Final Technical Report
Scaling the Microbial Ecology of Soil Carbon
Department of Energy, Genomic Sciences Program
DE-SC0016207
Lead PI: Bruce Hungate, Northern Arizona University

Summary

This project used tools from genomic and ecosystem sciences to advance techniques and understanding of the microbial ecology of soil carbon. The first major goal of the work was to explore the ecology of soil microbial responses to experimental warming, by quantifying growth, mortality, and C use and growth efficiency, as modulated by ecological stress, and on a taxon-specific basis. The work addressed a number of specific aims under this broad first goal. (1A) *To develop a fluxomics technique to determine taxon-specific growth efficiency and its response to warming*. This goal was a high-risk feature of our proposal, measuring taxon-specific growth efficiency using a variation on our existing fluxomics technique. The idea was to add position-specific isotopically labeled intermediates in the central metabolic network to soil incubations and to analyze nucleic acids for isotopic composition (instead of what we usually do, which is to analyze CO_2). Such an approach would enable gathering both biogeochemical information (^{13}C) and taxonomic information (nucleic acids) through our fluxomics model whereby we probe activity of the central metabolic network, including the partitioning between biosynthesis and energy generation, the foundation of growth efficiency. We conducted experiments, modeling, and sensitivity tests for this proposed approach. (1B) *To determine whether there are phylogenetic signals in bacterial growth and mortality that are conserved across biomes.* This builds on our past work that showed phylogenetic clustering within specific soils, and extends the comparison to test whether the phylogenetic signals are consistent among different ecosystems and climatic zones, an idea work from this project supported (Wang et al. 2021). (1C) *To determine responses of growth, mortality, and growth efficiency to temperature across biomes.* To meet this goal, we conducted lab and field experiments, including the first experiments where taxon-specific microbial growth rates were measured under field conditions (Purcell et al. 2021). The second major goal of the work was to integrate microbial ecology from the laboratory to long-term global warming experiments in the field, across multiple biomes, and through quantitative modeling, in order to scale –omics data to the field in understanding the C cycle in soil. Three specific aims were addressed under this second goal. (2A) *To develop a model of absolute rates of growth and mortality. This will extend our growth and mortality modeling from relative rates to population dynamics and absolute fluxes of elements.* This required two critical steps: including the number of copies of the 16S gene per cell, per taxon, in the model, and scaling to element fluxes using the element mass of bacterial cells. This goal was achieved and models of growth and mortality were developed, making possible studies of population dynamics in complex soil ecosystems (Koch et al. 2018). The scaling to element fluxes was also achieved, both in investigating phenomena like soil carbon priming (Morrissey et al. 2017) and nitrogen assimilation (Morrissey et al. 2018), and also quantitatively estimating taxon-specific fluxes to soil respiration (Stone et al. 2021). (2B) To conduct meta-analyses to synthesize information required for scaling: an analysis of bacterial cell size, mass, and element composition; and a synthesis of the literature on microbial productivity and turnover A graduate student supported on this project, Lindsey Jacobs, conducted a literature synthesis of bacterial cell size, mass, and element composition, having identified more than 50 papers with relevant data. Ms. Jacobs completed her MS degree with this subject as her primary focus (Jacobs, 2019). Dr. Ben Koch included a synthesis of soil

microbial growth and turnover estimates in his paper elucidating the population growth and mortality models (Koch et al. 2018), and PhD student Megan Foley conducted a larger synthesis, currently in preparation for publication. (2C) *To extend the qSIP model to RNA.* This requires dealing with the absence of any relationship between GC content and density of the RNA molecule. PhD student Katerina Papp published four papers demonstrating the use of qSIP in RNA. Dr. Papp is lead author on four publications articulating and developing this model published in the ISME Journal (Papp et al. 2018a), Applied and Environmental Microbiology (Papp et al. 2018b), Soil Biology and Biochemistry (Papp et al. 2019), and Ecology (Papp et al. 2020).

Accomplishments

Major Goal 1: Determine the ecology of soil microbial responses to experimental warming, by quantifying growth, mortality, and C use and growth efficiency, as modulated by ecological stress, and on a taxon-specific basis.

Goal 1A Develop a fluxomics technique to determine taxon-specific growth efficiency and its response to warming.

Part of this goal involved methods development for measuring growth, mortality, and growth efficiency. One experiment we conducted developed a "bulk-SIP" method for analyzing the isotopic composition of nucleic acids. We used combinations of substrates and isotopes, including ^{18}O -H_2O, position-specific ^{13}C labeled glucose (1-glucose, 2-glucose, and U-glucose), and ^{15}N-glutamine, in order to test the ability of the bulk-SIP approach to measure multiple isotopes simultaneously, and to test whether uptake rates of different substrates were correlated. The technique involves prepping a sample of nucleic acids in a concentrated spot which the NanoSIMS can then analyze. We analyzed extracted nucleic acids for bulk isotopic enrichment (Bulk-SIP), as a test of sensitivity (Figures A-C). We were able to detect significant ^{13}C enrichment in RNA within 4 hours of incubation and in DNA within 16 hours of incubation with [U-]^{13}C-Glucose (Figure A). Enrichment remained continuous after 16 hours indicating that all of the available labeled glucose had been consumed by this time. We also added ^{18}O-H_2O to measure growth by quantifying ^{18}O enrichment into DNA, and we added ^{15}N-glutamine as a supporting approach to measure potential de novo synthesis of nucleotides by measuring ^{15}N enrichment into DNA. Bulk- SIP results for ^{18}O and ^{15}N enrichment indicate that we can detect growth within 16

