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Final Project Report

- Project Title: The GREEN ‘omics of Nutrient Feedbacks to Soil Warming,
- Report Type: Final Project Report
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  - Pacific Northwest National Laboratory: Kirsten Hofmockel
- DOE Award Number: DE-SC0020172
- Project Duration: 15 September 2019 to 14 September 2024
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- Consortium Member Institutions:
  - Northern Arizona University (Lead)
  - West Virginia University
  - Lawrence Livermore National Laboratory
  - Pacific Northwest National Laboratory
- Distribution Limitations: This report contains no patentable material or protected data.

I. Executive Summary / Abstract

This project, *The GREEN 'omics of Nutrient Feedbacks to Soil Warming*, advanced the DOE Biological and Environmental Research (BER) mission by developing and applying novel isotope-enabled 'omics approaches to investigate microbial controls over nutrient cycling in terrestrial ecosystems. Guided by the Growth Rate, growth Efficiency, and stoichiometry of Essential Nutrients (GREEN 'omics) framework, our overarching goal was to achieve a predictive, systems-level understanding of how microbial traits regulate carbon and nutrient fluxes under climate warming.

Through a multi-institutional collaboration involving Northern Arizona University (lead), West Virginia University, Lawrence Livermore National Laboratory, and Pacific Northwest National Laboratory, we integrated quantitative stable isotope probing (qSIP), Chip-SIP, NanoSIMS, and genome-resolved metagenomics across long-term warming experiments in Arctic, boreal, temperate, and tropical ecosystems.

The project delivered three major advances:

1. Predictive links between microbial physiology and ecosystem processes. We showed that community-weighted temperature sensitivities of bacterial growth (Q10) accurately predict ecosystem-scale respiration responses across diverse soils.
2. Fundamental insights into ecological theory. Using qSIP, we provided the first in situ evidence for density-dependent population dynamics in soil bacteria and revealed that nutrient additions intensify competition, consolidating carbon use through fewer taxa.
3. Technical innovation in isotope-enabled 'omics. We characterized measurement error in qSIP to optimize experimental design, and extended SIP approaches to genome-resolved metagenomics, uncovering cross-kingdom interactions involving bacteria, fungi, and viruses.

Collectively, these advances demonstrate that a small number of microbial traits and taxa disproportionately control soil carbon and nutrient cycling. By directly connecting genomic features to realized ecosystem functions, this project provides foundational data and methods for improving representation of microbial processes in Earth system models.

II. Key Accomplishments: The GREEN 'omics of Nutrient Feedbacks to Soil Warming

DOE Award DE-SC0020172

- Developed robust isotope-enabled 'omics tools
  - Refined quantitative stable isotope probing (qSIP), including formal characterization of resolution, error, and statistical power.
  - Created "BulkSIP," a NanoSIMS-based method for multi-isotope analysis of nanogram-scale DNA.
  - Extended qSIP to genome-resolved metagenomics, enabling direct linkage of isotopic fluxes to microbial genomic traits.
  - Evaluated laboratory vs field based qSIP methods to for nitrogen assimilation
- Established predictive links between microbial traits and ecosystem processes
  - Demonstrated that community-weighted temperature sensitivities of bacterial growth (Q10) strongly predict ecosystem-scale soil respiration across four biomes.
  - Showed long-term soil warming decreases microbial growth rates, providing evidence of community adaptation to climate change.
- Advanced ecological theory through in situ tests
  - Delivered first direct evidence of density-dependent growth and mortality in soil bacterial populations.
  - Revealed that nutrient additions intensify competition, consolidating carbon use through fewer taxa.
  - Conducted a meta-analysis showing predatory bacteria assimilate carbon disproportionately, driving nutrient cycling.
- Generated high-impact publications and datasets
  - 10 peer-reviewed papers in leading journals (*Nature Communications, ISME Journal, Global Change Biology, mSystems*).
  - All sequence data and analysis code archived in public repositories (e.g., NCBI SRA, Bitbucket), in full compliance with DOE Data Management Plan.
- Trained the next generation of scientists
  - Supported X graduate students (Y completed Ph.D.s) and Z postdoctoral scholars.
  - Provided cross-disciplinary training in isotope geochemistry, 'omics, and ecological modeling.
  - Integrated project data into the "Visualization of Scientific Discovery" course at Northern Arizona University.
- Strengthened national lab–university collaboration
  - Northern Arizona University (lead) coordinated with West Virginia University, LLNL, and PNNL.
  - Delivered co-authored publications spanning institutions, bridging ecology, microbiology, and analytical chemistry.
- Advanced DOE BER mission and programmatic goals
  - Addressed FOA DE-FOA-0002059 priorities for systems biology studies of microbes in terrestrial biogeochemical cycles.

- Provided trait-based microbial data directly relevant to improving Earth system models of soil carbon–climate feedbacks.

III. Main Body

1. Project Goals and Objectives

The project's overarching goal was to develop and apply 'omics approaches to investigate microbial community processes involved in nutrient cycling through the GREEN 'omics framework.

