

University of Washington

Final Scientific/ Technical Report

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Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA)

David A. Stahl, Principal Investigator

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A final report including an abstract, and description of key objectives completed during the grant period, and publications resulting from research supported by the Department of Energy.

I. Abstract

The goal of the Stahl lab is to use a combination of isotopic, genomic, and activity-based analyses in the field, of selected field isolates, and defined synthetic communities to identify sources and sinks of different nitrogen species in the field, focusing on the FRC at the ORNL. The most general research objective is to characterize variants in the pathway for denitrification among field isolates and synthetic microbial assemblies as a foundation for predicting the biotic and abiotic controls of nitrogen and carbon transformations in the field. Specifically, we have focused on characterizing environmental controls of nitrous oxide emissions, an important greenhouse gas and ozone depleting molecule. Studies have mapped the flux of nitrous oxide and controlling genetic elements within the FRC subsurface, indicating the importance of different enzyme variants in nitrous oxide production and consumption in controlling surface emissions. We have developed methods to quantify and catalog the isotopic signatures imparted on nitrous oxide by different nitrous oxide reductase variants in isolated organisms and model synthetic communities. We have developed methods to quantify and catalog the nitrous oxide affinity of field relevant isolates and their biomass yield when respiring nitrous oxide on different carbon substrates. We continue to expand the collection of field-relevant microbial isolates within ENIGMA by focused enrichment and isolation from field material. These objectives have been facilitated by the development of new technologies, including: 1) the development of a multiplexed automated optical density measurement system for quantifying growth kinetics of microorganisms dependent on gaseous substrates in monoculture or gas exchange in synthetic communities and 2) the development of a microcalorimetric analysis format to quantify the impact of environmental stress on microbial maintenance energy. Since field relevant processes are mediated by assemblies of organisms interacting with each other and their local environment, we have also collaborated with other ENIGMA investigators on the assembly and characterization of synthetic communities. These studies have evaluated the role of organisms lacking a complete pathway for denitrification in the control of field processes, measuring process rates of assemblies of organisms that individually lack the full denitrification pathway but mediate the complete denitrification when growing together. These efforts have so far resulted in the publication of 18 peer reviewed papers ranging from ecological and physiological drivers of pathway optimization to field-scale process characterization.

II. Key Objectives

- 1. Compare and contrast community composition, dynamics, and activity of nitrate respiring populations.** This is a continuing effort as part of the Environmental Simulation group to understand the drivers of pathway partitioning and energy distribution within a community of interacting organisms, specifically the R12/3H11 synthetic community.
- 2. Collaborate with other ENIGMA investigators on the development and application of appropriate isolation, enrichment and single cell approaches to capture the relevant functional and phylogenetic diversity of nitrite respiring bacteria at the field site.** We collaborated with several labs across ENIGMA to quantify the nitrogen transformations of organisms that perform denitrification, nitrification, and dissimilatory nitrate reduction to ammonia and continue to develop methodologies and survey kinetic and isotopic signatures of these metabolisms^{1,5,10}.

3. **Quantify community dynamics using measures of resource allocation and energy flow.** We have demonstrated a modeling framework and methodology to quantify cellular energy partitioning in a sulfate-reducing isolate between growth and maintenance of viability² and continue to develop methods for quantifying the relative abundance of populations in a mixed community.
4. **Establish a framework to quantify “health” of a microbial community.** As part of this continued effort, kinetic and stoichiometric models of synthetic communities are being developed to assess the deviation from ideal behavior of a community and infer ecological drivers of community behavior.
5. **Use thermodynamic modeling and microcalorimetry to inform rules of community assembly, stability, and resilience.** We have demonstrated examples of thermodynamic “decision” points in metabolism and cellular responses at the cellular energy² and respiration scales^{10,11}.
6. **Characterize microbial community structure and how it emerges through co-evolution of microbial interactions.** Quantification of community structure remains a challenging and resource intensive process. However, we have examined coevolution and characterized the physiological adaptations that result using technologies developed in this project^{3,6}.

