Title

Systems biology approaches towards predictive microbial ecology

Journal

Environmental Microbiology, 20(12)

ISSN

1462-2912

Authors

Otwell, Anne E

de Lomana, Adrián López García

Gibbons, Sean M

et al.

Publication Date

2018-12-01

DOI

10.1111/1462-2920.14378

Peer reviewed

Systems biology approaches towards predictive microbial ecology

Anne E. Otwell[1], Adrián López García de Lomana[1], Sean M. Gibbons[1,2,3], Mónica V. Orellana[1,4] and Nitin S. Baliga[1,3,5,6]

[1] Institute for Systems Biology, Seattle, WA, USA
[2] eScience Institute, University of Washington, Seattle, WA, USA
[3] Molecular and Cellular Biology Program, University of Washington, Seattle, WA, USA
[4] Polar Science Center, Applied Physics Lab, University of Washington, Seattle, WA
[5] Departments of Biology and Microbiology, University of Washington, Seattle, WA, USA
[6] Lawrence Berkeley National Lab, Berkeley, CA, USA

Abstract

Through complex interspecies interactions, microbial processes drive nutrient cycling and biogeochemistry. However, we still struggle to predict specifically which organisms, communities, and biotic and abiotic processes are determining ecosystem function and how environmental changes will alter their roles and stability. While the tools to create such a predictive microbial ecology capability exist, cross-disciplinary integration of high-resolution field measurements, detailed laboratory studies, and computation is essential. In this perspective, we emphasize the importance of pursuing a multiscale, systems approach to iteratively link ecological processes measured in the field to testable hypotheses that drive high-throughput laboratory experimentation. Mechanistic understanding of microbial processes gained in controlled lab systems will lead to the development of theory that can be tested back in the field. Using N_2O production as an example, we review the current status of field and laboratory research and layout a plausible path to the kind of integration that is needed to enable prediction of how N-cycling microbial communities will respond to environmental changes. We advocate for the development of realistic and predictive gene regulatory network models for environmental responses that extend from single-cell resolution to ecosystems, which is essential to understand how microbial communities involved in N_2O production and consumption will respond to future environmental conditions.

The need for integrating microbial regulatory information into biogeochemical models

Microorganisms are majorly responsible for the cycling of essential nutrients through Earth's oceans, soils, and atmosphere. Each cycle is interconnected, and perturbations to a single biogeochemical cycle has permeating effects, ultimately leading to changes in ecosystem functioning. Current global nutrient models are invaluable and present fairly accurate depictions of the inputs and outputs of cycles. However, microbial gene regulatory information is mainly lacking from biogeochemical models. To gain a quantitative and predictive understanding of biogeochemical processes in a changing climate, we must integrate the fields of systems biology and biogeochemistry. Tools are now available for characterizing structure, function, and regulation of microorganisms and communities with relative ease, and technologies continue to expand rapidly. Systems-level analyses of key organisms and communities in the laboratory and the field can reveal regulatory mechanisms and environmental constraints determining microbial ecosystem processes. Integrating novel microbial insights gained from systems-level analyses into existing nutrient cycle models will greatly improve their predictive capabilities, which is essential for determining mitigation strategies and policies in a changing climate.

The nitrogen cycle is highly perturbed due to anthropogenic inputs of synthetic fertilizers. This global-scale perturbation has detrimental consequences including eutrophication (tied to dangerous algal blooms and dead zones) and groundwater contamination [1,2]. One of the most pressing issues is rapidly increasing levels of the greenhouse gas N_2O, which is linked to fertilizer usage. N_2O has a 100-year global warming potential 298 times higher than CO_2 [3] and is currently the most significant ozone-depleting emission [4]. Microorganisms drive transformations of nitrogen through various redox reactions (**Figure 1**). Microbial transformations of nitrogen, including the reactions that control the production of N_2O, are incredibly complex and determinants of these reactions are still being de-convoluted. In fact, a recent thematic issue in this journal focused on the microbial sources and sinks of N_2O (December 2017). In order to create an accurate model of the nitrogen cycle, including sources and sinks of N_2O, understanding the environmental and regulatory controls governing these microbial reactions at a mechanistic level is essential. Therefore, the nitrogen cycle serves as an exemplar for the need to integrate microbial regulatory information into current biogeochemical models.

Denitrification and nitrification (**Figure 1**) are widely accepted as the major contributors to N_2O production, as 70% of global N_2O emissions are thought to result from these processes occurring in managed and natural soils alone [3]. Enzymes involved in nitrification and denitrification are known (**Table 1** in **Figure 1**) and certain organisms involved in these processes are characterized, but linking this microbial information with ecosystem-level processes is extremely complex for various reasons. For one, organisms involved in nitrogen-cycling processes, particularly denitrification, tend to be metabolically versatile. Therefore, presence of genes involved in denitrification (e.g., Nor for the reduction of NO to N_2O) does not necessarily mean that the organism is utilizing this respiratory pathway for energy production. Instead, genes involved in the production and consumption of N_2O are regulated by various physical, chemical, and biological factors. In-depth studies relating gene expression to environmental conditions (e.g., pH, temperature, soil moisture, etc.) are necessary in order to predict N_2O dynamics in a

given environment. Adding additional complexity to the task of predicting ecosystem function (e.g., N_2O emissions) from microbial information is the fact that N-containing intermediates are likely readily transferred between organisms in the environment. For example, studies have shown that denitrification is a highly modular process and that incomplete denitrification pathways are common amongst organisms [5,6]. This means that products of NO_3^- reduction (NO_2^-) and partial denitrification (NO, N_2O) are likely produced by certain populations of organisms, and then transferred and transformed by other populations of organisms. Furthermore, N-containing compounds relevant to the production and consumption of N_2O can be both reduced and oxidized by microorganisms. For example, NO_2^- can be used as both an electron acceptor (reduced to NO in the denitrification pathway or reduced to ammonia in the DNRA pathway) and an electron donor (oxidized to NO_3^- by NO_2^- oxidizing organisms) (**Figure 1**).

A critical challenge we face today is the global perturbation of the nitrogen cycle, and the corresponding increase in N_2O emissions (IPCC 2007). Creating predictive models of N_2O, that incorporate microbial information, can help to inform educational campaigns and policy decisions regarding nitrogen fertilizer usage. In the following sections, we will describe advancements that have been made for characterizing microbial processes involved in N_2O production and consumption in both the field and the laboratory, additional work that is required for gaining a high-resolution understanding of N_2O dynamics (functional genomics, high resolution time series experiments, synthetic communities, etc.), and how these data can be integrated into predictive models for the nitrogen cycle and N_2O emissions.

Current and future methods for assessing sources and sinks of N_2O

Biogeochemical models of N_2O production

Modelling approaches to estimate N_2O emissions for different spatio-temporal scales are needed in order to predict the responses of the microbial biota to a changing climate. There are different types of models for nitrification/denitrification and N_2O prediction based on atmospheric N_2O measurements and anthropogenic activity [7]. These include single species models [8], field models [9], regional and global scales models, [10] bottom-up models [11], and top-down models [12]. N_2O simulation models are often used to predict the activity of nitrification/denitrification processes in soils [13–15]. One process-based model, the Landscape DeNitrification DeComposition (DNDC) model, predicts N_2O emissions from agricultural management variables like field observations of soil temperature, water content, plant growth and inorganic sources of nitrogen (NH_4^+, NO_3^-) including NO_3^- leaching from agricultural systems [16]. This model continues to be adapted and improved (reviewed by Gilheshy et al. [17]), including the incorporation of CO_2 measurements [18].

Isotopic labelling is another powerful strategy for creating models to describe the different nitrogen species transformed by microorganisms. These models use isotopic ratios to discriminate between different N_2O sources using $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O, including the intramolecular distribution of ^{15}N according to its site preference. This information has been successfully incorporated into biogeochemical compartmentalized or box models for marine environments [19] as well as for terrestrial

environments. This method of quantification has been applied at different scales ranging from regional [20] to global [21,22]. Global models incorporating isotopic information demonstrate that N_2O from marine sources is isotopically different from freshwater and continental soil [23]. This finding likely reflects differences in microbial community structure and function in these regions. For instance, ammonia-oxidizing archaea (AOA) are thought to be major contributors to N_2O in marine environments, whereas ammonia-oxidizing bacteria (AOB) and denitrifiers majorly contribute to N_2O emissions in certain soil environments [24–26]. Across environments, N_2O emissions are rising rapidly. Historically, marine N_2O emissions have had little effect on climate. However, the oxygen minimum zones (OMZs) are expanding and intensifying in the oceans due to global warming and eutrophication (IPCC 2017 [27] and [28–30]). For example, the Eastern Tropical South Pacific (ETSP) and the northern Indian Ocean as well as other anoxic areas, play increasingly critical roles in the net production of N_2O [9,31,32]. Thus, the production of N_2O in the OMZs may have an enormous impact on the global N_2O entering the atmosphere in the near future [9,33].