- Objective 1: Evaluate the microbial ecology of nutrient uptake, testing hypotheses about nutrient assimilation in response to temperature variation.

  Accomplishment: This objective was successfully met. We demonstrated that the temperature sensitivity (Q10) of growth varies significantly among bacterial taxa but is phylogenetically conserved. Crucially, the community-weighted average of bacterial growth Q10 was strongly predictive of the ecosystem-level soil respiration Q10 across four distinct biomes. Furthermore, we found that after 15 years of experimental field warming, the growth rates of wild soil microbes decreased, indicating a long-term community adaptation to climate change (Purcell et al., 2022).

- Objective 2: Evaluate the ecology of nutrient-use efficiency for soil microorganisms within a framework of ecological theory.

  Accomplishment: This objective was successfully met. Using qSIP, we tested fundamental ecological theory and found strong evidence for density-dependent growth and mortality in soil bacterial populations. We discovered that nutrient additions strengthen, rather than relax, this density dependence, suggesting antagonistic interactions are a key driver of competition (Stone et al., 2022). A meta-analysis of 82 qSIP datasets revealed that predatory bacteria have an outsized role in nutrient cycling, growing faster and assimilating carbon far more rapidly than non-predators (Hungate et al., 2021).

- Objective 3: Develop new isotope-enabled genomics and transcriptomics techniques that probe the microbial ecology of nutrient dissimilation.

  Accomplishment: This objective was successfully met through significant methodological advancements. We published a foundational paper characterizing the measurement error, resolution, and statistical power of qSIP, providing critical guidance for experimental design (Sieradzki et al., 2020). We also successfully extended qSIP to genome-resolved metagenomics, which allowed us to trace plant-derived carbon through bacteria, eukaryotes, and bacteriophages, identifying key genomic traits associated with cross-kingdom interactions (Starr et al., 2021).

Furthermore we developed and validated methods for insitu qSIP using 15NH4+ to quantify taxon specific nitrogen assimilation.

2. Summary of Project Activities and Approach

- Original Approach: The project's core approach was the integration of stable isotope tracers with ‘omics techniques to link the identity of microorganisms to their *in situ* functions. Our primary tools included:

  Quantitative Stable Isotope Probing (qSIP): Using tracers like ^{18}O-water to measure taxon-specific growth and mortality rates of bacteria and fungi in intact soil communities.

  Multi-Isotope Probing: Using tracers like ^{13}C, ^{14}C, and ^{15}N to measure substrate assimilation, nutrient-use efficiency, and metabolic pathways.

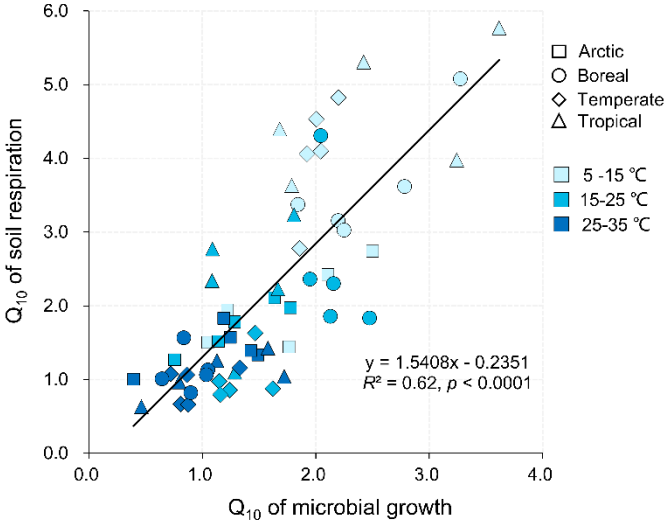
  Advanced Analytical Techniques: Employing Chip-SIP and NanoSIMS at Lawrence Livermore National Laboratory for high-precision, multi-isotope measurements on specific taxa and individual cells.

  Cross-Biome Field Experiments: The project leveraged long-term warming experiments in four distinct biomes (Arctic, boreal, temperate, and tropical) to test the generality of our findings.