III. List of publications

2022

1. Goff, J.L.; E.G. Szink, M.P. Thorgersen, A.D. Putt, K.A. Hunt, Y. Fan, L.M. Lui, T.N. Nielsen, J.P. Michael, Y. Wang, D. Ning, Y. Fu, F.L. Poole II, T.C. Hazen, D.A. Stahl, J. Zhou, A.P. Arkin, and M.W.W. Adams (2022) Ecophysiological and genomic analyses of a highly abundant *Bacillus cereus* strain reveal niche adaptation to a mixed waste contaminated subsurface environment. *Environmental Microbiology*. [DOI]: 10.1111/1462-2920.16173 [doi]:<https://kbase.us/n/112150/61/> OSTI:1889254
2. Hunt KA, von Netzer F, Gorman-Lewis D, Stahl DA. (2022) Microbial maintenance energy quantified and modeled with microcalorimetry. *Biotechnol Bioeng*. 2022 Jun 9; [DOI]:10.1002/bit.28155. {PMID}: 35680566 OSTI:1874049

2021

3. Hunt, K.A.; J. Forbes, F. Taub, N. Elliott, J. Hardwicke, R. Petersen, N. Stopnisek, D.A.C. Beck, and D.A. Stahl (2021) An automated multiplexed turbidometric and data collection system for measuring growth kinetics of anaerobes dependent on gaseous substrates. *Journal of Microbiological Methods*. [DOI]:[10.1016/j.mimet.2021.106294](https://doi.org/10.1016/j.mimet.2021.106294) {PMID}:[34333046](https://pubmed.ncbi.nlm.nih.gov/34333046/) OSTI: 1828000
4. Lui, Lauren M.; E. L. Majumder, H.J. Smith, H.K. Carlson, N.S. Baliga, F. von Netzer, M.W. Fields, D.A. Stahl, J-Z. Zhou, T.C. Hazen, P.D. Adams, A.P. Arkin (2021) Mechanism across scales a holistic modeling framework integrating laboratory and field studies for microbial ecology. *Frontiers in Microbiology*. [DOI]:[10.3389/fmicb.2021.642422](https://doi.org/10.3389/fmicb.2021.642422) OSTI:1773742
5. Otwell, A.E.; A.V. Carr, E.L.W Majumder, M. Ruiz, R.L. Wilpiseski, L.T. Hoang, B. Webb, S. Turkarslan, S.M. Gibbons, D.A. Elias, G. Siuzdak, D.A. Stahl, N.S. Baliga (2021) Sulfur metabolites play key system-level roles in modulating denitrification. *mSystems*. 6:e01025-20. [DOI]:10.1128/mSystems.01025-20. OSTI:1767862
6. Turkarslan, Serdar; N. Stopnisek, A.W Thompson, C.E Arens, J.J Valenzuela, J. Wilson, K.A Hunt, J. Hardwicke, S. Lim, Y.M Seah, Y. Fu, L. Wu, J-Z Zhou, K.L Hillesland, D.A Stahl, N.S Baliga (2021) Synergistic epistasis enhances cooperativity

of mutualistic interspecies interactions. ISME Journal [DOI]: 10.1038/s41396-021-00919-9 OSTI:1773759

2020

7. Kempfer, M.L.; X. Tao, R. Song, B. Wu, D. A. Stahl, J. D. Wall, A. P. Arkin, A. Zhou, J. Zhou (2020) Effects of genetic and physiological divergence on the evolution of a sulfate-reducing bacterium under conditions of elevated temperature. *mBio* 11, e00569-00520 [DOI]:10.1128/mBio.00569-20 OSTI:1647684
8. Moon J-W.; C.J. Paradis, D.C. Joyner , F. von Netzer, E.L. Majumder, E. Dixon, M. Podar, X. Ge, P.J. Walian, H.J. Smith, X. Wu, G.M. Zane, K.S. Walker, M.P. Thorgersen, F.L. Poole II, L.M. Lui , B.G. Adams, K.B. De León, S.S. Brewer, D.E. Williams, K.A. Lowe, M. Rodriguez, Jr., T.L. Mehlhorn, S.M. Pfiffner, R. Chakraborty, A.P. Arkin, J.D. Wall, M.W. Fields, M.W.W. Adams, D.A. Stahl, D.A. Elias a and T. C. Hazen* (2020) Integrated characterization of subsurface media from locations up- and down-gradient of a uranium-contaminated aquifer. *Chemosphere*. [DOI]:[10.1016/j.chemosphere.2020.126951](https://doi.org/10.1016/j.chemosphere.2020.126951) OSTI:1617965
9. Tian, R; D. Ning, Z. He, P. Zhang, S.J. Spencer, S. Gao, W. Shi, L. Wu, Y. Zhang, Y. Yang, B. G. Adams, A.M. Rocha, B.L. Detienne, K.A. Lowe, D.C. Joyner, D.M. Klingeman, A.P. Arkin, M.W. Fields, T.C. Hazen, D.A. Stahl, E.J. Alm, J. Zhou (2020) Small and mighty: adaptation of superphylum Patescibacteria to groundwater environment drives their genome simplicity. *Microbiome* 8, 51 [DOI]:1186/s40168-020-00825-w OSTI:1651273