Altogether, while current biogeochemical models are useful, they all are limited by a lack of microbial regulatory information. Models assume that all microbial populations contribute similarly to the production of N_2O or that they have a minor effect on N_2O production [34]. However, sequencing data have demonstrated high spatio-temporal dynamics of soil and marine microbial diversity [35] and different rates of N_2O production and consumption by microbial communities [36]. Recently, successful efforts to integrate environmental genomics, enzyme kinetics and biogeochemical models have been developed [37–39].

Field-based analysis of microbial communities involved in N_2O cycling

The characterization of nitrification and denitrification in the field has improved enormously, not only from the use of stable isotopes (discussed above) but also from the development of next generation sequencing (NGS) methods [40,41]. Combinations of ribosomal rRNA sequencing (16S rRNA), functional gene sequencing, metagenomics, and metatranscriptomics have been used to understand the roles of microorganisms in the nitrogen cycle [9,42–50]. The integration of these molecular tools with environmental and other biological measurements has significantly enhanced our understanding of microbial diversity and community dynamics in soils and marine areas. Sequencing methods have also identified a high temporal and spatial resolution of microbial communities involved in N_2O production and consumption, [51,52] which has important implications for modelling. Nitrifier and denitrifier communities vary regionally in relation to the different physico-chemical characteristics of the environment (soil/seawater/freshwater, oxygen/no oxygen). Thus, much effort has gone into understanding microbial community structure in various environments involved in N_2O emissions and responses to changes in environmental conditions, e.g., O_2 depletion, higher temperature, lower pH, etc. [49,53–57].

As it is now possible to quantify community composition through next generation sequencing methods, a natural next step is the inference of species interactions and activity. Several methods are

available for inferring potential community interactions from NGS data, including correlations between taxon abundances. Simulation studies have shown how certain types of interactions (e.g., strong mutualism or antagonism) are more easily detected using correlation methods than others (e.g., commensalism or amensalism [58]). Prior work has shown that environmental heterogeneity is often the strongest driver of correlation structure in microbial communities [59]. Indeed, cross-sectional correlations across space can either reflect environmental filtering or putative interactions between microbes, and telling the difference between the two is challenging. Therefore, understanding the overall spatial structure of an ecosystem is crucial to interpreting these analyses [60]. Spatial autocorrelation can be used to look for structured gradients in field systems, where proximate regions of space are more self-similar (e.g., patchy) than would be expected by chance [61]. This spatial patchiness in the biotic community could be related to physicochemical parameters in order to better assess whether these patterns are shaped by niche filtering or by biotic interactions. Similarly, at the temporal scale, we can take advantage of variable dynamics in time series observations by looking at temporal autocorrelation and cross-correlation structure. There are computationally tractable methods for rapidly inferring sparse, time-lagged cross-correlation networks between microbial phylotypes, which are equivalent to partial Granger causal interaction networks [62]. If the past abundance of one microbial species is linearly correlated with the future abundance of another species (i.e. Granger causal or linearly predictive), then perhaps there is some direct or indirect causal association between them or they are undergoing some sort of successional process. Many other spatiotemporal methods are available for inferring putative interactions from 'omics' data [63–65]. However, no matter how sophisticated these correlation-based methods are, inferred interactions must be validated by direct experimental evidence, including metabolite exchange assays, competition experiments, or microscopy before they are accepted as true [66]. Other methods like BONCAT (Bioorthogonal non-canonical amino acid tagging [67]) are capable of revealing metabolically active species. Once active taxa are defined, metabolic fluxes can be predicted using genome-scale metabolic models [68] combined with high-resolution measurements of N_2O consumption and production [9,69].

In the marine OMZs, metagenomics, metatranscriptomics and single-gene surveys have identified characteristic transitions in microbial community composition and metabolism with depth. Mainly, nitrification dominates in the oxygenated surface waters, whereas denitrification and anaerobic ammonium oxidation (anammox) are major processes in the suboxic OMZ core [31,52,70,71]. These studies in the OMZ have also revealed a remarkable richness of metabolic processes along the redox gradient, as well as novel linkages between community members [44]. Furthermore, stable isotope studies have provided enormous insight into understanding spatio-temporal patterns of production and consumption of different nitrogen species in the OMZ [72,73]. In these areas, the production of N_2O varies regionally [74] and seasonally [75–77]. Historically, consumption of N_2O (through NosZ activity) has been in balance with N_2O production. However, N_2O is now accumulating rapidly in the atmosphere (National Oceanographic and Atmospheric administration; [78]). While the OMZ is a clear oceanic region that shows highest N_2O production, patterns of N_2O 'hotspots' in soil are more complex. N_2O emissions

do appear to be tightly linked with environmental conditions (e.g., pH) as well as microbial community composition, but these complex relationships are not completely elucidated. One important factor that has been shown to control N_2O emissions in soils is the presence and activity of NosZ. Two distinct clades of this enzyme exist, which have been shown to have important differences and to be associated with different patterns of N_2O production [79–81]. Understanding the presence, activity, and regulation of genes like NosZ is essential for modelling N_2O production and consumption from a particular environment.

Laboratory-based studies to gain mechanistic understanding of N_2O production and consumption

In-depth understanding of denitrification and nitrification pathways is mainly based on pure culture studies in the laboratory with model organisms, such as the denitrifier *Paracoccus denitrificans*. Through dozens of studies that employ a range of techniques (e.g., transcriptomics, proteomics, enzyme kinetics, gas flux analysis), its denitrification phenotype, including biochemical pathways and regulatory networks involved, has been well-characterized [34,82–85]. For example, higher N_2O production is associated with low pH conditions, an observation that is consistent with field observations [86]. Certain ammonia oxidizers have been characterized in-depth, including the ammonia-oxidizing archaeon (AOA) *Nitrosopumilus maritimus* and the ammonia-oxidizing bacterium (AOB) *Nitrosomonas europaea [87]*. While the AOA and AOB carry out the same nitrogen transformation, the two groups show important differences with respect to their ammonia-oxidizing phenotype, including higher N_2O emissions by the AOB [88]. This observed difference between microbial groups that carry out the same function illustrates the limitations of incorporating only process-level information (e.g., ammonia oxidation) into global biogeochemical models. Rather, developing a mechanistic understanding of the distinct physiologies of active microorganisms (e.g., AOA versus AOB) and their role in biogeochemical processes in the context of environmental constraints is essential for creating robust and predictive models.

Studies with model organisms such as *P. denitrificans* and *N. maritimus* have provided a strong foundation for understanding denitrification and nitrification. However, there is currently a need to expand detailed lab-based studies to other organisms in order to better represent the diverse organisms known to carry out these processes. Tools for elevating uncharacterized field isolates to model organism status have expanded rapidly in recent years with the ease of sequencing and technologies for high-throughput functional analysis (e.g., RB-TnSeq [89]). New model organisms for representing processes related to N_2O production and consumption should be carefully selected based on relevance to the environment and unique metabolic features. For example, a large fraction of denitrification studies have focused on a handful of Gram-negative bacteria (including *P. denitrificans*). However, studies suggest that Gram-positive organisms are important in many environments and show distinct differences in denitrification phenotype and likely their regulatory pathways [90–93]. Furthermore, while pure culture studies have provided invaluable information, it is essential to increase complexity of laboratory-based study systems in order to better reflect the complexity of microbial community interactions occurring in the environment. Across the field of microbiology, there is a push to create model communities that better

represent processes of interest [94–97]. Model microbial communities provide a platform for detailed studies on community structure, function, dynamics, and evolution in controlled, tractable systems that are more environmentally-relevant than pure cultures. Model communities for denitrification and nitrification can be designed to represent environmentally important sinks and sources of N_2O (e.g., OMZ, specific soil types/conditions, etc.) and capture the diverse organisms involved in N transformation processes. As an example, a consortium can be developed that consists of an N_2O-producing and N_2O-consuming organism, and dynamics of N_2O can be tracked across time and various growth conditions, and eventually genetic perturbations. Communities can be established through both bottom-up methods (i.e., isolates assembled into synthetic communities) and top-down methods (i.e., simplified field communities selected under different conditions in reactor systems). Using a range of technologies from community-level systems analyses (e.g., metatranscriptomics, metaproteomics) to single-cell analytics (e.g., flow cytometry, single cell genomics), detailed characterization of these model communities across a range of conditions can reveal environmental and regulatory determinants of N_2O production.