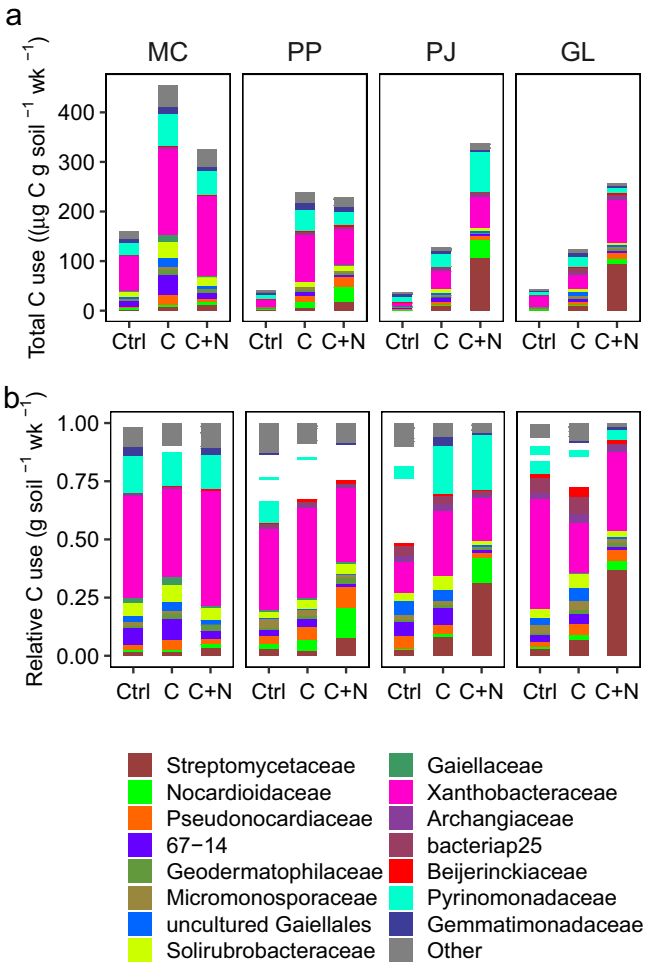
- Deviations and Challenges: The primary challenge encountered was the COVID-19 pandemic, which significantly limited access to laboratory facilities and field sites due to institutional and travel restrictions. This caused delays in some planned field experiments. To mitigate this, the project team pivoted to focus on the analysis and synthesis of existing data and conducted laboratory-based experiments that were less restricted, which successfully resulted in numerous high-impact publications.

3. Comprehensive Data and Results

- Temperature sensitivity of microbial growth predicts ecosystem respiration: The strong correlation between Q_{10} of microbial growth and Q_{10} of soil respiration. This finding demonstrates a direct link between the physiological traits of individual taxa and an emergent ecosystem-level process.



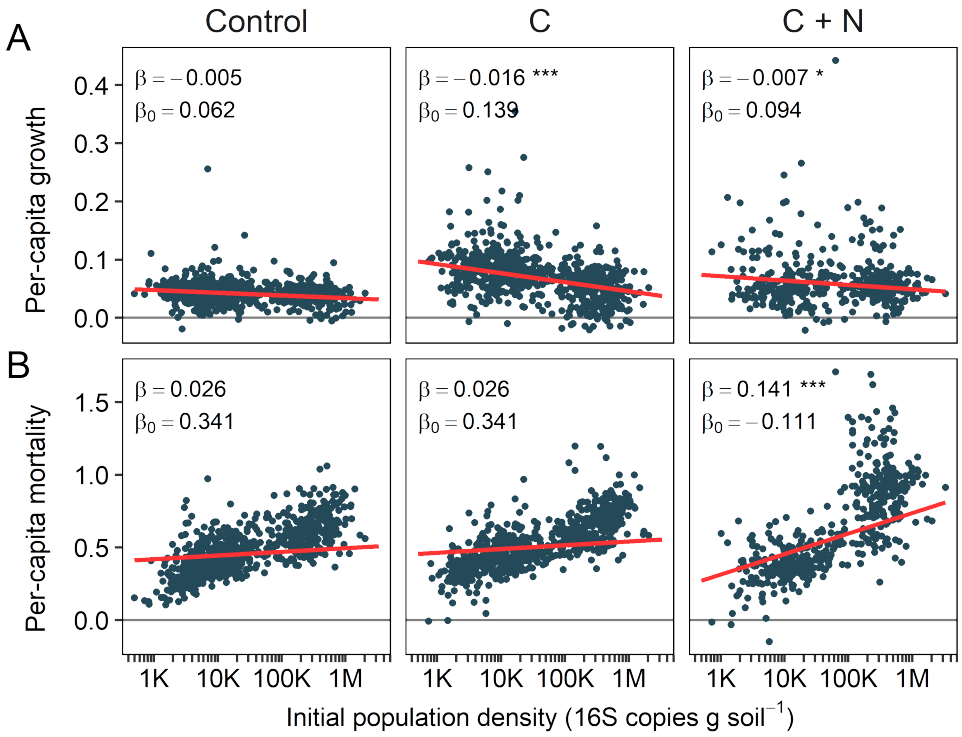
- Key Finding 2: Nutrients consolidate carbon flux through fewer taxa.



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 $[NH_4]_2SO_4$) combination (n = 3 experimental replicates). Bar color represents bacterial family (15 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.

While thousands of bacterial types exist in soil, only and handful of common genera accounted for over 50% of carbon use, a number that dropped further with nutrient addition.

- Key Finding 3: Density-dependent dynamics are shaped by nutrient availability.



