Feeding the World in the 21st Century: Grand Challenges in the Nitrogen Cycle

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The nitrogen (N) cycle is one of the most important biogeochemical cycles on Earth, as nitrogen is an essential nutrient for all known life forms. Natural processes, driven mostly by microbes in association with leguminous plants, fix and deliver around 120 megatons per year of bioavailable nitrogen to the biosphere. Humans have greatly augmented these processes, mostly through the industrial Haber-Bosch process and planting of leguminous crops, contributing at least the same amount. It is estimated that around 40% of the human population depends on the human contribution to the nitrogen cycle.

The purpose of this workshop, held November 9–10, 2015 at the National Science Foundation headquarters in Arlington, Virginia, was to identify ways that chemists can contribute to understanding and improving the nitrogen cycle in the context of agriculture and environmental management. Participants noted two major imbalances in the nitrogen cycle: insufficient bioavailable nitrogen in soils in the developing world, and too much inorganic nitrogen in the developed world.

Lack of bioavailable nitrogen in the developing world leads to low crop yields and diets with insufficient protein. Challenges involve finding ways to increase nitrogen availability and/or utilization in soils, and solutions may involve developing new crops or other technologies that can fix nitrogen *in situ*, since transporting nitrogen fertilizer is often costly and inefficient.

In the developed world by contrast, nitrogen fertilizer is cheap enough that farmers routinely add enough to fields to ensure that it is never a limiting nutrient. On average only 30% to 50% of this nitrogen is taken up by crop plants, and much of the rest is lost from soil, leading to multiple problems, including runoff into waterways with downstream eutrophication and release of nitrous oxide, a potent greenhouse gas. Challenges involve reducing these environmental impacts by better matching fertilizer applications to crop needs, developing methods to remove nitrates from soil to limit runoff, and inhibiting the production of nitrous oxide.

On the first day, workshop participants were divided into four groups based on different, though interrelated, processes (topic areas) within the nitrogen cycle: nitrogen fixation, nitrification, denitrification, and nitrogen assimilation. Participants were encouraged to identify big questions and brainstorm blue-sky research approaches without regard to resource or technological constraints.

On the second day, workshop participants regrouped in interdisciplinary teams focused around specific questions:

- How does N_2 reduction to NH_3 (ammonia) work at an atomic level in nitrogenase and in homogeneous and heterogeneous catalysts?

- How can biological nitrogen fixation systems be mobilized into a range of organisms?

- How can we effectively manage nitrification at an ecosystem level?

- How can we maximize nitrate reduction while simultaneously limiting nitrous oxide release?

- What are the chemical features of nitrogen signals that regulate changes in nitrogen assimilation in plants?

- How can we promote greater nitrogen use efficiency, and how is this influenced by changing environmental factors?

Within each of the four topic areas, participants identified big questions, knowledge gaps, expertise required, experimental approaches, technology needed to make progress, and specific desired outcomes. The rest of this document will summarize this discussion.

Nitrogen Fixation

The reduction of nitrogen (N_2) from the atmosphere to ammonia (NH_3) is called "nitrogen fixation" and represents the single largest input of fixed nitrogen into the global biogeochemical nitrogen cycle. Currently, two processes, roughly equal in magnitude, account for the majority of nitrogen fixation. These are biological nitrogen fixation in diazotrophic bacteria catalyzed by the enzyme nitrogenase and the industrial Haber-Bosch process. These two processes use very different conditions and mechanisms to achieve N_2 reduction. Gaining a molecular-level understanding of each process is a key foundation of research going forward.

Question 1: How does N_2 reduction to NH_3 (ammonia) work at an atomic level in nitrogenase and in homogeneous and heterogeneous catalysts?

Knowledge gaps
Foundational knowledge of how to activate and reduce N_2 by nitrogenase, homogeneous catalysts, and heterogeneous catalysts is essential, both to achieve this chemistry under milder conditions using less fossil fuel than is currently achievable, and to building distributed systems that allow for decentralized ammonia production from N_2 (e.g., in developing countries where transportation can be difficult or impossible). Significant knowledge gaps remain in all of these areas, and coordinated research is required to fill these gaps.