Towards predictive and mechanistic gene regulatory network models to uncover dynamic control of N_2O production and consumption by microbial communities

The ultimate goal of microbial ecology is phenotype prediction. Prediction rests on two pillars: quantification and theory. Current technologies are at a level of precision and recall that identification and quantification of all molecular species in a cell can be done at a relatively low cost and in a short timeframe—genomics, transcriptomics, proteomics, metabolomics and many more “omic” technologies are now available. Nevertheless, a major challenge of microbial ecology is the incorporation of high-throughput data into dynamic predictive models of molecular regulation at mechanistic resolution. While molecular data has never been so accessible to our community, we are not generally taking full advantage of these vast and detailed data to infer molecular regulatory mechanisms in microbial communities and their impact on the environment.

Data from various studies with model denitrifiers [83,84,98–101] and nitrifiers [102–108] is available for integration into models. However, soil and aquatic environments host complex microbial communities that could differ substantially in terms of the regulation and activity with respect to their nitrogen-cycling pathways [109]. We appreciate that it is essential to build upon current understanding based on studies with model organisms by performing systems-level analysis of a diverse range of environmentally-relevant denitrifiers and nitrifiers to reveal the vast universe of gene regulatory mechanisms around N_2O. We advocate for integrated microbial community models that include gene regulation across time. Along with other successful efforts in gene regulatory network inference [110–113], environmental gene regulatory influence network (EGRIN) models have broadly been used to predict cellular phenotypic states from single species [114–119]. The goal of creating EGRIN models for denitrifying organisms is to highlight key regulatory pathways and environmental determinants affecting the production and consumption of N_2O. This information could then be incorporated into global N models alongside taxa abundance and activity information. EGRIN models consist of genes organized in

conditionally co-regulated modules learned through semi-supervised biclustering of gene expression, guided by biologically informative priors and de novo cis-regulatory gene regulatory elements (e.g., transcription factor binding sites) [120,121]. Next, the underlying regulatory factors governing the co-regulation of genes within modules is deciphered through a combination of protein-DNA interaction mapping and a regression-based approach, to model transcriptional changes of genes within each module as a function of a linear combination of influences of transcription factors (TFs) and environmental variables [122]. Dynamic temporal response is naturally incorporated into EGRIN models, which enables the characterization of control regulatory mechanisms (feed-forward loops, toggle switches, fold-change detection) under varying environmental conditions like oxygen depletion and nutrient availability. These gene regulatory network models have also been successfully integrated with reconstructed metabolic models [68]. Probabilistic Regulation Of Metabolism (PROM; [123]) and Integrated Deduced REgulation And Metabolism (IDREAM; [124]) are approaches to integrate EGRIN models with reconstructed metabolic networks to predict how regulatory changes observed at a transcriptional level manifest at the phenotypic level. Ultimately, predictive and mechanistic-level EGRIN models need to be incorporated into global-scale biogeochemical N_2O production and consumption models, linking experimentally observed N fluxes in the field to biotic and abiotic factors, opening the possibility for testable hypothesis in laboratory conditions under simpler synthetic microbial communities.

Indeed, while pure culture studies allow for detailed, mechanistic studies of processes of interest, increasing complexity of study systems by developing experimental microbial communities is an important step towards increasing the environmental relevance of lab-based experimentation. Numerous studies have documented the prevalence of incomplete denitrification pathways in the genomes of organisms, strongly suggesting that N-intermediates are transferred across groups of interacting organisms [45]. These interactions can be recreated in the laboratory by pairing incomplete denitrifiers with N_2O-reducing organisms. EGRIN models can then be inferred through systems-level analysis of pure cultures and synthetic communities [68] by performing transcriptomics, metabolomics, and enzymatic assays on N-cycle enzymes across varied growth conditions of environmental relevance. In this way, the genetic, regulatory, and environmental determinants controlling the fate of N and N_2O fluxes can be elucidated. We envision first the development of such integrated models for relatively small communities under controlled environments in the lab, e.g., mutualistic syntrophic communities [125,126] or synthetic communities of microbial keystone taxa [127]. There have been multiple efforts to build such synthetic communities with simple interactions that result in predictive dynamical behavior ranging from predation, to competition and cooperation [128]. Uncovering the mechanistic underpinnings of resilience of microbial communities [129–131] will facilitate more accurate prediction of their response and adaptability to novel environments [132]. In the future, we foresee these models as useful tools to perturb microbial communities in the field such that metabolic sources and sinks are manipulated. For example, we would be able to predict the different impacts of introducing NosZ gene in specific taxa, or the effect of perturbing pH on community composition, physiological state and N_2O production [133]. Ultimately,

we would like to manipulate at the population level dynamical properties of microbial communities that are key for the production and consumption of N_2O.

Outlook

In summary, in order to answer fundamental questions about N_2O net production, we advocate for an iterative approach that interlaces field monitoring and laboratory studies (**Figure 1**). Field measurements allow us to understand temporal (daily, weekly, monthly, seasonal) and spatial (microenvironments to regional and global scales) patterns of microbial communities, which allow us to generate hypotheses for how species and environmental variables are interacting with one another. Furthermore, field measurements can track microbial community dynamics and activity [134], isotopic signatures, and the roles of symbiosis [135,136] and interacting biota [137–140] (e.g., viruses, other bacteria, protozoa) in shaping microbial community structure and function. However, it is difficult to discern mechanisms of regulation based on field studies, and this regulatory information is critical for determining and predicting activity of the microbial communities producing and consuming N_2O today and in a changing climate. Therefore, specific hypotheses relating microbial ecology to biogeochemistry can be formed based on field measurements, and then lab-based studies with field isolates can be designed in order to test these hypotheses in controlled, reproducible systems [102]. Laboratory investigations can provide mechanistic understanding of physiology and regulatory pathways of organisms and communities [129,130]. Through detailed studies, microbial dynamics, interactions, and evolution can be characterized in the laboratory and fundamental questions, including the response of communities to perturbation and the resilience of communities to climate change, can be answered [129–131]. Theories derived from lab-based studies (e.g., regulatory mechanisms involved in N_2O production across various conditions) can then be tested back in the field. The emergent knowledge of these iterative activities will allow for mechanistic understanding of field processes. Extending this framework to other environments around the globe has the potential to ultimately link microbial diversity to ecological and biogeochemical function. A Lawrence Berkeley National Laboratory-led effort is building this predictive ecology framework, by iteratively linking field and lab activities to understand the biotic and abiotic changes that have led to increased N_2O production from contaminated areas of the Oak Ridge National Laboratory Field Research Center. The ultimate goal of this effort is to be able to predict how environmental perturbations influence the fate of microbial processes in the natural environment. Answering these fundamental questions allows for an evolving predictive microbial ecology paradigm to emerge.