2019

10. Vuono, D.C.; R.W. Read, J. Hemp, B.W. Sullivan, J.A. Arnone, I. Neveux, R.R. Blank, E. Loney, D. Miceli, M.-K.H. Winkler, R. Chakraborty, D.A. Stahl and J.J. Grzymalski (2019) Resource concentration modulates the fate of dissimilated nitrogen in a dual-pathway Actinobacterium. *Frontiers in Microbiology*. [DOI]:[10.3389/fmicb.2019.00003](https://doi.org/10.3389/fmicb.2019.00003) {PMID}:[30723459](https://pubmed.ncbi.nlm.nih.gov/30723459/) PMCID:[PMC6349771](https://pubmed.ncbi.nlm.nih.gov/PMC6349771/) OSTI:1560586

2018

11. Flowers, J.; M. Richards, B. Meyer, N.S. Baliga and D.A. Stahl (2018) Constraint-Based Modeling Captures the Metabolic Versatility of *Desulfovibrio vulgaris*. *Environmental Microbiology and Environmental Microbiology Reports*. [DOI]:[10.1111/1758-2229.12619](https://doi.org/10.1111/1758-2229.12619) {PMID}:[29377633](https://pubmed.ncbi.nlm.nih.gov/29377633/) OSTI:1777375
12. Ge, X.; B.J. Vaccaro, M.P. Thorgersen, F.L. Poole, E.L. Majumder, G.M. Zane, K.B. De Leon, A.L. Lancaster, J. Moon, K.A. Lowe, C.J. Paradis, D.C. Joyner, F. von Netzer, D.A. Stahl, P.D. Adams, A.P. Arkin, J.D. Wall, T.C. Hazen and M.W.W. Adams (2018) Iron- and Aluminum-Induced Depletion of Molybdenum in Acidic Environments Impedes the Nitrogen Cycle. *Environmental Microbiology*. [DOI]:[10.1111/1462-2920.14435](https://doi.org/10.1111/1462-2920.14435) {PMID}:[30289197](https://pubmed.ncbi.nlm.nih.gov/30289197/) OSTI:1528691
13. Zhou, A.; R. Lau, R. Baran, J. Ma, F. von Netzer, W. Shi, D. Gorman-Lewis, M.L. Kempfer, Z. He, Y. Qin, Z. Shi, G.M. Zane, L. Wu, B.P. Bowen, T.R. Northen, K.L. Hillesland, D.A. Stahl, J.D. Wall, A.P. Arkin and J. Zhou (2018) Key metabolites and mechanistic changes for salt tolerance in an experimentally evolved sulfate-reducing bacterium, *Desulfovibrio vulgaris*. *mBio*. [DOI]:[10.1128/mBio.01780-17](https://doi.org/10.1128/mBio.01780-17) {PMID}:[29138306](https://pubmed.ncbi.nlm.nih.gov/29138306/) PMCID:[PMC5686539](https://pubmed.ncbi.nlm.nih.gov/PMC5686539/) OSTI:1432221

2017

14. Thompson, A.W.; S. Turkarlan, C.E. Arens, A. López García de Lomana, A.V. Raman, D.A. Stahl and N.S. Baliga (2017) Robustness of a model microbial community emerges from population structure among single cells of a clonal