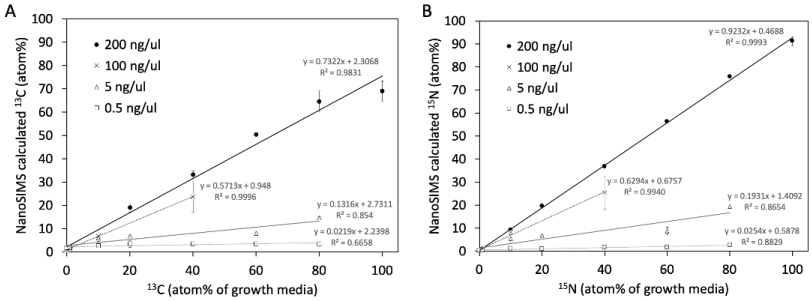

Figure A. DNA standard curves created using Pseudomonas stutzeri DNA grown in defined media. 27/26 data. Standard curves with different concentrations of DNA for A) ^{13}C and B) ^{15}N.

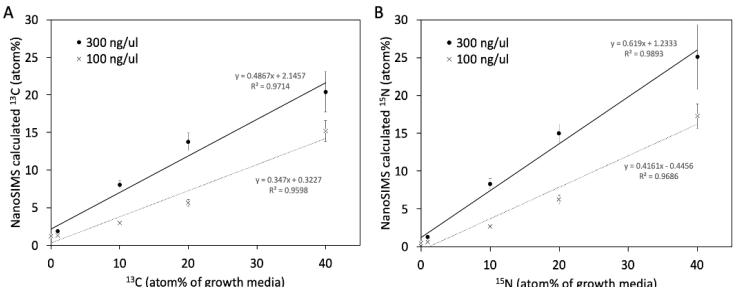

Figure B. RNA standard curves created using Pseudomonas stutzeri RNA grown in defined media. 27/26 data. Standard curves with different concentrations of RNA for A) ^{13}C and B) ^{15}N.

hours and the majority of growth occurred by 52 hours (Figure A). Correlation between ^{18}O and ^{15}N enrichment is linear (Figure B), supporting the use of ^{18}O-Bulk-SIP as a metric for growth.

Analysis of bulk RNA and DNA by spot-NanoSIMS shows promise, shown in the attached (Figures A-C). The steady increase over time in ^{15}N of DNA shows the soil community grew during the incubation, utilizing N from added glutamine and O from labeled water for DNA synthesis. The ^{13}C time course indicates that glucose was utilized rapidly for growth, and then leveled off. In RNA, the ^{13}C signal was remarkably strong, indicating high use of the added glucose for RNA synthesis, whereas the ^{15}N (panel D) and ^{18}O (panel F) signals were considerably lower. RNA recovery from this soil was very low, and it's possible that low yield contributed to a variable and non-representative isotope signature. We are comparing extraction techniques between the NAU and LLNL teams to see if this can be optimized. In addition to establishing the time course and appropriate sampling times for this soil, which duplicate those we plan for the upcoming experiment (August 2017), the bulk isotope data provide a new and improved means for qSIP quantification, a technique we have just worked out in the past several months. The new calibration approach has reduced variance in qSIP isotope enrichment estimates by approximately a factor of two. qSIP analyses are underway for multiple temperature treatments, a test of our ability to detect temperature effects on growth rate using qSIP. For growth in particular, the spot-NanoSIMS IRMS analysis showed excellent promise (Figure 1, panel C), as the ^{18}O composition of DNA increased linearly over 48 hours before the rate of increase declined between 48 and 72 hours, likely indicating turnover. qSIP analyses of these same time points are underway, enabling comparing the methods. qSIP requires stronger isotope labeling, but has the advantage of providing taxon-specific information. The spot-NanoSIMS analysis certainly suggests that taxon-specific resolution will be possible, even over this short time scale. This work is currently in preparation for publication (Blazewicz et al., in preparation).