Acknowledgements

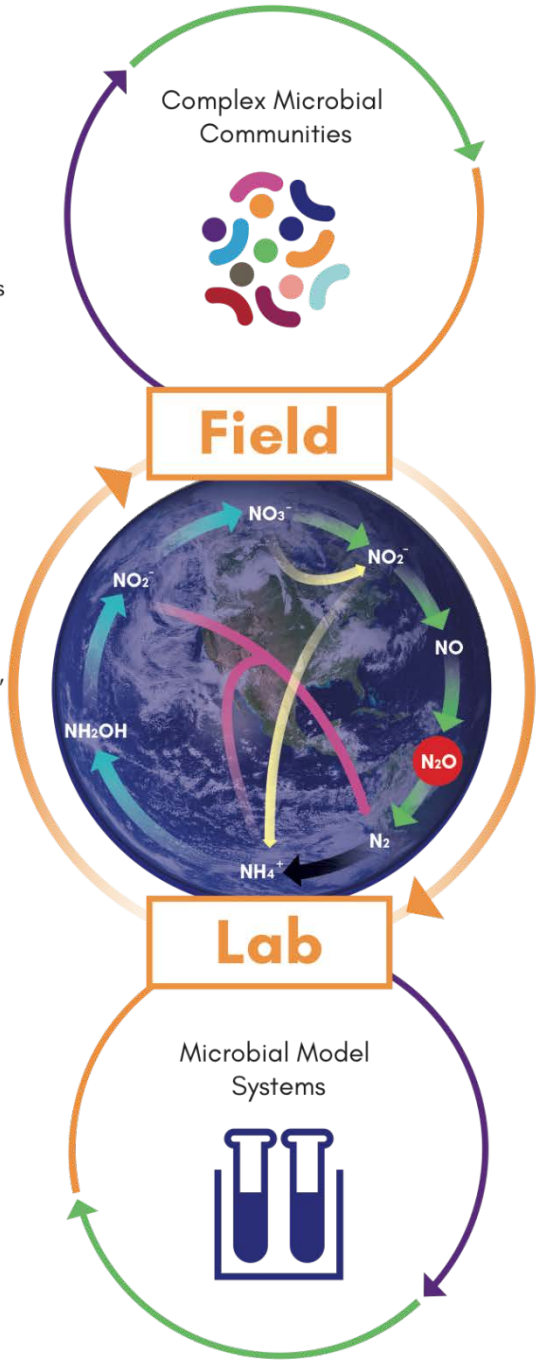
We thank the National Science Foundation (grants MCB-1316206 to M.V.O and N.S.B.; DBI-1565166 and MCB-1616955 to N.S.B.). This work was supported by Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA; http://enigma.lbl.gov), a Scientific Focus Area Program at Lawrence Berkeley National Laboratory supported by the U.S. Department of Energy's Office of Science, Office of Biological & Environmental Research under contract number DE-AC02-05CH11231. S.M.G.

was supported by an award from the Washington Research Foundation. We would like to thank Allison Kudla of ISB for drawing the figure.

Figure 1. *Systems biology approaches towards predictive microbial ecology.* The nitrogen cycle includes an intricate set of reactions, which are mainly driven by microorganisms. Major microbial processes include denitrification, nitrogen fixation, nitrification, anammox, and dissimilatory nitrate reduction to ammonia (DNRA) (see Table 1 for details). These microbial activities, along with geochemical parameters, determine the fate of nitrogen, for instance whether a given environment is a source of the greenhouse gas N_2O. Through an iterative approach that integrates detailed field measurements with high-throughput laboratory experimentation, predictive models for N_2O production and consumption can be built. This approach requires application of current technologies (from singlecell to multi-omic analyses) and advanced computation (e.g., gene regulatory network inference algorithms). This framework can be extended to other biogeochemical cycles in order to predict how microbial communities and ecosystem function will be altered in a changing climate.

Predictive Microbial Ecology

N_2O Case Study



TECHNOLOGY

Field reactors

In situ probes

Isotope fractionation

Multiomics technologies

Remote sensing

COMPUTATION

Community composition

OTUs

Species interactions

Biogeochemical constraints

ECOLOGICAL OUTCOME

Signatures of resilience, resistance, robustness

BIOLOGY

Observations, phenomena, hypothesis

Environmental constraints

Microbial isolates

Microbial synthetic communities

COMPUTATION

Predictive gene network models

TECHNOLOGY

Lab reactors

Lab evolution

Multiomics technologies

Single cell analysis

Table 1. *Major microbial processes driving the nitrogen cycle.* Microorganisms transform nitrogen compounds through various oxidation and reduction reactions. Specific nitrogen transformations and the enzymes known to catalyze these reactions are shown.

	N transformation	Enzyme(s)
Denitrification	NO_3^- to NO_2^-	Nar, Nap
	NO_2^- to NO	Nir
	NO to N_2O	Nor
	N_2O to N_2	Nos
Nitrogen Fixation	N_2 to NH_4^+	Nif
Nitrification	NH_4^+ to NH_2OH	Amo
	NH_2OH to NO_2^-	Hao
	NO_2^- to NO_3^-	Nxr
Anammox	NO_2^- to NO	Nir
	NO + NH_4^+ to N_2H_4	Hzs
	N_2H_4 to N_2	Hdh
DNRA	NO_3^- to NO_2^-	Nir
	NO_2^- to NH_4^+	Nrf

References

1. Shibata H, Galloway JN, Leach AM, Cattaneo LR, Cattell Noll L, Erisman JW, et al. Nitrogen footprints: Regional realities and options to reduce nitrogen loss to the environment. Ambio. 2017;46: 129–142. doi:10.1007/s13280-016-0815-4

2. Canfield DE, Glazer AN, Falkowski PG. The evolution and future of Earth's nitrogen cycle. Science. 2010;330: 192–196. doi:10.1126/science.1186120

3. Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos Trans R Soc Lond B Biol Sci. 2013;368: 20130122. doi:10.1098/rstb.2013.0122

4. Ravishankara AR, Daniel JS, Portmann RW. Nitrous oxide (N2O): the dominant ozone-depleting substance emitted in the 21st century. Science. 2009;326: 123–125. doi:10.1126/science.1176985

5. Graf DRH, Jones CM, Hallin S. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N2O emissions. PLoS One. 2014;9: e114118. doi:10.1371/journal.pone.0114118

6. Roco CA, Bergaust LL, Bakken LR, Yavitt JB, Shapleigh JP. Modularity of nitrogen-oxide reducing soil bacteria: linking phenotype to genotype. Environ Microbiol. Wiley Online Library; 2017;19: 2507–2519. Available: http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13250/full

7. Davidson EA. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nat Geosci. Nature Publishing Group; 2009;2: 659. doi:10.1038/ngeo608

8. Barford C, Montoya J, Altabet M, Mitchell R. Steady-State Oxygen Isotope Effects of N2O Production in Paracoccus denitrificans. Microb Ecol. 2017;74: 507–509. doi:10.1007/s00248-017-0965-3

9. Babbin AR, Bianchi D, Jayakumar A, Ward BB. Rapid nitrous oxide cycling in the suboxic ocean. Science. American Association for the Advancement of Science; 2015;348: 1127–1129. doi:10.1126/science.aaa8380

10. Bouwman AF, Boumans LJM, Batjes NH. Modeling global annual N2O and NO emissions from fertilized fields. Global Biogeochem Cycles. Wiley Online Library; 2002;16: 28–21. Available: https://nph.onlinelibrary.wiley.com/doi/abs/10.1029/2001GB001812

11. Bergamaschi P, Corazza M, Karstens U, Athanassiadou M, Thompson RL, Pison I, et al. Top-down estimates of European CH 4 and N 2 O emissions based on four different inverse models. Atmos Chem Phys. Copernicus GmbH; 2015;15: 715–736. Available: https://www.atmos-chem-phys.net/15/715/2015/acp-15-715-2015.html

12. Crutzen PJ, Mosier AR, Smith KA, Winiwarter W. N2O Release from Agro-biofuel Production

Negates Global Warming Reduction by Replacing Fossil Fuels. In: Crutzen PJ, Brauch HG, editors. Paul J Crutzen: A Pioneer on Atmospheric Chemistry and Climate Change in the Anthropocene. Cham: Springer International Publishing; 2016. pp. 227–238. doi:10.1007/978-3-319-27460-7_12

13. Del Grosso SJ, Parton WJ, Mosier AR, Walsh MK, Ojima DS, Thornton PE. DAYCENT national-scale simulations of nitrous oxide emissions from cropped soils in the United States. J Environ Qual. 2006;35: 1451–1460. doi:10.2134/jeq2005.0160

14. Butterbach-Bahl K, Stange F, Papen H, Li C. Regional inventory of nitric oxide and nitrous oxide emissions for forest soils of southeast Germany using the biogeochemical model PnET-N-DNDC. J Geophys Res D: Atmos. Wiley Online Library; 2001;106: 34155–34166. Available: http://onlinelibrary.wiley.com/doi/10.1029/2000JD000173/full

15. Henault C, Bizouard F, Laville P, Gabrielle B, Nicoullaud B, Germon JC, et al. Predicting in situ soil N2O emission using NOE algorithm and soil database. Glob Chang Biol. 2005;11: 115–127. doi:10.1111/j.1365-2486.2004.00879.x