Relationship between initial population densities and per-capita growth and mortality rates in each treatment measured with ^{18}O-water qSIP. *Stone et al., 2022, ISME).* Single points represent *per-capita* population rates (day^{-1}) by size of each bacterial population, prior to stable isotope incubation, in each individual soil samples per treatment of replicate soils collected from four ecosystems along an elevational transect in AZ. Soil treatments are water-only (Control), glucose-amended (C), and glucose and ammonium-sulfate amended (C + N). A single taxon may appear repeatedly in each panel, from 1 to 12 times depending on its abundance and frequency. A: *per-capita* growth rates. B: *per-capita* mortality rates. Lines represent the relationship between *per-capita* rates and population density in each treatment calculated from linear mixed models. Slope coefficients (β) provided have asterisks to represent significant difference of slopes from zero. β_0 values represent vertical axis intercepts.This figure supports the conclusion that nutrient addition intensifies competition.

4. Assessment of Impact

A. Technical Effectiveness and Innovation

This project significantly advanced the DOE Genomic Science Program's systems biology toolkit. We refined qSIP into a robust, quantitative platform with documented resolution and error properties, developed a NanoSIMS-based BulkSIP method for multi-isotope analysis of nanogram-scale DNA, and extended SIP approaches to genome-resolved metagenomics. Together, these tools enable precise mapping of microbial traits—including growth, nutrient-use efficiency, and dissimilation pathways—onto taxonomic and genomic frameworks in situ.

B. Scientific Contributions

Our results demonstrated that:

- Microbial evolutionary history is a stronger determinant of growth and nutrient assimilation than environment, providing a trait-based foundation for prediction.
- Nutrient addition narrows microbial control of carbon fluxes, with 3–6 dominant genera accounting for the majority of soil carbon use.
- Predatory bacteria and active viral populations play outsized roles in nutrient cycling, broadening the scope of organisms recognized as central to soil biogeochemistry.

These findings establish generalizable principles that connect microbial traits to ecosystem processes across biomes, directly addressing FOA DE-FOA-0002059 priorities for systems biology studies of microbes in biogeochemical cycling.

C. Alignment with DOE BER Mission

The work aligns with BER's mandate to understand and predict the roles of biological systems in the Earth system. By demonstrating how microbial traits and interactions govern soil carbon responses to warming, we generated mechanistic insights essential for improving Earth system models, thereby enhancing the predictive capacity of climate projections.

D. Broader Impacts

- Training and Workforce Development: The project supported four graduate students and three postdoctoral scholars, producing dissertations, peer-reviewed publications, and career advancement into academic and national lab positions.
- Interdisciplinary Collaboration: Scientists across ecology, microbiology, and analytical chemistry collaborated, producing integrative publications with multi-institutional authorship.
- Education and Outreach: Project data and visualizations were incorporated into a "Visualization of Scientific Discovery" course at Northern Arizona University, training students in both science and communication.

- Workshop on Quantitative Stable Isotope Probing, hosted by the Joint Genome Institute, and co-organized by NAU, Lawrence Livermore National Lab, Lawrence Berkely National Lab, and the Joint Genome Institute.

E. Future Research Directions

This work lays the foundation for:

- Applying qSIP-metatranscriptomics to quantify taxon-specific gene expression and dissimilation rates in situ.
- Expanding studies of soil viral activity and its role in nutrient cycling.
- Integrating trait-based microbial data into Earth system and ecosystem models to improve projections of soil carbon-climate feedbacks.

F. Products & Accomplishments

i. Peer-Reviewed Publications

1.
2. Purcell, A. M., Dijkstra, P., Finley, B., Hayer, M., Koch, B. J., Mau, R. L., Morrissey, E. M., Papp, K., Schwartz, E., Stone, B. W. G., & Hungate, B. A. (2020). Quantitative stable isotope probing with $H_2{}^{18}O$ to measure taxon-specific microbial growth. *Soil Science Society of America Journal*, 84(4), 1256–1273. https://doi.org/10.1002/saj2.20084
3.

Products & Accomplishments

1. Peer-Reviewed Publications

1. Chao, W; 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. *The ISME Journal*. Sep;15(9):2738-2747. doi: 10.1038/s41396-021-00959-1. Epub 2021
2. Stone, B. W., Morrissey, E. M., Mau, R. L., Dijkstra, P., Schwartz, E., Koch, B. J., Hofmockel, K. S., Pett-Ridge, J., Blazewicz, S. J., & Hungate, B. A. (2021). Nutrients cause consolidation of soil carbon flux to a small proportion of the bacterial

community. *Nature Communications*, 12, 3384. https://doi.org/10.1038/s41467-021-23676-4