Expertise
The key to success will be organizing teams of chemists, biochemists, and engineers to work on common challenges. Organized research teams operated in the 1980s at the Nitrogen Fixation Laboratory (Sussex, UK) and the Kettering Laboratory (Yellow Springs, Ohio) and made significant strides in our understanding. There will once again be great value in organizing cross-disciplinary teams to move the field forward.

Question 2: How can biological nitrogen fixation systems be mobilized into a range of organisms?

Knowledge gaps
The complexities of nitrogen fixation must be elucidated to allow the targeted deployment of this process into systems of interest (e.g., plants). Tremendous knowledge gaps remain in many core areas, including understanding how to mobilize all genes needed for nitrogen fixation into new prokaryotes and eukaryotes, and how to direct energy flow toward the introduced nitrogen fixation system in the modified organisms. Furthermore, it is essential to explore a wide variety of natural systems to better understand the range of approaches used by biology. A better understanding of all of the factors needed to achieve nitrogen fixation in both prokaryotes and eukaryotes is also essential.

Expertise
Best progress will be made by assembling a team that includes biologists, chemists, biochemists, systems biologists, synthetic biologists, and ecologists. Building teams with knowledge across nitrogen fixation and eukaryotic genetics will be valuable.

Nitrification

Question: How can we effectively manage nitrification at an ecosystem level?

Nitrification, the oxidation of ammonium to nitrate, is the main process that connects chemically reactive nitrogen to other processes of the nitrogen cycle. Specifically, nitrification provides nitrate (and nitrite) to denitrifying bacteria, and determines the relative availability of ammonium and nitrate for assimilation into plants. In addition, leakage of nitrogen oxide intermediates NO and N_2O during nitrification is a major source of these deleterious gases to the environment.

Microbial nitrification is also the main process that competes with plants for applied anhydrous ammonium fertilizer. Rapid conversion of ammonium to nitrate by nitrification causes coastal oxygen minimum zones ("dead zones"), eutrophication of freshwater, and accumulation of greenhouse gases in the atmosphere via off-gassing of nitric and nitrous oxide.

To effectively manage nitrification, participants proposed a two-pronged approach: 1) discover and develop comprehensive, specific, environmentally benign, and cost-effective nitrification inhibitors using directed approaches; and 2) develop large-scale, predictive ecosystem nitrogen flux models to monitor plant and soil nitrogen status for data-driven application of fertilizer/inhibitor formulations. These flux models will be useful for all areas of the nitrogen cycle, including nitrification, denitrification, nitrogen fixation, and assimilation.

The success of this approach requires an interdisciplinary combination of expertise and methodologies, and also relies on engagement with policy makers and industry.

Knowledge gaps
Although the first ammonia- and nitrite-oxidizing bacteria were isolated 125 years ago, the enzymology and physiology of these chemolithotrophic microbes (microbes able to use inorganic reduced compounds as energy sources) remain largely mysterious. One of the principal

enzymes involved in ammonia oxidation, ammonia monooxygenase, has defied purification, preventing an understanding of the catalytic mechanism and active site structure.

In addition, the discovery of ammonia-oxidizing archaea greatly expanded the realm of possible enzymology involved in the ammonia oxidation process. Because of this enzymatic and organismal diversity, a comprehensive and specific nitrification inhibitor is not yet known.

Nitrapyrin is the main inhibitor applied to agricultural soils in the U.S. today, and has been in wide use since 1974. Even so, the mechanism of inhibition by nitrapyrin is not defined and is not even known to be specific to ammonia oxidizers; it might target nitrite oxidizers, methane oxidizers (as they express a homologous monooxygenase), or multiple groups of microorganisms.

There are a tremendous number of open questions in nitrification. One area of need is to better understand the enzymes involved. We have identified the need for investigation into structure-function correlation and molecular mechanism of key enzymes involved in nitrification, interactions and integration between enzymes in nitrification pathways, specific mechanisms and targets of nitrification inhibitors in nitrification enzymes, and the diversity of enzymology in ammonia- and nitrite-oxidation pathways.