16. Molina-Herrera S, Haas E, Klatt S, Kraus D, Augustin J, Magliulo V, et al. A modeling study on mitigation of N2O emissions and NO3 leaching at different agricultural sites across Europe using LandscapeDNDC. Sci Total Environ. 2016;553: 128–140. doi:10.1016/j.scitotenv.2015.12.099

17. Gilhespy SL, Anthony S, Cardenas L, Chadwick D, del Prado A, Li C, et al. First 20 years of DNDC (DeNitrification DeComposition): Model evolution. Ecol Modell. 2014;292: 51–62. doi:10.1016/j.ecolmodel.2014.09.004

18. Houska T, Kraus D, Kiese R, Breuer L. Constraining a complex biogeochemical model for CO 2 and N 2 O emission simulations from various land uses by model--data fusion. Biogeosciences. Copernicus GmbH; 2017;14: 3487. Available: http://search.proquest.com/openview/9db71795a5d39c7c07b8c305738e2e68/1?pq-origsite=gscholar&cbl=105740

19. Somes CJ, Schmittner A, Galbraith ED, Lehmann MF, Altabet MA, Montoya JP, et al. Simulating the global distribution of nitrogen isotopes in the ocean. Global Biogeochem Cycles. Wiley Online Library; 2010;24. Available: https://onlinelibrary.wiley.com/doi/abs/10.1029/2009GB003767

20. Buchwald C, Santoro AE, Stanley RHR, Casciotti KL. Nitrogen cycling in the secondary nitrite maximum of the eastern tropical North Pacific off Costa Rica. Global Biogeochem Cycles. Wiley Online Library; 2015;29: 2061–2081. Available: http://onlinelibrary.wiley.com/doi/10.1002/2015GB005187/full

21. de Vries FT, Thébault E, Liiri M, Birkhofer K, Tsiafouli MA, Bjørnlund L, et al. Soil food web properties explain ecosystem services across European land use systems. Proc Natl Acad Sci U S A. 2013;110: 14296–14301. doi:10.1073/pnas.1305198110

22. Somes CJ, Schmittner A, Galbraith ED, Lehmann MF, Altabet MA, Montoya JP, et al. Simulating

the global distribution of nitrogen isotopes in the ocean: NITROGEN ISOTOPES IN THE GLOBAL OCEAN. Global Biogeochem Cycles. 2010;24. doi:10.1029/2009GB003767

23. Snider DM, Venkiteswaran JJ, Schiff SL, Spoelstra J. From the ground up: global nitrous oxide sources are constrained by stable isotope values. PLoS One. 2015;10: e0118954. doi:10.1371/journal.pone.0118954

24. Löscher CR, Kock A, Könneke M, LaRoche J, Bange HW, Schmitz RA. Production of oceanic nitrous oxide by ammonia-oxidizing archaea. Biogeosciences. Copernicus GmbH; 2012;9: 2419–2429. doi:10.5194/bg-9-2419-2012

25. Meinhardt KA, Stopnisek N, Pannu MW, Strand SE, Fransen SC, Casciotti KL, et al. Ammonia-oxidizing bacteria are the primary N2O producers in an ammonia-oxidizing archaea dominated alkaline agricultural soil. Environ Microbiol. Wiley Online Library; 2018; Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.14246

26. Hink L, Nicol GW, Prosser JI. Archaea produce lower yields of N2O than bacteria during aerobic ammonia oxidation in soil. Environ Microbiol. Wiley Online Library; 2017;19: 4829–4837. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13282

27. Intergovernmental Panel on Climate Change. Working Group 1. Climate Change 2007: The Physical Science Basis : Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change : Summary for Policymakers and Technical Summary and Frequently Asked Questions [Internet]. 2007. Available: http://books.google.com/books/about/Climate_Change_2007.html?hl=&id=TZxbjgEACAAJ

28. Diaz RJ, Rosenberg R. Spreading dead zones and consequences for marine ecosystems. Science. 2008;321: 926–929. doi:10.1126/science.1156401

29. Wright JJ, Konwar KM, Hallam SJ. Microbial ecology of expanding oxygen minimum zones. Nat Rev Microbiol. 2012;10: 381–394. doi:10.1038/nrmicro2778

30. Codispoti LA. Oceans. Interesting times for marine N2O. Science. 2010;327: 1339–1340. doi:10.1126/science.1184945

31. Ulloa O, Canfield DE, DeLong EF, Letelier RM, Stewart FJ. Microbial oceanography of anoxic oxygen minimum zones. Proc Natl Acad Sci U S A. 2012;109: 15996–16003. doi:10.1073/pnas.1205009109

32. Arévalo-Martínez DL, Kock A, Löscher CR, Schmitz RA, Bange HW. Massive nitrous oxide emissions from the tropical South Pacific Ocean. Nat Geosci. Nature Publishing Group; 2015;8: 530. doi:10.1038/ngeo2469

33. Battaglia G, Joos F. Marine N2O emissions from nitrification and denitrification constrained by modern observations and projected in multimillennial global warming simulations. Global Biogeochem Cycles. Wiley Online Library; 2018;32: 92–121. Available:

https://onlinelibrary.wiley.com/doi/abs/10.1002/2017GB005671

34. Bakken LR, Bergaust L, Liu B, Frostegård A. Regulation of denitrification at the cellular level: a clue to the understanding of N2O emissions from soils. Philos Trans R Soc Lond B Biol Sci. 2012;367: 1226–1234. doi:10.1098/rstb.2011.0321

35. Shade A, Caporaso JG, Handelsman J, Knight R, Fierer N. A meta-analysis of changes in bacterial and archaeal communities with time. ISME J. 2013;7: 1493–1506. doi:10.1038/ismej.2013.54

36. Levine UY, Teal TK, Robertson GP, Schmidt TM. Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. ISME J. 2011;5: 1683–1691. doi:10.1038/ismej.2011.40

37. Li M, Qian W-J, Gao Y, Shi L, Liu C. Functional Enzyme-Based Approach for Linking Microbial Community Functions with Biogeochemical Process Kinetics. Environ Sci Technol. 2017;51: 11848–11857. doi:10.1021/acs.est.7b03158

38. Reed DC, Algar CK, Huber JA, Dick GJ. Gene-centric approach to integrating environmental genomics and biogeochemical models. Proc Natl Acad Sci U S A. 2014;111: 1879–1884. doi:10.1073/pnas.1313713111

39. Zhu Y-G, Xue X-M, Kappler A, Rosen BP, Meharg AA. Linking Genes to Microbial Biogeochemical Cycling: Lessons from Arsenic. Environ Sci Technol. 2017;51: 7326–7339. doi:10.1021/acs.est.7b00689

40. Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-Cohen L. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. MBio. 2015;6. doi:10.1128/mBio.02288-14

41. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20: 1297–1303. doi:10.1101/gr.107524.110

42. Ganesh S, Parris DJ, DeLong EF, Stewart FJ. Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone. ISME J. 2014;8: 187–211. doi:10.1038/ismej.2013.144

43. Orellana LH, Chee-Sanford JC, Sanford RA, Löffler FE, Konstantinidis KT. Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Appl Environ Microbiol. 2017; doi:10.1128/AEM.01646-17

44. Plominsky AM, Trefault N, Podell S, Blanton JM, De la Iglesia R, Allen EE, et al. Metabolic potential and in situ transcriptomic profiles of previously uncharacterized key microbial groups involved in coupled carbon, nitrogen and sulfur cycling in anoxic marine zones. Environ Microbiol. 2018; doi:10.1111/1462-2920.14109

45. Masuda Y, Itoh H, Shiratori Y, Isobe K, Otsuka S, Senoo K. Predominant but Previously-overlooked Prokaryotic Drivers of Reductive Nitrogen Transformation in Paddy Soils, Revealed by Metatranscriptomics. Microbes Environ. 2017;32: 180–183. doi:10.1264/jsme2.ME16179

46. Lüke C, Speth DR, Kox MAR, Villanueva L, Jetten MSM. Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. PeerJ. 2016;4: e1924. doi:10.7717/peerj.1924

47. Zehr JP, Kudela RM. Nitrogen cycle of the open ocean: from genes to ecosystems. Ann Rev Mar Sci. 2011;3: 197–225. doi:10.1146/annurev-marine-120709-142819

48. Hawley AK, Brewer HM, Norbeck AD, Paša-Tolić L, Hallam SJ. Metaproteomics reveals differential modes of metabolic coupling among ubiquitous oxygen minimum zone microbes. Proc Natl Acad Sci U S A. 2014;111: 11395–11400. doi:10.1073/pnas.1322132111