3. Hungate, B. A., Mau, R. L., Schwartz, E., Caporaso, J. G., Dijkstra, P., van Gestel, N., Koch, B. J., Liu, C. M., Morrissey, E. M., Stone, B. W., & Pett-Ridge, J. (2021). The functional significance of bacterial predators. *mBio*, 12(2), e00466-21. https://doi.org/10.1128/mBio.00466-21
4. Starr, E. P., Shi, S., Blazewicz, S. J., Koch, B. J., Probst, A. J., Hungate, B. A., Pett-Ridge, J., & Firestone, M. K. (2021). Stable-isotope-informed, genome-resolved metagenomics uncovers potential cross-kingdom interactions in rhizosphere soil. *mSphere*, 6(2), e00086-21. https://doi.org/10.1128/mSphere.00086-21
5. Stone, B. W. G., Morrissey, E. M., Mau, R. L., Dijkstra, P., Schwartz, E., Koch, B. J., Hofmockel, K. S., Pett-Ridge, J., Blazewicz, S. J., & Hungate, B. A. (2022). Nutrients strengthen density dependence of per-capita growth and mortality rates across all major phyla of soil bacteria. *The ISME Journal*, 16, 2224–2234. https://doi.org/10.1038/s41396-022-01265-0
6. Li J, Mau RL, Dijkstra P, Koch BJ, Schwartz E, Liu XJA, Morrissey EM, Blazewicz SB, PettRidge 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
7. Purcell, A. M., Dijkstra, P., Finley, B., Hayer, M., Koch, B. J., Mau, R. L., Morrissey, E., Papp, K., Schwartz, E., Stone, B. W. G., & Hungate, B. A. (2022). Decreased growth of wild soil microbes after fifteen years of warming. *Global Change Biology*, 28(18), 5453–5467. https://doi.org/10.1111/gcb.16300
8. Sieradzki, E. T., Koch, B. J., Greenlon, A., Sachdeva, R., Malmstrom, R. R., Mau, R. L., Blazewicz, S. J., Firestone, M. K., Hofmockel, K., Schwartz, E., Hungate, B. A., & Pett-Ridge, J. (2020). Measurement error and resolution in quantitative stable isotope probing: implications for experimental design. *mSystems*, 5(3), e00151-20.
9. Foley, M.M., Stone, B.W.G., Caro, T.A., Sokol N.W., Koch, B.J., Blazewicz, S.J., Dijkstra, P., Hayer, M., Hofmockel, K. Finley, B.K., Mack, M., Marks, J.C., Mau, R.L., Monsaint-Queeney, V., Morrissey, E., Propster, J., Purcell, A.M., Schwartz, E. Pett-Ridge, J., Fierer, N. Hungate, B.A., 2024. Growth rate as a link between microbial diversity and soil biogeochemistry. *Nat Ecol Evol* **8**, 2018–2026 (2024). https://doi.org/10.1038/s41559-024-02520-7
10. 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
11. SL Bell, AE Zimmerman, BW Stone, CH Chang, M Blumer, RS Renslow, ... 2023. Effects of warming on bacterial growth rates in a peat soil under ambient and elevated CO2. Soil Biology and Biochemistry 178, 108933
12. Stone, BWG, Paul Dijkstra, Brianna K Finley, Raina Fitzpatrick, Megan M Foley, Michaela Hayer, Kirsten S Hofmockel, Benjamin J Koch, Junhui Li, Xiao Jun A Liu, Ayla Martinez, Rebecca L Mau, Jane Marks, Victoria Monsaint-Queeney, Ember M Morrissey, Jeffrey Propster, Jennifer Pett-Ridge, Alicia M Purcell, Egbert Schwartz, Bruce A Hungate, 2023. Life history strategies among soil bacteria—dichotomy for few, continuum for many. The ISME Journal 17 (4), 611-619

13. Jeth Walkup, Chansotheary Dang, Rebecca L Mau, Michaela Hayer, Egbert Schwartz, Bram W Stone, Kirsten S Hofmockel, Benjamin J Koch, Alicia M Purcell, Jennifer Pett-Ridge, Chao Wang, Bruce A Hungate, Ember M Morrissey, 2023. The predictive power of phylogeny on growth rates in soil bacterial communities. ISME communications 3 (1), 73, https://doi.org/10.1038/s43705-023-00281-1
14. Dang Chansotheary, Jeth GV Walkup, Bruce A Hungate, Rima B Franklin, Egbert Schwartz, Ember M Morrissey, 2021. Phylogenetic organization in the assimilation of chemically distinct substrates by soil bacteria. Environmental Microbiology. https://doi.org/10.1111/14622920.15843
15. Mau RL, M Hayer, AM Purcell, S Geisen, BA Hungate, E Schwartz, 2024. Measurements of soil protist richness and community composition are influenced by primer pair, annealing temperature, and bioinformatics choices. Applied and Environmental Microbiology 90 (7), e00800-24.
16. Finley Brianna K, Rebecca L Mau, Michaela Hayer, Bram W Stone, Ember M Morrissey, Benjamin J Koch, Craig Rasmussen, Paul Dijkstra, Egbert Schwartz, Bruce A Hungate, 2021. Soil minerals affect taxon-specific bacterial growth. The ISME Journal, 1-9, https://doi.org/10.1038/s41396-021-01162-y
17. JR Propster, E Schwartz, M Hayer, S Miller, V Monsaint-Queeney, BJ Koch, EM Morrissey, MC Mack, BA Hungate, 2023. Distinct Growth Responses of Tundra Soil Bacteria to Short-Term and Long-Term Warming. Applied and Environmental Microbiology 89 (3), e01543-22 10.1128/aem.01543-22. Epub 2023 Feb 27.
18. Reed, K., Dang, C., Walkup, J., Purcell, A., Hungate, B., & Morrissey, E. (2025). Comparing field and lab quantitative stable isotope probing for nitrogen assimilation in soil microbes. *Applied and Environmental Microbiology*, *91*(2), e01849-24.