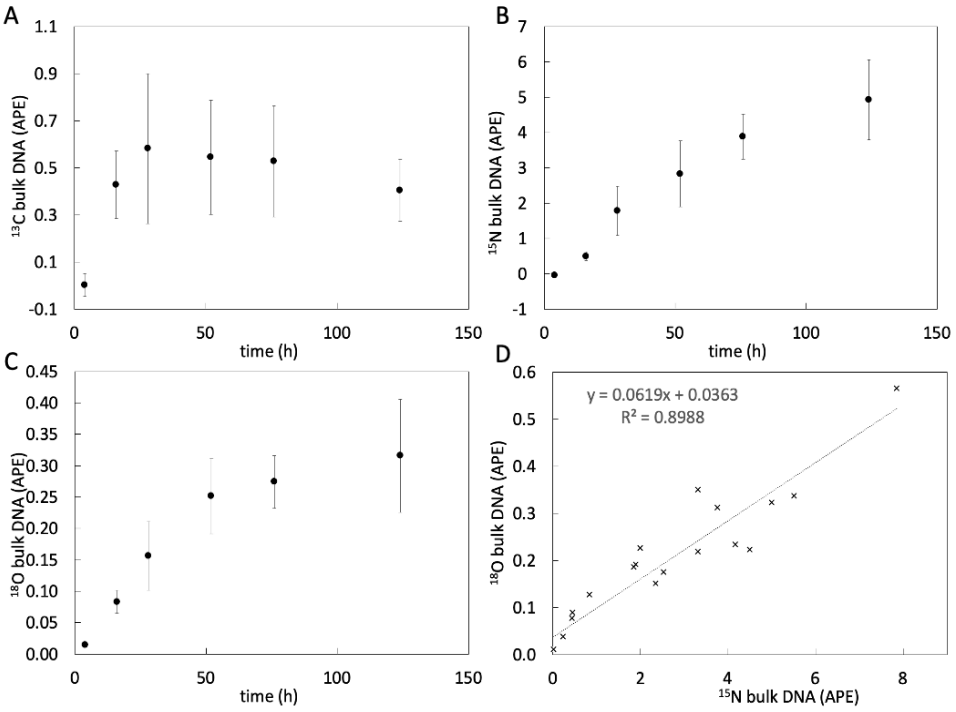

Figure C. Isotopic enrichment of DNA (top row) over time from soil incubations using A) ^{13}C, B) ^{15}N, C) and ^{18}O. D) Correlation between ^{18}O and ^{15}N enrichment.

We built the theoretical model (Figure D) and ran sensitivity tests based on our initial measurements using qSIP (Hungate et al. 2015), bulk SIP (Blazewicz et al., in preparation) and Chip-SIP (Mayali et al. 2012). We evaluated sensitivity by calculating the relationship between what we can measure as isotope recovery in nucleic acids and corresponding changes in growth efficiency that cause variation. Highly sensitive methods will involve very small changes in growth efficiency that can be detected by large shifts in the recovery of carbon atoms. We evaluated this by describing the full range of possible recovery patterns of the 6 different carbon positions in glucose as growth efficiency varied (Figure E). We found a much higher range of possible patterns of recovery in CO2 from each of the different carbon positions in the glucose molecule compared to RNA. The range of possible patterns of recovery in RNA was a factor of ~5 lower (Figure E). This does not rule out the utility of the RNA-based method, but it does indicate that the technique may not be sufficiently sensitive to resolve taxon-specific variation in CUE.

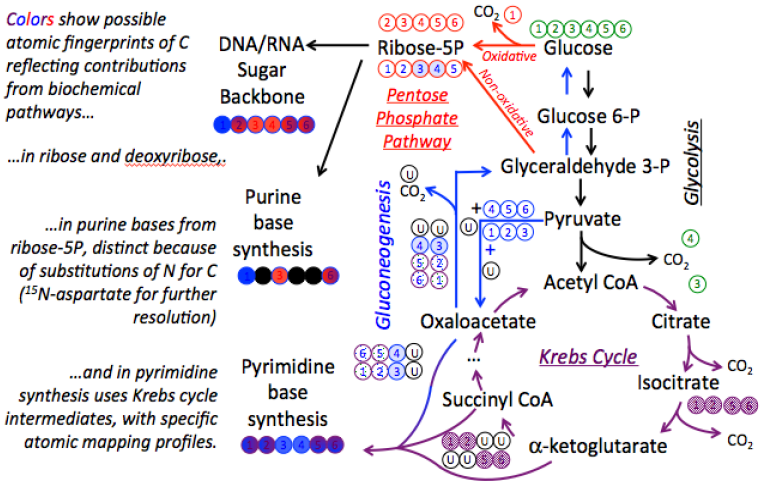

Figure D. Atom mapping concept for metabolic flux analysis of isotopes recovered in nucleic acids. The final sets of colored circles in the sugar backbone, the purine bases, and the pyrimidine bases, show the fates of the 6 C atoms from glucose (even though the products are, at most, 5-C compounds, we show how C from each of the 6 positions in glucose can end up in these molecules). Colored circles show the distinct imprint of metabolism on the atomic fingerprints on glucose atoms during the formation of the sugar backbone, purine bases, and pyrimidine bases.

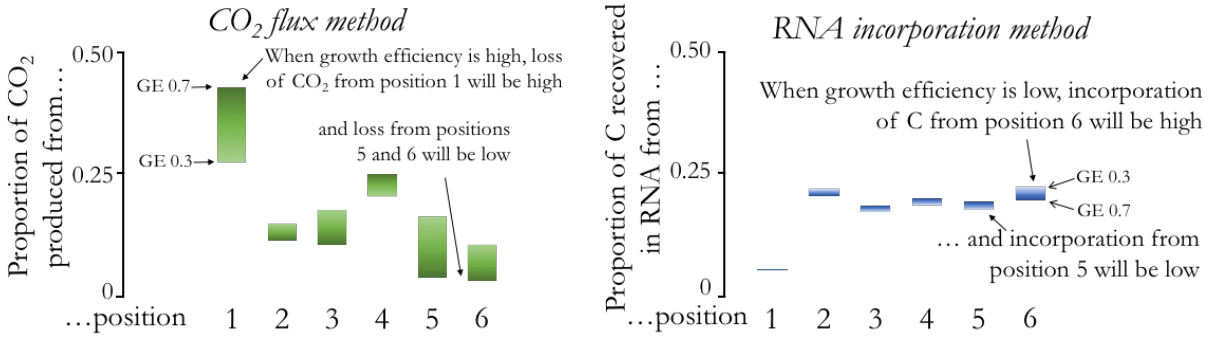

Figure E. Left panel: The range of variation in CO2 recovery from isotopomer addition, using the CO2 technique (Dijkstra et al. 2011). The tall bars show the range of variation in CO2 recovery from which variation in growth efficiency can be inferred. Right panel: the reduced range of variation in isotopomer recovery from RNA indicates far less sensitivity for detecting changes in growth efficiency.