49. Fuchsman CA, Devol AH, Saunders JK, McKay C, Rocap G. Niche Partitioning of the N Cycling Microbial Community of an Offshore Oxygen Deficient Zone. Front Microbiol. 2017;8: 2384. doi:10.3389/fmicb.2017.02384

50. Nelson MB, Martiny AC, Martiny JBH. Global biogeography of microbial nitrogen-cycling traits in soil. Proc Natl Acad Sci U S A. 2016;113: 8033–8040. doi:10.1073/pnas.1601070113

51. Lauber CL, Hamady M, Knight R, Fierer N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol. 2009;75: 5111–5120. doi:10.1128/AEM.00335-09

52. Peng X, Jayakumar A, Ward BB. Community composition of ammonia-oxidizing archaea from surface and anoxic depths of oceanic oxygen minimum zones. Front Microbiol. 2013;4: 177. doi:10.3389/fmicb.2013.00177

53. Chen J, Hanke A, Tegetmeyer HE, Kattelmann I, Sharma R, Hamann E, et al. Impacts of chemical gradients on microbial community structure. ISME J. 2017;11: 920–931. doi:10.1038/ismej.2016.175

54. Giles ME, Daniell TJ, Baggs EM. Compound driven differences in N2 and N2O emission from soil; the role of substrate use efficiency and the microbial community. Soil Biol Biochem. 2017;106: 90–98. doi:10.1016/j.soilbio.2016.11.028

55. Wu B, Liu F, Weiser MD, Ning D, Okie JG, Shen L, et al. Temperature determines the diversity and structure of N2O-reducing microbial assemblages. Funct Ecol. Wiley Online Library; 2018; Available: https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1365-2435.13091

56. Yin C, Fan F, Song A, Fan X, Ding H, Ran W, et al. The response patterns of community traits of N2O emission-related functional guilds to temperature across different arable soils under inorganic fertilization. Soil Biol Biochem. 2017;108: 65–77. doi:10.1016/j.soilbio.2017.01.022

57. Čuhel J, Šimek M, Laughlin RJ, Bru D, Chèneby D, Watson CJ, et al. Insights into the Effect of Soil pH on N2O and N2 Emissions and Denitrifier Community Size and Activity. Appl Environ Microbiol. 2010;76: 1870–1878. doi:10.1128/AEM.02484-09

58. Freilich MA, Wieters E, Broitman BR, Marquet PA, Navarrete SA. Species co-occurrence networks: Can they reveal trophic and non-trophic interactions in ecological communities? Ecology. Wiley Online Library; 2018;99: 690–699. Available: https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1002/ecy.2142

59. Berry D, Widder S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front Microbiol. 2014;5: 219. doi:10.3389/fmicb.2014.00219

60. Smith WPJ, Davit Y, Osborne JM, Kim W, Foster KR, Pitt-Francis JM. Cell morphology drives spatial patterning in microbial communities. Proc Natl Acad Sci U S A. 2017;114: E280–E286. doi:10.1073/pnas.1613007114

61. O'Brien SL, Gibbons SM, Owens SM, Hampton-Marcell J, Johnston ER, Jastrow JD, et al. Spatial scale drives patterns in soil bacterial diversity. Environ Microbiol. 2016;18: 2039–2051. doi:10.1111/1462-2920.13231

62. Gibbons SM, Kearney SM, Smillie CS, Alm EJ. Two dynamic regimes in the human gut microbiome. PLoS Comput Biol. 2017;13: e1005364. doi:10.1371/journal.pcbi.1005364

63. Martin-Platero AM, Cleary B, Kauffman K, Preheim SP, McGillicuddy DJ, Alm EJ, et al. High resolution time series reveals cohesive but short-lived communities in coastal plankton. Nat Commun. 2018;9: 266. doi:10.1038/s41467-017-02571-4

64. Sugihara G, May R, Ye H, Hsieh C-H, Deyle E, Fogarty M, et al. Detecting causality in complex ecosystems. Science. 2012;338: 496–500. doi:10.1126/science.1227079

65. Fisher CK, Mehta P. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. PLoS One. 2014;9: e102451. doi:10.1371/journal.pone.0102451

66. Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, et al. Challenges in microbial ecology: building predictive understanding of community function and dynamics. ISME J. 2016;10: 2557–2568. doi:10.1038/ismej.2016.45

67. Hatzenpichler R, Connon SA, Goudeau D, Malmstrom RR, Woyke T, Orphan VJ. Visualizing in situ translational activity for identifying and sorting slow-growing archaeal−bacterial consortia. Proc Natl Acad Sci U S A. National Academy of Sciences; 2016;113: E4069–E4078. doi:10.1073/pnas.1603757113

68. Mellbye BL, Giguere AT, Murthy GS, Bottomley PJ, Sayavedra-Soto LA, Chaplen FWR. Genome-Scale, Constraint-Based Modeling of Nitrogen Oxide Fluxes during Coculture of Nitrosomonas europaea and Nitrobacter winogradskyi. mSystems. 2018;3. doi:10.1128/mSystems.00170-17

69. Wen Y, Chen Z, Dannenmann M, Carminati A, Willibald G, Kiese R, et al. Disentangling gross N2O production and consumption in soil. Sci Rep. 2016;6: 36517. doi:10.1038/srep36517

70. Stewart FJ, Ulloa O, DeLong EF. Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ Microbiol. 2012;14: 23–40. doi:10.1111/j.1462-2920.2010.02400.x

71. Zaikova E, Walsh DA, Stilwell CP, Mohn WW, Tortell PD, Hallam SJ. Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. Environ Microbiol. 2010;12: 172–191. doi:10.1111/j.1462-2920.2009.02058.x

72. Sun X, Jayakumar A, Ward BB. Community Composition of Nitrous Oxide Consuming Bacteria in the Oxygen Minimum Zone of the Eastern Tropical South Pacific. Front Microbiol. 2017;8: 1183. doi:10.3389/fmicb.2017.01183

73. Ji Q, Babbin AR, Peng X, Bowen JL, Ward BB. Nitrogen substrate–dependent nitrous oxide cycling in salt marsh sediments. J Mar Res. 2015;73: 71–92. Available: https://www.ingentaconnect.com/content/jmr/jmr/2015/00000073/f0020003/art00002

74. Ji Q, Babbin AR, Jayakumar A, Oleynik S, Ward BB. Nitrous oxide production by nitrification and denitrification in the Eastern Tropical South Pacific oxygen minimum zone. Geophys Res Lett. Wiley Online Library; 2015;42: 10–755. Available: https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1002/2015GL066853

75. Farías L, Besoain V, García-Loyola S. Presence of nitrous oxide hotspots in the coastal upwelling area off central Chile: an analysis of temporal variability based on ten years of a biogeochemical time series. Environ Res Lett. IOP Publishing; 2015;10: 044017. Available: http://iopscience.iop.org/article/10.1088/1748-9326/10/4/044017/meta

76. Torres-Beltrán M, Hawley AK, Capelle D, Zaikova E, Walsh DA, Mueller A, et al. A compendium of geochemical information from the Saanich Inlet water column. Sci Data. 2017;4: 170159. doi:10.1038/sdata.2017.159

77. Capelle DW, Hawley AK, Hallam SJ, Tortell PD. A multi-year time-series of N2O dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. Limnol Oceanogr. Wiley Online Library; 2018;63: 524–539. Available: https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.1002/lno.10645

78. US Department of Commerce, NOAA, Laboratory ESR. ESRL Global Monitoring Division - Halocarbons and other Atmospheric Trace Species. 2005; Available: https://www.esrl.noaa.gov/gmd/hats/combined/N2O.html

79. Domeignoz-Horta LA, Philippot L, Peyrard C, Bru D, Breuil M-C, Bizouard F, et al. Peaks of in situ N2o emissions are influenced by N2o-producing and reducing microbial communities across arable soils. Glob Chang Biol. Wiley Online Library; 2018;24: 360–370. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/gcb.13853

80. Sanford RA, Wagner DD, Wu Q, Chee-Sanford JC, Thomas SH, Cruz-García C, et al. Unexpected

nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc Natl Acad Sci U S A. 2012;109: 19709–19714. doi:10.1073/pnas.1211238109

81. Stein LY. Resolving N2O respiration pathways: a tale of two NosZ clades. Environ Microbiol. Wiley Online Library; 2017;19: 4806–4807. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13969