2. Dissertations and Manuscripts in Preparation

19. Monsaint-Queeney, V. (2024). *Taxon-specific patterns of microbial N assimilation and growth rates in response to three different N additions in soil*. Ph.D. Dissertation, Northern Arizona University.
    1. Main Findings: This dissertation work used dual ^{18}O and ^{15}N qSIP to investigate microbial responses to ammonium, nitrate, and glutamine additions . Key findings include that N addition generally decreased the growth rate of most microbial taxa; N assimilation was highest when N was supplied as glutamine (a combined C and N source) ; and the relationship between growth and N assimilation was strongest for reduced N forms (ammonium, glutamine) compared to nitrate.
    2. Main Contributions: This represents a major training accomplishment of the grant, directly supporting a graduate student through to her Ph.D. . The research provides a highly detailed, taxon-specific dataset on microbial N source preference, directly addressing

       Project Goal 1 by elucidating microbial responses to different nutrient forms.

20. Reed Close, Kinsey (Expected Fall 2025) Microbial and plant biodiversity in Appalachian agroecosystems and their implications for soil health. Ph.D. Dissertation, West Virginia University.
    1. Main Findings: A chapter of this dissertation work used 15N qSIP to measure taxon specific NH4+ nitrogen assimilation in the laboratory and *in-situ* in an agricultural system. This allowed us to determine whether qSIP results are impacted by the laboratory environment which involves the removal of live plant roots. The results indicated that most bacteria exhibited similar rates of nitrogen assimilation in the laboratory as in the field. However, rates for ~10% of taxa differed between methods.
    2. Main Contributions: The grant provided partial support for this PhD student and developed methods and results that will increase the application qSIP to better understand taxon specific nitrogen assimilation.
21. Walkup, Jeth (2023) Evaluating innovative methods of quantitatively linking microbial community structure to ecosystem function. Ph.D. Dissertation, West Virginia University.

    Main Findings: A chapter of this dissertation work examined the predictive poser of phylogeny on growth rates in soil. Specifically using phylogenetic modeling this work demonstrated that trait prediction can be performed both within and across ecosystems which may facilitate the integration of microbial communities into ecosystem models.

    Main Contributions: The grant provided partial support for this PhD student. The work demonstrates that taxon specific bacterial growth rates can be predicted from the traits of phylogenetic related taxa.

22. Stone, B. W. G., Zimmerman, A. E., et al. (Manuscript in Preparation). Temperature response of taxon-specific nitrogen use in a peatland soil microbiome.

    Main Findings: This study, conducted on peat soils from the SPRUCE experimental site, used dual-isotope qSIP to measure growth and N assimilation under different temperature and N amendment scenarios . It found that growth and assimilation of reduced N (ammonium, glutamate) were strongly correlated, but this was not the case for nitrate. The genus *Paraburkholderia* was found to be a dominant N assimilator, accounting for up to 60% of N use. Critically, the analysis showed that evolutionary history (phylogeny) was a far stronger predictor of N assimilation and growth than temperature.

    Main Contributions: This work directly achieves a key planned activity of the project: conducting experiments at the DOE-supported SPRUCE site to test hypotheses about microbial responses to warming in a critical ecosystem. The findings provide strong support for a central project hypothesis (H2) that phylogenetic history is a better predictor of microbial function than environmental factors like temperature.

23. Blazewicz, S. J., Weber, P., et al. (Manuscript in Preparation). BulkSIP: Isotopic Analysis of Nanogram Quantities of Bulk DNA via NanoSIMS elucidates community level microbial activities.

    Main Findings: This work details the development of a novel method, "BulkSIP," that uses a NanoSIMS isotope imaging mass spectrometer to rapidly and simultaneously analyze isotopic enrichment (^{13}C, ^{15}N, ^{18}O) in nanogram quantities of bulk DNA and RNA. The approach is quantitative across a wide range of concentrations and was successfully applied to a soil incubation using three simultaneous isotopic tracers. This method requires substantially less sample material than traditional Isotope Ratio Mass Spectrometry (IRMS) and can analyze multiple isotopes at once.

    Main Contributions: This manuscript represents a significant technical achievement under Project Goal 3 (develop new isotope-enabled techniques). BulkSIP serves as a powerful, efficient screening tool that complements the more intensive qSIP fractionation, allowing researchers to confirm bulk community enrichment with minimal sample loss before proceeding with more detailed analyses. This work was led by the LLNL partners, demonstrating a key collaborative outcome.

24. Hayer, M; Pett-Ridge J, et al., Hungate BA In preparation. Bacterial growth rate responses to temperature in a subtropical forest.