Also needed is a better understanding of the organismal diversity involved in nitrification. Specifically, study is needed of the diversity of nitrification processes in an organismal and ecosystem context, the genomic diversity of nitrifying microorganisms, signaling mechanisms between nitrifying microorganisms and other microbes and plants, interactions between nitrifiers and plants, natural nitrification inhibitors in plants and their biosynthesis pathways, and the influence of changing environments on communities and activities of nitrifying organisms.

At a basic chemistry level, research is needed into the molecular mechanisms of the involved enzymes, and also into mechanisms that facilitate coupling and uncoupling of ammonia to nitrite oxidation as well as factors that govern the leakage of intermediate molecules during nitrification. In the environmental chemistry realm, we find a need for research into the fate of nitrification inhibitors in the environment, and an understanding of how global change and local changes influence field parameters that influence application of fertilizer and inhibitors.

With regard to technology, we recommend the development of robust, large-scale sensor networks for assessing nitrogen and nutrient status of soils and plants.

Expertise

Progress will require collaboration between chemists with a variety of subspecialties as well as experts from outside chemistry. Within chemistry, expertise is required in biochemistry, bioinorganic and synthetic chemistry, analytical chemistry and engineering, computational chemistry, and biogeochemistry. Additional expertise is needed from plant biology and plant breeding, chemical and microbial ecology, microbial physiology, molecular genetics, and biophysics.

Denitrification

Denitrification, the process by which microbes use nitrogen oxide compounds as terminal electron acceptors under anaerobic conditions (e.g., in the reduction of NO_3^- to N_2), is a critical aspect of the global nitrogen cycle. The final step in the denitrification pathway is the reduction of N_2O to N_2. Certain organisms lack the enzyme required for this final step, and in other instances N_2O escapes prior to reduction. Approximately 75% of anthropogenic N_2O results from microbial activity in agricultural soil.

Problem: Both NO_3^- and N_2O cause serious environmental problems, including drinking water pollution, water eutrophication, ozone depletion, and global warming.

Question: How can we maximize nitrate reduction while simultaneously limiting nitrous oxide release?

Knowledge gaps
To achieve this goal we will need to have better understandings of the denitrification and dissimilatory nitrate reduction to ammonium (DNRA) pathways, especially with respect to microbial diversity, cellular physiology, pathway regulation, enzyme coupling, enzyme maturation, and enzyme mechanism.

Expertise
Progress will require collaboration between chemists with a variety of subspecialties as well as experts from other fields. Within chemistry, expertise is required in biochemistry, bioinorganic chemistry, synthetic chemistry, bioanalytical chemistry, and isotopic biogeochemistry. Additional expertise is needed from molecular biology, enzymology, microbial ecology, microbial physiology, biophysics, crystallography, and structural biology.

Nitrogen assimilation

Nitrogen assimilation involves the biochemical linkage of both oxidized (nitrate) or reduced (ammonia) nitrogen forms to carbon-containing compounds, forming the organic nitrogen sources essential for life and biomass accumulation at all ecosystem levels. These include amino acids, nucleic acids, proteins, chlorophyll, and a wide diversity of secondary metabolites. The nitrogen assimilation pathway is a major consumer of biochemical energy, and thus a key balance point in managing biological tradeoffs within and between organisms that drive the nitrogen cycle. Better answers to three important questions were considered essential to improving the outputs of nitrogen assimilation.

Question 1: What are the biochemical mechanisms for enzymes that catalyze nitrogen reduction, nitrosylation, and amination?

Knowledge gaps

The initial reduction of nitrate in plants shares mechanistic features with microbial denitrification, described above, so advances in understanding and modulation of denitrification are also relevant to nitrogen assimilation. Scientists now recognize that nitrite is a key intermediate for interconversion among nitrogen forms, with similarities to molecules involved in the oxidation and reduction of carbon and sulfur. However, in plants, nitrate reduction to nitrite and then ammonia operates in chemical contexts distinct from those of soil microbes, and is coordinated between different organelles. Furthermore, plants can alter reaction equilibria via subcellular localization or export and input of substrates, products, or cofactors. The mechanisms of electron transfer associated with these nitrogen interconversions under variable biological conditions are not fully understood.