82. Qu Z, Bakken LR, Molstad L, Frostegård Å, Bergaust LL. Transcriptional and metabolic regulation of denitrification in Paracoccus denitrificans allows low but significant activity of nitrous oxide reductase under oxic conditions. Environ Microbiol. 2016;18: 2951–2963. doi:10.1111/1462-2920.13128

83. Giannopoulos G, Sullivan MJ, Hartop KR, Rowley G, Gates AJ, Watmough NJ, et al. Tuning the modular Paracoccus denitrificans respirome to adapt from aerobic respiration to anaerobic denitrification. Environ Microbiol. 2017;19: 4953–4964. doi:10.1111/1462-2920.13974

84. Olaya-Abril A, Hidalgo-Carrillo J, Luque-Almagro VM, Fuentes-Almagro C, Urbano FJ, Moreno-Vivián C, et al. Exploring the Denitrification Proteome of Paracoccus denitrificans PD1222. Front Microbiol. 2018;9: 1137. doi:10.3389/fmicb.2018.01137

85. Bergaust L, Mao Y, Bakken LR, Frostegård A. Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrous [corrected] oxide reductase in Paracoccus denitrificans. Appl Environ Microbiol. 2010;76: 6387–6396. doi:10.1128/AEM.00608-10

86. SImek M, Cooper JE. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. Eur J Soil Sci. 2002;53: 345–354. doi:10.1046/j.1365-2389.2002.00461.x

87. Hink L, Lycus P, Gubry-Rangin C, Frostegård Å, Nicol GW, Prosser JI, et al. Kinetics of NH3-oxidation, NO-turnover, N2O-production and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers. Environ Microbiol. Wiley Online Library; 2017;19: 4882–4896. Available: http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13914/full

88. Hink L, Gubry-Rangin C, Nicol GW, Prosser JI. The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. ISME J. 2018;12: 1084–1093. doi:10.1038/s41396-017-0025-5

89. Liu H, Deutschbauer AM. Rapidly moving new bacteria to model-organism status. Curr Opin Biotechnol. 2018;51: 116–122. doi:10.1016/j.copbio.2017.12.006

90. Verbaendert I, De Vos P, Boon N, Heylen K. Denitrification in Gram-positive bacteria: an underexplored trait. Biochem Soc Trans. 2011;39: 254–258. doi:10.1042/BST0390254

91. No RMS. Denitrification and Non-Denitrifier Nitrous Oxide Emission in Gram-Positive Bacteria. Metalloenzymes in Denitrification: Applications and Environmental Impacts. Royal Society of

Chemistry; 2016;9: 349. Available: https://books.google.com/books?hl=en&lr=&id=srKADQAAQBAJ&oi=fnd&pg=PA349&dq=Denitrification+and+Non-Denitrifier+Nitrous+Oxide+Emission+in+Gram-Positive+Bacteria&ots=OwGskjSM6h&sig=e5Ma_SVkT-D6v8ZnLjeg8d2v80A

92. Mania D, Heylen K, van Spanning RJM, Frostegård Å. The nitrate-ammonifying and nosZ-carrying bacterium B acillus vireti is a potent source and sink for nitric and nitrous oxide under high nitrate conditions. Environ Microbiol. Wiley Online Library; 2014;16: 3196–3210. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.12478

93. Mania D, Heylen K, van Spanning RJM, Frostegård Å. Regulation of nitrogen metabolism in the nitrate-ammonifying soil bacterium B acillus vireti and evidence for its ability to grow using N2O as electron acceptor. Environ Microbiol. Wiley Online Library; 2016;18: 2937–2950. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13124

94. Hillesland KL, Lim S, Flowers JJ, Turkarslan S, Pinel N, Zane GM, et al. Erosion of functional independence early in the evolution of a microbial mutualism. Proc Natl Acad Sci U S A. 2014;111: 14822–14827. doi:10.1073/pnas.1407986111

95. De Roy K, Marzorati M, Van den Abbeele P, Van de Wiele T, Boon N. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. Environ Microbiol. 2014;16: 1472–1481. doi:10.1111/1462-2920.12343

96. Miller LD, Mosher JJ, Venkateswaran A, Yang ZK, Palumbo AV, Phelps TJ, et al. Establishment and metabolic analysis of a model microbial community for understanding trophic and electron accepting interactions of subsurface anaerobic environments. BMC Microbiol. 2010;10: 149. doi:10.1186/1471-2180-10-149

97. Venturelli OS, Carr AC, Fisher G, Hsu RH, Lau R, Bowen BP, et al. Deciphering microbial interactions in synthetic human gut microbiome communities. Mol Syst Biol. EMBO Press; 2018;14: e8157. Available: http://msb.embopress.org/content/14/6/e8157.abstract

98. Gaimster H, Chalklen L, Alston M, Munnoch JT, Richardson DJ, Gates AJ, et al. Genome-Wide Discovery of Putative sRNAs in Paracoccus denitrificans Expressed under Nitrous Oxide Emitting Conditions. Front Microbiol. 2016;7: 1806. doi:10.3389/fmicb.2016.01806

99. Gaimster H, Alston M, Richardson DJ, Gates AJ, Rowley G. Transcriptional and environmental control of bacterial denitrification and N2O emissions. FEMS Microbiol Lett. 2018;365. doi:10.1093/femsle/fnx277

100. Kohlmann Y, Pohlmann A, Schwartz E, Zühlke D, Otto A, Albrecht D, et al. Coping with anoxia: a comprehensive proteomic and transcriptomic survey of denitrification. J Proteome Res. 2014;13: 4325–4338. doi:10.1021/pr500491r

101. Thorgersen MP, Lancaster WA, Ge X, Zane GM, Wetmore KM, Vaccaro BJ, et al. Mechanisms

of Chromium and Uranium Toxicity in Pseudomonas stutzeri RCH2 Grown under Anaerobic Nitrate-Reducing Conditions. Front Microbiol. 2017;8: 1529. doi:10.3389/fmicb.2017.01529

102. Qin W, Amin SA, Lundeen RA, Heal KR, Martens-Habbena W, Turkarslan S, et al. Stress response of a marine ammonia-oxidizing archaeon informs physiological status of environmental populations. ISME J. 2018;12: 508–519. doi:10.1038/ismej.2017.186

103. Cho CM-H, Yan T, Liu X, Wu L, Zhou J, Stein LY. Transcriptome of a Nitrosomonas europaea mutant with a disrupted nitrite reductase gene (nirK). Appl Environ Microbiol. 2006;72: 4450–4454. doi:10.1128/AEM.02958-05

104. Wei X, Yan T, Hommes NG, Liu X, Wu L, McAlvin C, et al. Transcript profiles of Nitrosomonas europaea during growth and upon deprivation of ammonia and carbonate. FEMS Microbiol Lett. 2006;257: 76–83. doi:10.1111/j.1574-6968.2006.00152.x

105. Mellbye BL, Giguere A, Chaplen F, Bottomley PJ, Sayavedra-Soto LA. Steady-State Growth under Inorganic Carbon Limitation Conditions Increases Energy Consumption for Maintenance and Enhances Nitrous Oxide Production in Nitrosomonas europaea. Appl Environ Microbiol. 2016;82: 3310–3318. doi:10.1128/AEM.00294-16

106. Pérez J, Buchanan A, Mellbye B, Ferrell R, Chang JH, Chaplen F, et al. Interactions of Nitrosomonas europaea and Nitrobacter winogradskyi grown in co-culture. Arch Microbiol. 2015;197: 79–89. doi:10.1007/s00203-014-1056-1

107. Lu H, Ulanov AV, Nobu M, Liu W-T. Global metabolomic responses of Nitrosomonas europaea 19718 to cold stress and altered ammonia feeding patterns. Appl Microbiol Biotechnol. 2016;100: 1843–1852. doi:10.1007/s00253-015-7095-y

108. Carini P, Dupont CL, Santoro AE. Patterns of thaumarchaeal gene expression in culture and diverse marine environments. Environ Microbiol. 2018; doi:10.1111/1462-2920.14107

109. Lycus P, Lovise Bøthun K, Bergaust L, Peele Shapleigh J, Reier Bakken L, Frostegård Å. Phenotypic and genotypic richness of denitrifiers revealed by a novel isolation strategy. ISME J. 2017;11: 2219–2232. doi:10.1038/ismej.2017.82