    Preliminary results indicate that warming appeared to suppress bacterial relative growth rate. Preliminary analyses suggest that few active populations contribute disproportionately to biogeochemical processes compared to dormant taxa, and their growth dynamics may shift under stress caused by warming. These findings, while promising, remain provisional pending completion of statistical analyses and validation.

ii. Presentations

- Pett-Ridge, J. (2022). *Invited Lecture: Analyses on the edge of our comfort zone: NanoSIMS studies of soil organic matter, microbial interactions and viruses* 2022 NanoSIMS Workshop, National Physical Laboratory, Teddington, UK, Oct 2022
- Pett-Ridge, J. (2023). Keynote Lecture: *Pursuing Wild Microbes: How Microbiome Interactions and Ecophysiological Traits Shape the Persistence of Soil Carbon*, DOE Biological Systems Science Division Annual PI Meeting, Washington DC, April 2023

- Zimmerman A.E. (2024) et al. 11/06/2024. "Nitrogen assimilation and biosynthetic allocation in peat microbiomes enabled by SIP-metaproteomics." Connecting Microbiome Communities (CMiC) Meeting, San Diego, California. IR: PNNL-SA-205871.
- Stone, B. W. (2023) et al. Nitrogen addition and temperature shifts alter the chemical composition, thermodynamics, and stoichiometry of the DOM pool of Sphagnum peat. Ecological Society of America (ESA) Annual Meeting, Portland, OR, August.
- Bell1, Sheryl L (2023) et al. Impacts of altered climate on microbial growth and nutrient assimilation in an ombitrophic peat bog. DOE GSP Meeting, April.
- Stone, Sheryl Bell, Kaitlin Rempfert, Amy Zimmerman, Kirsten Hofmockel. (2022). Short term temperature increase promotes the growth of generalist bacterial taxa in boreal peatland soils. SES Biennial Meeting, May.
- Bram W Stone1, Sheryl L Bell1, Steven J Blazewicz2, Paul Dijkstra3, Michaela Hayer3, Benjamin J Koch3, Michelle Mack3, Jane C Marks3, Samantha N Miller3, Ember M Morrissey4, Juan Pineiro4, Jeffrey S Propster3, Alicia M Purcell3, Egbert Schwartz3, Chao Wang4, Kirsten S Hofmockel1, Jennifer Pett-Ridge2, Bruce A Hungate3 (2022). Temperature sensitivity of soil bacterial networks from the Arctic to the Tropics. DOE GSP Meeting, April.

iii. Datasets and Technologies

- Software: R code for qSIP data analysis, including growth and mortality modeling. A script for estimating 16S rRNA gene copy number has been publicly archived on Bitbucket.
- Datasets: All sequencing data and associated metadata from publications have been deposited in publicly accessible repositories (e.g., NCBI SRA) as required by the Data Management Plan.
- Techniques: Refined protocols for qSIP analysis, multi-isotope detection via NanoSIMS, and cell extraction for single-cell analysis are detailed in the project's publications.

iv. Synergistic Research Developments

- FICUS proposal 60039; Zimmerman AE, Hofmockel KS, Bell, SL, Stone B, Hungate B, Schwartz E, Monsaint-Queeney V, Pett-Ridge J Resolving Taxon-specific contributions to nutrient cycling in soil microbial communities through stable isotope enabled multi-omics.

4. Educational and Outreach Activities

- Human Resources: The project supported the training of four graduate students (Propster, Monsaint-Queeney, Purcell, Foley) and three postdoctoral scholars (Stone, Wang, Nevado) .
- Course Development: Material and artistic visualizations from this project were used in the "Visualization of Scientific Discovery" course at Northern Arizona University, taught by Victor Leshyk, to train science students in effective communication.

- Quantitative SIP Metagenomics Workshop: New Frontiers in Quantitative Approaches to Stable Isotope Probing, Sept, 2024, Joint Genome Institute This workshop, co-hosted by NAU, LLNL and LBNL, brought together an international group of experts in Stable Isotope Probing to discuss how to develop internal standards, accepted metadata, and using qSIP results for ecosystem-scale modeling

5. Participants and Collaborating Organizations

Lead Institution: Northern Arizona University (NAU)

- Bruce Hungate – PI, overall project leadership, qSIP development and synthesis.
- Michelle Mack – Co-PI, ecosystem ecology, Arctic and boreal sites.
- Paul Dijkstra – Co-PI, stable isotope methods, metabolic modeling.
- Egbert Schwartz – Co-PI, microbial ecology and isotopic analyses.
- Benjamin Koch – Co-PI, quantitative microbial ecology, data synthesis.

Postdoctoral Scholars

- Bram Stone – Postdoctoral Research Associate (*also listed below with PNNL, as Dr. Stone transitioned from postdoc to staff scientist during the grant period)

Graduate Students

- Victoria Monsaint-Queeney – Ph.D., microbial N assimilation, dissertation research.
- Alicia Purcell – Ph.D., long-term warming adaptation study.
- Matthew Foley – Ph.D., microbial turnover under warming.
- Jeff Propster – Ph.D., poster presentation AGU 2020.

Other Staff / Technicians

- Michaela Hayer – Research Scientist, qSIP lab (e.g., DNA prepping, fractionation, sequencing, data analysis and interpretation).