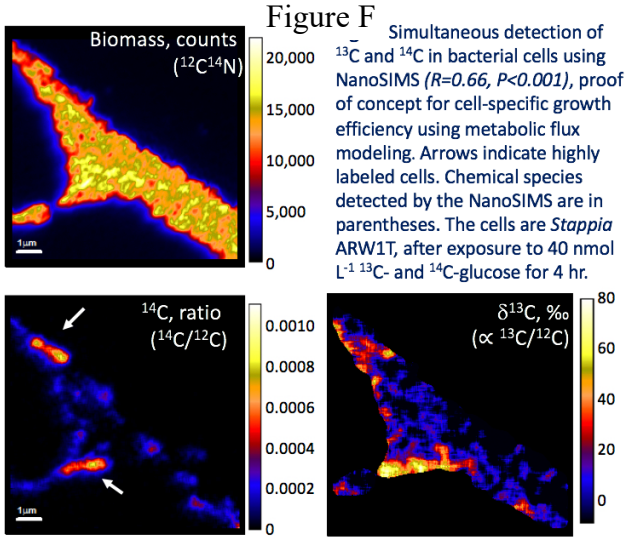

Figure F Simultaneous detection of ^{13}C and ^{14}C in bacterial cells using NanoSIMS *(R=0.66, P<0.001)*, proof of concept for cell-specific growth efficiency using metabolic flux modeling. Arrows indicate highly labeled cells. Chemical species detected by the NanoSIMS are in parentheses. The cells are *Stappia* ARW1T, after exposure to 40 nmol L^{-1} ^{13}C- and ^{14}C-glucose for 4 hr.

We also conducted Nano-SIMS measurements on cells after exposure to ^{13}C and ^{14}C isotope tracers, testing the possibility of applying different isotopomers simultaneously to the same sample, resolving recovery of distinct labels and improving the sensitivity of the technique. This approach showed promise, in that we were able to resolve both ^{13}C and ^{14}C from different substrates in the same incubation (Figure F). We further explored the idea of labeling with ^{13}C and ^{14}C onto different positions in the same molecule, which would open the possibility of metabolic flux analysis using two position-specific tracers on individual cells using Nano-SIMS. This is clearly possible, but the synthesis cost of the compounds was prohibitive.

Goal 1B. Determine whether there are phylogenetic signals in bacterial growth and mortality that are conserved across biomes. We showed a strong phylogenetic signal in bacterial growth rate (Morrissey et al. 2016), carbon assimilation (Finley et al. 2020, Morrissey et al. 2017), and nitrogen assimilation (Morrissey et al. 2018). And we found some support for a general phylogenetic signal in our cross-system experiment (Wang et al. 2021), which showed evidence for phylogenetic signals in the four biomes assessed (tropical, temperate, boreal, and arctic). From the abstract of Wang et al. 2021, where these results are reported: “Differences in the temperature sensitivities of taxa and the taxonomic composition of communities determined community-assembled bacterial growth Q_{10}, which was strongly predictive of soil respiration Q_{10} within and across biomes. Our results suggest community-assembled traits of microbial taxa may enable enhanced prediction of carbon cycling feedbacks to climate change in ecosystems across the globe.” In other words, taxa that tended to grow rapidly in one ecosystem tended to exhibit the same patterns in others.