- population. *Environmental Microbiology*. [DOI]:[10.1111/1462-2920.13764](https://doi.org/10.1111/1462-2920.13764) {PMID}:[28419704](https://pubmed.ncbi.nlm.nih.gov/28419704/) OSTI:1375466
15. Turkarslan, S.; A.V. Raman, A.W. Thompson, C.E. Arens, M.A. Gillespie, F. von Netzer, K.L. Hillesland, S. Stolyar, A. López García de Lomana, D.J. Reiss, D. Gorman-Lewis, G.M. Zane, J.A. Ranish, J.D. Wall, D.A. Stahl and N.S. Baliga (2017) Mechanism for microbial population collapse in a fluctuating resource environment. *Molecular Systems Biology*. [DOI]:[10.15252/msb.20167058](https://doi.org/10.15252/msb.20167058) {PMID}:[28320772](https://pubmed.ncbi.nlm.nih.gov/28320772/) PMID:[PMCID:PMC5371734](https://pubmed.ncbi.nlm.nih.gov/28320772/) OSTI:1546850
 16. Zeng, L.; E. Wooton, D.A. Stahl and P.J. Walian (2017) Identification and Characterization of the Major Porin of *Desulfovibrio vulgaris* Hildenborough. *Journal of Bacteriology*. [DOI]:[10.1128/JB.00286-17](https://doi.org/10.1128/JB.00286-17) {PMID}:[28874410](https://pubmed.ncbi.nlm.nih.gov/28874410/) PMID:[PMCID:PMC5686591](https://pubmed.ncbi.nlm.nih.gov/28874410/) OSTI:1766617
 17. Zhang, P.; Z. He, J.D. Van Nostrand, Y. Qin, Y. Deng, L. Wu, Q. Tu, J. Wang, C.W. Schadt, M.W. Fields, T.C. Hazen, A.P. Arkin, D.A. Stahl and J. Zhou (2017) Dynamic succession of groundwater sulfate-reducing communities during prolonged reduction of uranium in a contaminated aquifer. *Environmental Science and Technology*. [DOI]:[10.1021/acs.est.6b02980](https://doi.org/10.1021/acs.est.6b02980) {PMID}:[28300407](https://pubmed.ncbi.nlm.nih.gov/28300407/) OSTI:1394592
 18. Zhou, A.; R. Lau, R. Baran, J. Ma, F. von Netzer, W. Shi, D. Gorman-Lewis, M.L. Kempher, Z. He, Y. Qin, Z. Shi, G.M. Zane, L. Wu, B.P. Bowen, T.R. Northen, K.L. Hillesland, D.A. Stahl, J.D. Wall, A.P. Arkin and J. Zhou (2017) Key metabolites and mechanistic changes for salt tolerance in an experimentally evolved sulfate-reducing bacterium, *Desulfovibrio vulgaris*. *mBio*. [DOI]:[10.1128/mBio.01780-17](https://doi.org/10.1128/mBio.01780-17) {PMID}:[29138306](https://pubmed.ncbi.nlm.nih.gov/29138306/) PMID:[PMCID:PMC5686539](https://pubmed.ncbi.nlm.nih.gov/29138306/) OSTI:1432221

IV. Computer Modeling

We developed a genome scale model of *Desulfovibrio vulgaris* (iJF744) to assess its metabolic plasticity¹¹ and continue to develop this and other models for the simulation and interrogation of interacting organisms in SynComs. We also developed microcalorimetry as a method to quantify cellular energy allocation in a single organism or community under varying environmental conditions¹⁵. That work focused on the development of a modeling framework to quantify the cellular allocation of energy dissipation to growth and maintenance in response to varying environmental stress, using temperature stress for initial studies².

V. Technology Developed and Implemented

We have developed and demonstrated an automated optical density monitoring system to increase the throughput and resolution of time resolved growth data from cultures with a defined gas headspace³. This has been used to quantify physiological changes in a coevolved SynCom of *Desulfovibrio vulgaris* and *Methanococcus maripaludis*⁶ and the ongoing description of a denitrifying SynCom of *Rhodanobacter* R12 and *Acidovorax* 3H11.

VI. Training (* = current members)

Graduate Students (GS) and Postdocs (PD):

Jeffrey Gabster	(GS)	Sergey Stolyar	(PD)	Birte Meyer	(PD)
Christopher Walker	(GS)	Kristina Hillesbrand	(PD)	Jason Flowers	(PD)

David Vuono	(PD)	Frederick von Netzer	(PD)	Kristopher Hunt	(PD*)
Nejc Stopnisek	(PD)	Anne Otwell	(PD)		

Research Associates (RA):

Nicholas Elliot	(RA)	Madelyn Shapiro	(RA)	JeanMarie Calvillo	(RA)
Kelley Meinhardt	(RA)	Jessica Hardwicke	(RA)		

Research Scientists (RS):

Thomas Lie	(RS*)
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