110. Desai JS, Sartor RC, Lawas LM, Jagadish SVK, Doherty CJ. Improving Gene Regulatory Network Inference by Incorporating Rates of Transcriptional Changes. Sci Rep. 2017;7: 17244. doi:10.1038/s41598-017-17143-1

111. Chan TE, Stumpf MPH, Babtie AC. Gene Regulatory Network Inference from Single-Cell Data Using Multivariate Information Measures. Cell Syst. 2017;5: 251–267.e3. doi:10.1016/j.cels.2017.08.014

112. von Dassow G, Meir E, Munro EM, Odell GM. The segment polarity network is a robust developmental module. Nature. 2000;406: 188–192. doi:10.1038/35018085

113. Liu F, Zhang S-W, Guo W-F, Wei Z-G, Chen L. Inference of Gene Regulatory Network Based on Local Bayesian Networks. PLoS Comput Biol. 2016;12: e1005024. doi:10.1371/journal.pcbi.1005024

114. Yoon SH, Turkarslan S, Reiss DJ, Pan M, Burn JA, Costa KC, et al. A systems level predictive model for global gene regulation of methanogenesis in a hydrogenotrophic methanogen. Genome Res. 2013;23: 1839–1851. doi:10.1101/gr.153916.112

115. Ashworth J, Coesel S, Lee A, Armbrust EV, Orellana MV, Baliga NS. Genome-wide diel growth state transitions in the diatom Thalassiosira pseudonana. Proc Natl Acad Sci U S A. 2013;110: 7518–7523. doi:10.1073/pnas.1300962110

116. Bonneau R, Facciotti MT, Reiss DJ, Schmid AK, Pan M, Kaur A, et al. A predictive model for transcriptional control of physiology in a free living cell. Cell. 2007;131: 1354–1365. doi:10.1016/j.cell.2007.10.053

117. Peterson EJR, Reiss DJ, Turkarslan S, Minch KJ, Rustad T, Plaisier CL, et al. A high-resolution network model for global gene regulation in *Mycobacterium tuberculosis*. Nucleic Acids Res. 2014;42: 11291–11303. doi:10.1093/nar/gku777

118. López García de Lomana A, Schäuble S, Valenzuela J, Imam S, Carter W, Bilgin DD, et al. Transcriptional program for nitrogen starvation-induced lipid accumulation in *Chlamydomonas reinhardtii*. Biotechnol Biofuels. 2015;8: 207. doi:10.1186/s13068-015-0391-z

119. Brooks AN, Reiss DJ, Allard A, Wu W-J, Salvanha DM, Plaisier CL, et al. A system-level model for the microbial regulatory genome. Mol Syst Biol. 2014;10: 740. doi:10.15252/msb.20145160

120. Reiss DJ, Baliga NS, Bonneau R. Integrated biclustering of heterogeneous genome-wide datasets for the inference of global regulatory networks. BMC Bioinformatics. 2006;7: 280. doi:10.1186/1471-2105-7-280

121. Reiss DJ, Plaisier CL, Wu W-J, Baliga NS. cMonkey2: Automated, systematic, integrated detection of co-regulated gene modules for any organism. Nucleic Acids Res. 2015;43: e87. doi:10.1093/nar/gkv300

122. Bonneau R, Reiss DJ, Shannon P, Facciotti M, Hood L, Baliga NS, et al. The Inferelator: an algorithm for learning parsimonious regulatory networks from systems-biology data sets de novo. Genome Biol. 2006;7: R36. doi:10.1186/gb-2006-7-5-r36

123. Chandrasekaran S, Price ND. Probabilistic integrative modeling of genome-scale metabolic and regulatory networks in Escherichia coli and Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2010;107: 17845–17850. doi:10.1073/pnas.1005139107

124. Wang Z, Danziger SA, Heavner BD, Ma S, Smith JJ, Li S, et al. Combining inferred regulatory and reconstructed metabolic networks enhances phenotype prediction in yeast. PLoS Comput Biol. Public Library of Science; 2017;13: e1005489. doi:10.1371/journal.pcbi.1005489

125. Stolyar S, Van Dien S, Hillesland KL, Pinel N, Lie TJ, Leigh JA, et al. Metabolic modeling of a mutualistic microbial community. Mol Syst Biol. 2007;3: 92. doi:10.1038/msb4100131

126. Mee MT, Collins JJ, Church GM, Wang HH. Syntrophic exchange in synthetic microbial communities. Proc Natl Acad Sci U S A. 2014;111: E2149–56. doi:10.1073/pnas.1405641111

127. Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol. 2018; doi:10.1038/s41579-018-0024-1

128. Grosskopf T, Soyer OS. Synthetic microbial communities. Curr Opin Microbiol. 2014;18: 72–77. doi:10.1016/j.mib.2014.02.002

129. Turkarslan S, Raman AV, Thompson AW, Arens CE, Gillespie MA, von Netzer F, et al. Mechanism for microbial population collapse in a fluctuating resource environment. Mol Syst Biol. 2017;13: 919. doi:10.15252/msb.20167058

130. Thompson AW, Turkarslan S, Arens CE, López García de Lomana A, Raman AV, Stahl DA, et al. Robustness of a model microbial community emerges from population structure among single cells of a clonal population. Environ Microbiol. 2017;19: 3059–3069. doi:10.1111/1462-2920.13764

131. Valenzuela JJ, López García de Lomana A, Lee A, Armbrust EV, Orellana MV, Baliga NS. Ocean acidification conditions increase resilience of marine diatoms. Nat Commun. 2018;9: 2328. doi:10.1038/s41467-018-04742-3

132. López García de Lomana A, Kaur A, Turkarslan S, Beer KD, Mast FD, Smith JJ, et al. Adaptive Prediction Emerges Over Short Evolutionary Time Scales. Genome Biol Evol. 2017;9: 1616–1623. doi:10.1093/gbe/evx116

133. Bakken LR, Frostegård Å. Sources and sinks for N2 O, can microbiologist help to mitigate N2 O emissions? Environ Microbiol. Environmental Microbiology; 2017;19: 4801–4805. Available: https://www.researchgate.net/profile/Lars_Bakken/publication/322008554_Sources_and_sinks_for_N2_O_can_microbiologist_help_to_mitigate_N2_O_emissions/links/5a44d3c7458515f6b0531765/Sources-and-sinks-for-N2-O-can-microbiologist-help-to-mitigate-N2-O-emissions

134. Frias-Lopez J, Thompson A, Waldbauer J, Chisholm SW. Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. Environ Microbiol. Wiley Online Library; 2009;11: 512–525. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1462-2920.2008.01793.x

135. Skennerton CT, Chourey K, Iyer R, Hettich RL, Tyson GW, Orphan VJ. Methane-Fueled Syntrophy through Extracellular Electron Transfer: Uncovering the Genomic Traits Conserved within Diverse Bacterial Partners of Anaerobic Methanotrophic Archaea. MBio. 2017;8. doi:10.1128/mBio.00530-17

136. Glass JB, Chen S, Dawson KS, Horton DR, Vogt S, Ingall ED, et al. Trace Metal Imaging of Sulfate-Reducing Bacteria and Methanogenic Archaea at Single-Cell Resolution by Synchrotron X-

Ray Fluorescence Imaging. Geomicrobiol J. Taylor & Francis; 2018;35: 81–89. doi:10.1080/01490451.2017.1321068

137. Oloo F, Valverde A, Quiroga MV, Vikram S, Cowan D, Mataloni G. Habitat heterogeneity and connectivity shape microbial communities in South American peatlands. Sci Rep. 2016;6: 25712. doi:10.1038/srep25712

138. Caron DA, Connell PE, Schaffner RA, Schnetzer A, Fuhrman JA, Countway PD, et al. Planktonic food web structure at a coastal time-series site: I. Partitioning of microbial abundances and carbon biomass. Deep Sea Res Part I. 2017;121: 14–29. doi:10.1016/j.dsr.2016.12.013

139. Needham DM, Sachdeva R, Fuhrman JA. Ecological dynamics and co-occurrence among marine phytoplankton, bacteria and myoviruses shows microdiversity matters. ISME J. 2017;11: 1614–1629. doi:10.1038/ismej.2017.29

140. Parada AE, Fuhrman JA. Marine archaeal dynamics and interactions with the microbial community over 5 years from surface to seafloor. ISME J. 2017;11: 2510–2525. doi:10.1038/ismej.2017.104