using qSIP-informed growth rates and community-level carbon use efficiency (CUE). CUE estimates were based on the incorporation of 18O-water into DNA as a measure of gross biomass production and measured CO2 in headspace gas from soil incubations. We calculated the production of 18O-labeled biomass carbon (18P) at the community-level for each sample by summing the products of per-taxon 18O enrichment (excess atom fraction, EAF) and relative abundance:

\[ 18P = \sum_{i=1}^{n} (\text{EAF}_i \cdot y_i) \cdot \text{DNA}_i \cdot f \cdot (\text{MBC}_0 \sim \text{DNA}_i) \]

where \( P_i \) indicates production of biomass carbon (\( \mu g \ C \ g \) dry soil\(^{-1}\) week\(^{-1}\)) and \( C_i \) indicates 16S copy number per cell for taxon \( i \). The 0.2 coefficient represents an estimate that 20% of cellular mass is composed of carbon.

Fig. 6 Change in functional evenness across soil nutrient amendments and major bacterial phyla. Cumulative contribution of bacteria to total relativized carbon (C) use (the sum of \( \mu g \ C \cdot CO_2 \) respired and \( \mu g \) MBC produced per taxon relativized by total C use across all taxa, both per g dry soil per week [wk]) across soil amendment. Points represent averages across soil replicates from four ecosystems (mixed conifer forest, ponderosa pine forest, piñon pine-juniper scrubland, and desert grassland) \( n = 3 \) experimental replicates). Color indicates soil treatment (Control = no amendment, C = carbon—glucose—only, C + N = carbon and nitrogen—\( \left(NH_4\right)_2SO_4 \)). Taxa were ranked by individual contribution to C use.

where \( R \) indicates the total CO2 respired per gram dry soil per week.

We used the community CUE values from each sample (Eq. 5) to constrain/as upper and lower limits our estimates of per-taxon CUE. For a group of three replicates from a given ecosystem and treatment, we used the minimum and maximum observed community-level CUE values as the acceptable range of per-taxon CUE values. These constraints were used to control the shape of the function of per-taxon CUE and growth rate, though functions were modeled both with and without constraints (i.e., per-taxon CUE values were bounded only by 0 and 0.7). The range of community-level CUE values for each treatment were 0.18–0.53 for control soils, 0.04–0.13 for carbon amended soils and 0.03–0.08 for carbon and nitrogen amended soils and did not vary much between ecosystems. As a result of uncertainty in the literature about the relationship between growth rate and CUE, several different relationships were postulated to model per-taxon CUE as a function of per-taxon growth rate: linear increase, linear decrease, exponential decrease, unimodal with peak CUE at growth rate of 0.05 (the median of all per-taxon growth rates in the data). Comparisons between functions were made by calculating AIC values from per-taxon respiration, summed, and regressing against measured respiration values. Likewise, for each function, we tested how well per-taxon CUE estimates reconstructed community-level CUE by weighting the CUE value of each taxon by its relative abundance, summing, and regressing against community-level CUE. To select the best per-taxon CUE function, AIC values from both scaling efforts were combined. To make AIC values comparable, all respiration and CUE terms were z-transformed prior to regression scaling. To reflect our priority of estimating per-taxon respiration, AIC values from the respiration scaling regression models were multiplied by two and summed with AIC values from CUE scaling such that CUE C0 = 2(AICresp) + AICc1. Across these comparisons, the best estimate of per-taxon CUE was the unimodal function of growth rate, constrained by...
community-level CUE and peaking at growth rates of 0.5 (Table 1), such that:

$$\text{CUE}_i = -4 \left( \text{CUE}_{\text{E:T}} - \text{CUE}_{\text{E:T:range}} \right) \left( g_i - 0.5 \right)^2 + \left( \text{CUE}_{\text{E:T:max}} \right).$$

where CUE indicates per-taxon CUE, CUE_{E:T:range} indicates the maximum CUE values observed for a group of replicates within a given ecosystem and treatment (E:T). With this function, higher per-capita growth rate values were parameterized to produce higher CUE values initially and then decrease reflecting a growth-CUE tradeoff. Here, bound by the difference in maximum and minimum CUE values.