West Virginia University (WVU)

- Ember Morrissey – Co-PI, microbial community assembly, phylogenetic analysis.

Graduate Students / Postdocs

- Jen Kane – Postdoctoral Scholar, nitrogen cycling and microbial ecology
- Kinsey Reed – PhD Student, soil microbial ecology and ^{15}N qSIP in the field
- Chao Wang – Visiting Scholar, microbial temperature sensitivity, respiration coupling.

- Chansotheary Dang –Ph.D. Student, microbial growth and carbon cycling
- Jeth Walkup - PhD student, phylogenetic modeling and trait prediction
- Juan Pineiro- Postdoctoral Scholar, microbial trait null modeling

Lawrence Livermore National Laboratory (LLNL)

- Jennifer Pett-Ridge – Co-PI, NanoSIMS, multi-isotope tracing, bulk-SIP.
- Steven Blazewicz – Co-PI, microbial population dynamics, isotope-enabled viromics.
- Xavier Mayali – Co-PI, microbial interactions, Chip-SIP development.
- Peter Weber – Co-PI, NanoSIMS instrumentation and isotopic analysis.

Postdoctoral Scholars / Staff

- Wei Li – postdoctoral scholar (now staff scientist), NanoSIMS data collection and analysis
- George Michael Allen – technician, stable isotope probing sample fractionation
- Aaron Chew– post-grad technician, molecular lab analyses
- Rina Estera-Molina – project manager, technician involved in BulkSIP development

P Pacific Northwest National Laboratory (PNNL)

- Kirsten Hofmockel – Co-PI, microbial traits, boreal SPRUCE field site, 0.7 CM.

Postdoctoral Scholars / Staff

- Bram (B.W.) Stone, staff scientist – density dependence studies, nutrient-use efficiency.
- Amy Zimmerman, staff scientist – multi-‘omics and quantitative. 6.2 CM
- Sheryl Bell, Research Scientist, 15N qSIP data analysis and interpretation 5.9 CM total
- Kaitlin Rempfert – postdoctoral scholar, 15N qSIP data analysis 4.9 CM

Total Person-Months

Northern Arizona University (NAU)

- Hungate – PI, 0.25 mo/y
- Mack – Co-PI, 0.25 mo/y
- Dijkstra – Co-PI, 3 mo/y
- Schwartz – Co-PI, 0.5 mo/y
- Koch – Co-PI, 3.5 mo/y
- Bram Stone – Postdoctoral Research Associate, 12 mo/y for year 1
- Victoria Monsaint-Queeney – 6 mo/y

- Alicia Purcell – Ph.D., 3 mo/y
- Foley – Ph.D., 6 mo/y
- Jeff Propster – Ph.D., 3 mo/y
- Hayer – Research Scientist, 8 mo/y

Pacific Northwest National Laboratory (PN NL)

- Hofmockel – Co-PI, 0.7 CM total
- Stone, staff scientist – 6.0 CM total
- Zimmerman, staff scientist –6.2 CM total
- Bell, Research Scientist – 5.9 CM total
- Rempfert – postdoctoral scholar, 4.9 CM

West Virginia University (WVU)

- Ember Morrissey – Co-PI, 1 mo/y
- Kane – Postdoctoral Scholar, 5 mo/y
- Reed – PhD Student, 6 mo/y
- Wang – Visiting Scholar, 6 mo/y
- Dang –Ph.D. Student, 6 mo/y
- Walkup - PhD student, 4 mo/y
- Pineiro- Postdoctoral Scholar, 3 mo/y

Lawrence Livermore National Laboratory (LLNL)

- Pett-Ridge, 0.2 months
- Blazewicz, 8.3 months
- Weber, 0.4 months
- Mayali, 0.05 months
- Mike Allen (HT-SIP pipeline), 2.3 months
- Aaron Chew (molec lab tech), 0.1 months
- Rina Estera (lab and field tech), 0.1 months
- Wei Li (NanoSIMS lab postdoc), 1.4 months

Additional Criteria

- Link to BER Program Goals: This project directly addressed the goals of the FOA DE-FOA-0002059 by conducting "systems biology studies on... microbes... involved in biogeochemical cycling" and leading the "development and application of -omics approaches to investigate microbial community processes".
- Interdisciplinary Collaboration: The project's success was built on a deep, interdisciplinary collaboration between ecologists, microbiologists, modelers, and analytical chemists across four institutions, as evidenced by the co-authorship across all major publications.

Future Research Directions:

Application of qSIP-metatranscriptomics to quantify taxon-specific gene expression and rates of dissimilatory processes *in situ*.

Further investigation into the role of viruses in soil nutrient cycling, building on the discovery of highly active phages.

Integration of quantitative, trait-based microbial data generated by this project into ecosystem and Earth system models to improve climate projections.

- Data Management and Transparency: The project adhered to the Data Management Plan submitted with the original proposal, as required by the DOE Office of Science. All publications include data availability statements, and data and analysis code have been made public through appropriate archives.