N assimilation in plants is also limited by transport between organelles, between tissues, and across membrane systems at the rhizosphere interface. The biochemical basis for selectivity and flux of membrane proteins that mediate transport of different nitrogen forms is poorly understood. Similarly, detailed knowledge is lacking for many of the reaction mechanisms that catalyze the breakage and reformation of nitrogen-containing bonds in organic nitrogen compounds with key roles in nitrogen transport, utilization, and remobilization (e.g., amino acids and ureides in nitrogen-fixing legumes).

Expertise
Progress will require collaboration between synthetic, bioanalytical, and bioinorganic chemists as well as structural biologists, biochemists, and enzymologists, particularly those who study oxotransfer reactions by metalloenzymes.

Question 2: What are the chemical features of nitrogen signals that regulate changes in nitrogen assimilation?

Knowledge gaps
All living organisms possess nitrogen sensing and response systems, which are reasonably well understood in model microbes. Importantly, in multicellular plants, nitrogen compounds are both chemical metabolites and signaling molecules that promote a number of genetically programmed response modules. The biochemical details for some aspects of nitrogen signaling pathways have recently been elucidated. This includes the identification of the first transceptor protein that functions to both transport nitrogen and induce responses to sensed nitrogen, an emerging role of small signaling peptides, and key hubs within transcriptional regulatory networks.

However, many important questions remain. Among the many inorganic and organic nitrogen forms with signaling roles, which are the most potent in programming desired responses? How does sensitivity to signals and response outputs vary during plant development? How does the exchange of signals between plants and symbiotic nitrogen-fixing microbes balance the conflict between mutualism and fitness, limit partner choice, and impose sanctions on the allocation of plant carbon to poor performing nodules? At the level of agronomic productivity, how much of plant biomass response to nitrogen is due to nitrogen signaling and acquisition versus bottlenecks in nitrogen metabolism, assimilation, and remobilization (e.g., to seeds)?

Expertise
Understanding the chemical features of nitrogen signaling in multicellular plants will benefit greatly from enhanced collaboration of interdisciplinary teams of chemists, microbial and plant biologists, agricultural scientists and engineers, and quantitative systems scientists.

Question 3: How can we promote environmentally resilient nitrogen use efficiency (NUE), especially in the light of changing environmental factors?

Knowledge gaps
Promoting environmentally resilient nitrogen use efficiency is an important goal for future food security. Plant NUE is naturally low—less than half of applied nitrogen is taken up by crops—and the chemical drivers for nitrogen availability and assimilation are subject to a variety of changing environmental factors. Climate change will have very different impacts on agricultural systems where nitrogen is typically adequate or in excess compared to those where nitrogen is limiting, and the interconnection between nitrogen and water availability will intensify. Although environmental conditions may modulate the strength and amplitude of nitrogen signals or nitrogen-response modules, the biochemical targets and underlying mechanisms are poorly characterized.

Rising levels of greenhouse gases are predicted to increase temperatures; change the timing, frequency, and intensity of seasonal rainfall (causing both droughts and flooding); and alter soil chemistry. In addition to these abiotic factors, rising CO_2 has been shown to inhibit plant nitrate assimilation and constrain nitrogen cycling, shift the distribution of species within ecosystems, and interfere with carbon sequestration into biomass. Indeed, information is sparse as to how the environmental conditions anticipated in the near future will alter what nitrogen forms organisms assimilate.

Rising atmospheric CO_2 concentrations often inhibit the biochemical reactions for rhizobial nitrogen fixation and nitrate assimilation, and these may shift the dependence of legumes on nitrate, ammonia, or organic nitrogen forms. Uptake systems for organic nitrogen become more important in cooler soils or in soil with low organic matter. It is evident that plant nitrogen signaling systems affect root architecture and hence a plant's capacities for drought tolerance and nutrient acquisition, but empirical data on the mechanistic interactions among these stresses are sparse.

Expertise
This effort will require interdisciplinary teams of chemists, microbial and plant biologists, agricultural scientists and engineers, and quantitative systems scientists. Also needed will be modelers of climatic, ecological, and crop productivity to integrate new "big data" and better estimate impacts of various nitrogen sources to net primary productivity in various locations.