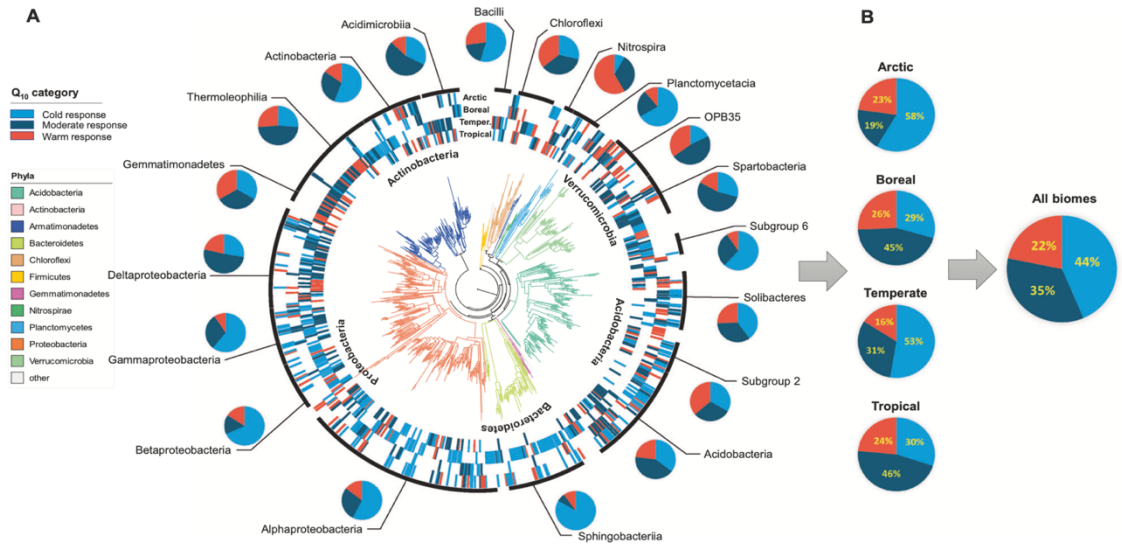

Fig G Temperature sensitivity of microbial growth in relation to bacterial phylogeny. A Each phylotype was categorized based on its maximum Q_{10} among 5–15, 15–25, and 25–35 °C as "cold," "moderate," or "warm" responders. The pie charts represent proportion of growth Q_{10} values in each response category at class level (with exception for *Chloroflexi* phyla) using data from all four sites. **B** Total proportion of growth Q_{10} response for each biome and across all biomes. Phylogenetic statistical analysis for growth Q_{10} was provided in Supplementary Table 4.

Analysis of data gathered to date indicates a strong phylogenetic signal across a suite of bacterial traits, from growth to glucose uptake to ammonium assimilation (Morrissey et al. 2018, Figure H). This analysis suggests that there may be suites of traits, clustered phylogenetically, that summarize bacterial resource acquisition and growth strategies. If correct, this has the potential to be a powerfully organizing feature of bacterial metabolism. The major contribution of our work to this goal is that all the assays through which bacterial activity is assessed are done under realistic conditions, representing actual growth and assimilation rates of organisms in complex communities.

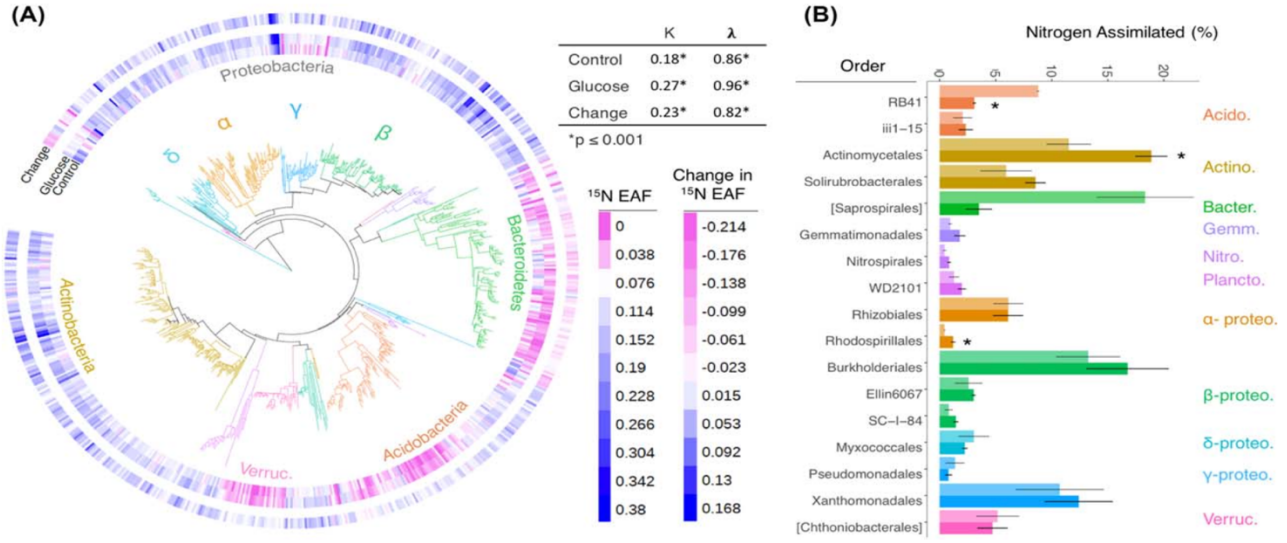

Fig H Nitrogen assimilation by prokaryotic taxa.
A. Phylogenetic tree and heat map of ^{15}N isotope incorporation of prokaryotic taxa. Inner circles correspond to the excess atom fraction (EAF) ^{15}N in each taxon's DNA after incubation with $^{15}NH_4^+$ (control) or glucose and $^{15}NH_4^+$ (glucose). 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 ($\alpha = 0.05$).

Preliminary sequencing data show that this response at 45 degrees appears to be strongly driven by

individual taxa, especially at 5 days. Figure 7 shows atom % 18O enrichment in response to temperature for the SPRUCE experiment (note, day 10, 5 degree treatment, and day 5, 15 degree treatment, are still missing sequencing replicates, so the variance is unusually high). Even at the coarse level of phylum, taxon-specific responses are apparent.

Major Goal 2: Integrate from the laboratory to long-term global warming experiments in the field, across multiple biomes, and through quantitative modeling, in order to scale –omics data to the field in understanding the C cycle in soil.