We applied per-taxon CUE estimates from Eq. 6 to per-taxon growth rates to yield estimates of per-taxon respiration:

$$r_i = r_{i_p} + r_{m_i} = \left( \frac{g_i}{\text{CUE}_i} \cdot \frac{g_i}{g} \right) + \left( \frac{g_i}{\text{CUE}_i} - g_i \right) \cdot \beta,$$

where $r_i$ indicates per-capita respiration for taxon $i$, $r_{i_p}$ indicates growth-related respiration, $r_{m_i}$ indicates maintenance-related respiration, and $\beta$ is a constant of 0.01 that represents the maintenance requirements as a proportion of total energy use. We used these values of per-taxon, per-capita respiration rates to estimate per-taxon respiration per gram of dry soil per week:

$$R_i = P_i \cdot r_{i_p} + P_i \cdot r_{m_i},$$

where $R_i$ indicates respiration of CO$_2$-C (µg C g dry soil$^{-1}$ week$^{-1}$) for taxon $i$. In addition to per-taxon respiration estimates based on $^{13}$C enrichment, we used another model for comparison. Here, respiration was calculated based on 16S abundance alone:

$$R_i = N_i \cdot f(R \sim N \sim 0),$$

where $N_i$ indicates final 16S abundance for taxon $i$, $R$ indicates microbial respiration of CO$_2$-C (µg C g dry soil$^{-1}$ week$^{-1}$) and $N$ indicates total 16S abundance at the end of the incubation. Here, the $R \sim N$ function indicates the linear relationship, with an intercept of 0, between CO$_2$ respiration and 16S gene concentration across all samples.

Diversity, compositional, and statistical analysis. For patterns of evenness in bacterial carbon use and relative abundance, we used Pielou’s evenness which is the quotient of Shannon’s diversity and the observed richness. For each sample, we applied Pielou’s evenness to bacterial abundances as well as bacterial carbon use (relativized to sum to one, in both cases).

We created a linear mixed model to test the relationship between the carbon use (the sum of biomass production and respiration) and relative abundance of bacterial genera from the dominant phyla, which accounted for >90% of all C flux. Here, we averaged carbon use and relative abundance for all replicates in a given ecosystem and treatment. We used the lm4 R package (version 1.1-20) and obtained $p$-values using the Satterthwaite method in the InterTest R package (version 3.1-0) to limit pseudo-replication. We accounted for differences in carbon use across ecosystems and due to bacterial Genus by implementing random intercepts. We selected for the optimal random and fixed components by dropping individual terms and comparing models with likelihood ratio tests, disregarding models that failed to converge. Our final model fit was:

$$\log_{10}(C_i) \sim \log_{10}(y_j)^T + (1) + (1)(\text{Genus}),$$

where $C_i$ indicates the relative carbon use for taxon $i$ (averaged across all three replicates in a given ecosystem and treatment), $y_j$ indicates the relative abundance of taxon $i$ (averaged across all three replicates), $T$ indicates soil treatment, and $E$ indicates ecosystem.

For differences in composition, we created species abundance tables using the traditional abundances, as well as measures of carbon use (growth and maintenance respiration) of each ASV in each sample. To account for differences in absolute abundances and flux rates between sites, we relativized all abundance tables. We summarized compositional differences using Bray–Curtis dissimilarities, then identified multivariate centroids for all replicates in a site by treatment group. We tested the effect of site and nutrient amendment on the resulting group centroids using PERMANOVA tests implemented with the adonis function in vegan. Here, the $R \sim N$ function indicates the linear relationship, with an intercept of 0, between CO$_2$ respiration and 16S gene concentration across all samples.

**Data availability**

Sequence data and sample metadata have been previously deposited in the NCBI Sequence Read Archive under the project number PRJNA521534. All other data have been made available at https://github.com/bramstone/bacterial-carbon-flux-qSIP (https://doi.org/10.5281/zenodo.4529585).

**Code availability**

All statistical and modelling analyses have been made available at https://github.com/bramstone.

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Author contributions

B.W.S. analyzed data and wrote first draft of manuscript. J.L. performed bioinformatic analyses on prokaryote genomes. B.J.K., S.J.B., P.D., K.S.H., M.H., X.J.L., R.L.M., J.P.R., S.J.B., E.S. and B.A.H. contributed to project scope, experimental design, and interpreted findings. P.D., E.M.M. and B.A.H. contributed to organization and framing of manuscript. X.J.L., M.H. and R.L.M. collected and processed samples. M.H. and R.L.M. performed laboratory work and generated data. B.W.S. created supplemental Fig. 1. All authors contributed meaningfully to revisions.

Competing interests

The authors declare no competing interests.

Additional information

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