Goal 2A. Develop a model of absolute rates of growth and mortality. This model has been developed, using simple assumptions about the numbers of copies of the 16S gene among bacterial taxa. We will use this model to incorporate a more sophisticated treatment of 16S copy number, using taxon-specific information.
We developed and published an extension of our modeling approach to qSIP, an approach that converts isotope composition to taxon-specific vital rates of bacterial populations (Koch et al. 2018, Ecosphere). Figure I shows population abundances of bacterial taxa (panel A) and vital rates of growth and mortality (panel B) for the same taxa. This is the first estimate of in situ bacterial growth and mortality rates, and the high rates we observed are likely to upend current thinking about soil microbial ecology, namely the notion that most taxa are dormant in soil. Our results show that most taxa, in fact, are growing (and dying), suggesting the possibility that simultaneous growth and turnover conceal the fact that these organisms are cycling elements at rates faster than we previously

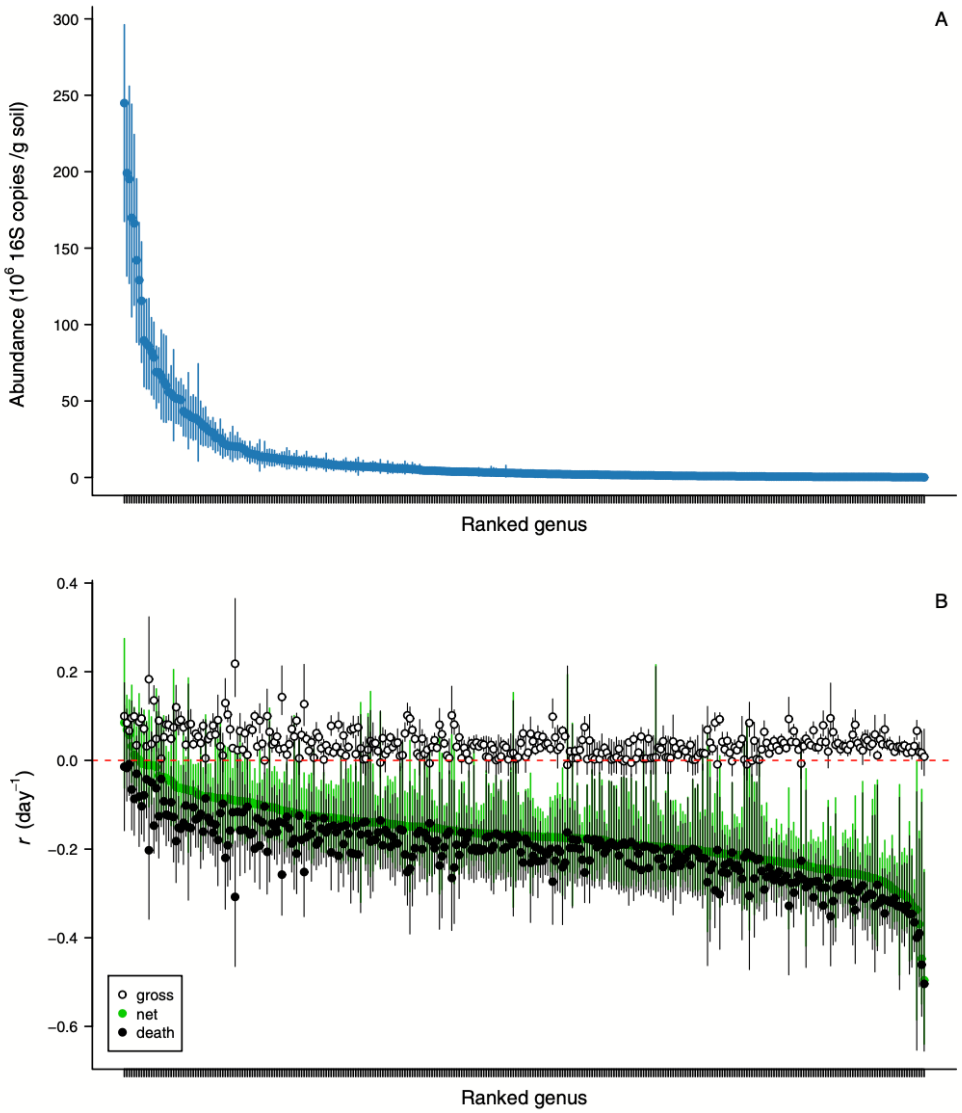

Fig I (A) Taxon-specific abundances of 16S rRNA gene copies at the end of the rewetting incubation (day 10) were approximately lognormally distributed, whereas (B) population growth rates (r) followed a normal distribution across genera. Nearly all taxa declined in abundance in response to soil rewetting (net population growth rate, $r < 0$; green circles) because mortality rates (d, filled black circles) outweighed rates of reproduction (b, open circles). Points indicate bootstrapped medians; bars are 90% confidence intervals. Genera are ranked independently in each panel.

thought. This work extends our growth and mortality modeling from relative rates to population dynamics and absolute fluxes of elements.

Goal 2B. Conduct meta-analyses to synthesize information required for scaling: i) an analysis of bacterial cell size, mass, and element composition, and ii) a synthesis of the literature on microbial productivity and turnover. A graduate student funded on this project, Lindsey Jacobs, conducted a literature synthesis of bacterial cell size, mass, and element composition, having identified more than 50 papers with relevant data. Ms. Jacobs completed her MS degree with this subject as her primary focus (Jacobs, 2019).

We have conducted the very first application of qSIP to undisturbed soils, in the field. This effort was also our first foray into measuring bacterial growth rates in an intact ecosystem-scale warming experiment. The figure below shows average density shift in the experiment, and treatments are labeled across the top. They included the control ("MC") and warmed ("PP") sites. We also included a laboratory qSIP treatment, in which field samples were compared to those in the lab, asking the basic question, do patterns measured in the lab diverge from those in nature? And, can we measure quantitative and ecological traits of microorganisms in nature, with minimal manipulation? Density shifts (Figure 9) occur because of ^{18}O incorporation from the labeled water, and the results are very encouraging in that we can measure density shifts of DNA after exposure to ^{18}O-H_2O for 10 days in the field. These shifts are of similar magnitude to those observed in the lab, suggesting at least no massive divergence at the level of the whole community response. Warming appeared to reduce aggregate community-level growth rate. PhD student Megan Foley and research scientist Ben Koch conducted synthesis work on microbial growth and turnover, using a literature-based dataset as well as qSIP measurements from our own work under this proposal. This work is ongoing and will result in a synthesis paper about microbial growth in soil.

Goal 2C. Extend the qSIP model to RNA. As part of her PhD dissertation, Dr. Katerina Papp used our quantitative stable isotope probing to determine the isotopic composition of RNA on a taxon-specific basis. Dr. Papp is lead author on four publications articulating and developing this model published in the ISME Journal (Papp et al. 2018a), Applied and Environmental Microbiology (Papp et al. 2018b), Soil Biology and Biochemistry (Papp et al. 2019), and Ecology (Papp et al. 2020). The main findings from this work are that: 1) RNA turns over at a slower rate than previously thought, with some evidence pointing to the stability of ribosomal RNA over time (e.g., 8-10 days). 2) RNA synthesis is strongly correlated with DNA synthesis, suggesting that, at the level of transcription, there is little decoupling between metabolic activity and cellular growth, and that instead these appear to proceed in lock step. 3) mRNA turns over faster than DNA, but they are strongly correlated, and the difference in turnover is not as large as previously thought. These are major advancements in our understanding of the turnover rates of these essential biological molecules.

Goal 2D. Derive new qSIP calibration using IRMS or Nano-SIMS measurements of the isotopic composition of the total nucleic acid pool (or of specific gene targets). We have developed a new correction technique for qSIP that substantially increases precision (and validates accuracy), where internal standards are included in each spin. The standards are known sequences, distinct from those found in soil, and of known isotope composition. This provides a sound calibration which will also enable cross-experiment comparisons. This work is currently in preparation for publication (Schwartz et al., in preparation).

Training and professional development

This project has offered training and professional development opportunities for a number of undergraduate students, graduate students, and postdocs. Graduate students trained included: Lindsey Jacobs (MS), Robert Woodruff (MS), Alicia Purcell (PhD), Katerina Papp (PhD), Megan Foley (PhD), Brianna Finley (PhD), Victoria Monsaint-Queeney (PhD). These students learned quantitative stable isotope probing, a very new technique that is poised to advance the field by merging mathematical models with molecular tools in microbial ecology. Undergraduate students involved in the project included: Ryan Lancione, undergraduate student investigating sigma factors; Dylan Verdi, undergraduate student studying fast metabolic flux processes and modeling; Katheryn Nantz,

undergraduate student extracting DNA for qSIP analysis of soils incubated at different temperatures; John Codon, undergraduate student conducting qPCR on DNA extracted from Toolik soils; Shavindi Ediriarachchi, undergraduate student comparing methods to quantify bacteria in soils from two elevation gradient sites in northern Arizona; Monica Long, undergraduate student conducting a culture-based calibration of qSIP to NanoSIMS. Junhui Liu was supported by the project as a Postdoctoral Research Associate in quantitative molecular microbial ecology.

4. How have the results been disseminated to communities of interest?
We have disseminated results from this work through publications, presentations at national and international scientific meetings, and invited lectures around the world.

Publications that resulted from this project include the following:

Morrissey EM, Mau RL, Schwartz E, Koch BJ, Hayer M, Hungate BA, 2018. Taxonomic patterns in the nitrogen assimilation of soil prokaryotes. Environmental Microbiology. 10.1111/1462-2920.14051

Koch BJ, McHugh TA, Hayer M, Schwartz E, Blazewicz SJ, Dijkstra P, van Gestel N, Marks JC, Mau RL, Morrissey EM, Pett-Ridge J, Hungate BA, 2018. Estimating taxon-specific population dynamics in diverse microbial communities. Ecosphere (9) e02090, DOI: 10.1002/ecs2.2090

Papp, K, Hungate BA, Schwartz E, 2018. Comparison of Microbial Ribosomal RNA Synthesis and Growth through Quantitative Stable Isotope Probing with $H_2{}^{18}O$. Applied and Environmental Microbiology, DOI: 10.1128/AEM.02441-17

Papp K, Mau R, Hayer M, Koch B, Hungate BA, Schwartz E, 2018. Quantitative Stable Isotope Probing with $H_2{}^{18}O$ reveals that most bacterial taxa in soil synthesize new ribosomal RNA. ISME Journal, doi.org/10.1038/s41396-018-0233-7

Finley BK, Dijkstra P, Rasmussen C, Schwartz E, Mau RL, Liu XJA, van Gestel N, Hungate BA, 2018. Soil mineral assemblage and substrate quality effects on microbial priming. Geoderma 322:38-47

Papp K, Hungate BA, Schwartz E, 2019. mRNA, rRNA and DNA quantitative stable isotope probing with $H_2{}^{18}O$ indicates use of old rRNA among soil Thaumarchaeota. Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2018.12.016, 130, 159-166

Li J, Mau RL, Dijkstra P, Koch BJ, Schwartz E, Liu XJA, Morrissey EM, Blazewicz SB, Pett-Ridge J, Stone BW, Hayer M, Hungate BA, 2019. Predictive genomic traits for bacterial growth in culture versus actual growth in soil. The ISME Journal. doi.org/10.1038/s41396-019-0422-z

Morrissey EM, Mau RL, Hayer M, Liu XJA, Schwartz E, Dijkstra P, Koch BJ, Allen K, Blazewicz SJ, Hofmockel K, Pett-Ridge J, Hungate BA, 2019. Evolutionary history constrains microbial traits across environmental variation. Nature Ecology and Evolution

Papp KS, Hungate BA, Schwartz E, 2020. Glucose triggers strong taxon-specific responses in microbial growth and activity, insights from DNA and RNA qSIP. Ecology, 101, e02887

Blazewicz SJ, Hungate BA, Koch BJ, Nuccio EE, Morrissey EM, Brodie EL, Schwartz E, Pett-Ridge J, Firestone MK, 2020. Taxon-Specific Microbial Growth and Mortality Patterns Reveal Distinct Temporal Population Responses to Rewetting in a California Grassland Soil. https://doi.org/10.1038/s41396-020-0617-3

Sieradzki ET, Koch BJ, Greenlon A, Sachdeva R, Malmstrom RR, Mau RL, Blazewicz SJ, Firestone MK, Hofmockel K, Schwartz E, Hungate BA, Pett-Ridge J, 2020. Measurement error and resolution in quantitative stable isotope probing: implications for experimental design. mSystems 5:e00151-20. https://doi.org/ 10.1128/mSystems.00151-20

Wang Chao, Ember M. Morrissey, Rebecca L. Mau, Michaela Hayer, Juan Piñeiro, Michelle C. Mack, Jane C. Marks, Sheryl L. Bell, Samantha N. Miller, Egbert Schwartz, Paul Dijkstra, Benjamin J. Koch c,e, Bram W. Stone, Alicia M. Purcell, Steven J. Blazewicz, Kirsten S. Hofmockel, Jennifer Pett-Ridge, Bruce A. Hungate, 2021. The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization. ISME J 15, pages2738–2747

Purcell AM, Dijkstra P, Finley B, Hayer M, Koch BJ, Mau RL, Morrissey E, Papp K, Schwartz E, Stone BW, Hungate BA, 2019. Quantitative Stable Isotope Probing with $H_2{}^{18}O$ to Measure Taxon-Specific Microbial Growth. Methods of Soil Analysis, 4(1). Soil Science Society of America

Schwartz E, Hayer M, Hungate BA, Mau RL, 2019. Stable Isotope Probing of Microorganisms in Environmental Samples with $H_2{}^{18}O$. In Dumont MG, García MH, editors, Stable Isotope Probing, Methods and Protocols, Methods in Molecular Biology, volume 2046, Springer, pages 129-136.

Jacobs LL. 2019. Potential Ecological Indicators of Soil Prokaryotic Cell Size. M.S. thesis, Northern Arizona University Flagstaff, AZ USA.

Results from this work have also been disseminated through multiple meeting presentations, including at the European Geosciences Union, the Ecological Society of America, the Joint Genome Institute Annual Meeting, and the American Geophysical Union. Additionally, various lectures were presented that featured prominently data from this project, including as the Graduate Student Invited Speaker at the University of Colorado, the University of Chicago, the University of New Hampshire, as a President's Invited Speaker at the American Chemical Society's Annual Meeting, and as a Keynote Speaker of the "Multi- Omics and the Microbial Ecology of Element Cycling in Ecosystems", Multi-Omics for Microbiomes Conference, held at Pacific Northwest National Laboratory.

Impact of the Research

Biological diversity – the variety of life – has profound influence on ecosystems, yet the ability to describe that influence for microbial biodiversity is weak. Despite the progress of the molecular revolution in revealing microbial biodiversity, we are not yet able to describe, quantitatively, how individual members of those complex communities influence ecosystem processes. The diversity of soil microorganisms and their influences on the soil carbon (C) cycle are vast, yet we are currently unable to assess how the diversity of microorganisms shapes responses of terrestrial ecosystems to global environmental change. The major impact of the project is that it used and developed new techniques defining a new frontier at the interface between molecular biology and ecosystem science in order to close the knowledge gap between microbial biodiversity and ecosystem science. Progress made during this project has contributed to a broad understanding of the relationship between microbial phylogenetic relationships and biogeochemical processes across ecosystems and in response to climate forcing, new mathematical models for powerful interpretations of molecular data generated in microbial ecology, in particular that utilizing stable isotope probing and metabolic flux modeling. More generally, this project advanced an understanding of the importance of quantitative approaches to microbial ecology, and the importance of estimating microbial traits, in situ, in order to understanding their relevance to ecosystem processes.

The core discipline of this research is Microbial Ecology, yet the implications of the work completed extend to other fields as well, such as Ecosystem Science, Biogeochemistry, and Global Change Ecology. The work contributed to a stronger and more mechanistic inclusion of microorganisms in these fields, in particular means to represent, quantitatively, the relationships between taxonomic biodiversity and large-scale fluxes of elements.