U.S. Department of Energy
Advanced Research Projects Agency – Energy (ARPA-E)

Request for Information
DE-FOA-0003199
on
Biological Approaches for Developing a New Nitrogen Cycle in Agriculture for Bioenergy Crops

Introduction:

The purpose of this Request for Information (RFI) is to solicit input for a potential ARPA-E program focused on the development of technologies to reduce nitrogen inputs and soil-based greenhouse gas emissions of nitrous oxide (N_2O) from the cultivation of bioenergy crops (i.e., corn, soybean, and sorghum). The goals for this programmatic concept include the evaluation of technologies capable of: 1) providing high-efficacy, low energy-intensive alternatives to synthetic fertilizer in agricultural production by advancing microbial fertilization approaches, enhancing plant nitrogen (N) use efficiency-related traits, and developing enabling tools for autogenic N_2 fixation; and 2) reducing soil-based N_2O emissions in growing these crops by altering the microbial N-cycle either through microbial and/or plant approaches.

ARPA-E seeks input from molecular biologists, biochemists, microbiologists, bioengineers, soil scientists, crop breeders, crop geneticists, plant scientists, and others with potentially relevant expertise. Additionally, ARPA-E is seeking input from prospective end-users of such technologies, including corn/soybean/sorghum growers, seed producers, farm monitoring and equipment companies, agronomists, biofuel stakeholders, and nitrogen fertilizer supply chains. This RFI is focused on soliciting input about biological approaches, rather than management-based approaches, to reduce N-inputs and soil-based emissions of N_2O. The questions towards the end of this document are intended to assist relevant stakeholders in providing input on:

1. Biological approaches to reducing synthetic N fertilizer and mitigating N_2O emissions, including approaches to genetic modifications of microbes and crops as well as methods to predict and control the activities of soil and root microbiomes.
2. Success metrics to quantify the impact of alternative technological approaches compared to synthetic nitrogen supply or mitigating N_2O emissions from the cultivation of bioenergy crops.
3. Approaches to evaluating technical and technology-to-market feasibility of these strategies.
4. Economic factors for large-scale adoption of these new technologies.

Areas Not of Interest for Responses to this RFI:

- Work focused on basic research aimed purely at discovery and fundamental knowledge generation.
- Efforts to use microbial fertilization and alter plant nitrogen use efficiency to improve crop yields at current applied N-levels.
- Land management or agronomic practices that can reduce N-inputs and field greenhouse gas emissions.

RFI Guidelines:

CAREFULLY REVIEW ALL RFI GUIDELINES BELOW.

Note that the information you provide will be used by ARPA-E solely for program planning, without attribution. **THIS IS A REQUEST FOR INFORMATION ONLY. THIS NOTICE DOES NOT CONSTITUTE A FUNDING OPPORTUNITY ANNOUNCEMENT (FOA). NO FOA EXISTS AT THIS TIME.**

The purpose of this RFI is solely to solicit input for ARPA-E consideration to inform the possible formulation of future research programs. ARPA-E will not provide funding or compensation for any information submitted in response to this RFI, and ARPA-E may use information submitted to this RFI without any attribution to the source. This RFI provides the broad research community with an opportunity to contribute views and opinions.

No material submitted for review will be returned and there will be no formal or informal debriefing concerning the review of any submitted material. ARPA-E may contact respondents to request clarification or seek additional information relevant to this RFI. All responses provided will be considered, but ARPA-E will not respond to individual submissions or publish publicly a compendium of responses. **Respondents shall not include any information in the response to this RFI that could be considered proprietary or confidential**.

Responses to this RFI should be submitted in PDF format to the email address **ARPA-E-RFI@hq.doe.gov** by **5:00 PM Eastern Time on Monday, November 27.** Emails should conform to the following guidelines:

- Insert "Response to A New Nitrogen Cycle - <your organization name>" in the email subject line.
- In the body of your email, include your name, title, organization, type of organization (e.g., university, non-governmental organization, small business, large business, federally funded research and development center [FFRDC], government-owned/government-operated [GOGO]), email address, telephone number, and area of expertise.
- Responses to this RFI are limited to no more than 10 pages in length (12-point font size).
- Responders are strongly encouraged to include preliminary results, data, and figures that describe their potential materials, designs, or processes.

Technical Background:

To achieve high yields for bioenergy crops, growers use N-containing fertilizers in excess of crop N use efficiency due to the environmental loss of N. This practice has pushed the N-cycle out of balance such that the rate of N-inputs into a soil does not equal the amount of complete de-nitrification as it exists in nature (Figure 1). This application causes numerous environmental effects including the evolution of potent greenhouse gases such as N_2O. To reduce N demand and field-based N_2O emissions associated with bioenergy production, ARPA-E is seeking information on developing biological solutions to the cost, energy, and emissions challenges associated with current agricultural N-cycle. ARPA-E is interested in technologies that can be applied to crops currently used for biofuel production without negatively impacting the yield of soybean, corn, and sorghum. Though soybean does not require high N-inputs, the emissions of N_2O are higher than corn and sorghum (Table 1). To reduce the N demand and emissions of N_2O from bioenergy crop production, ARPA-E is requesting information to evaluate biological approaches to meet the goals of this programmatic concept.

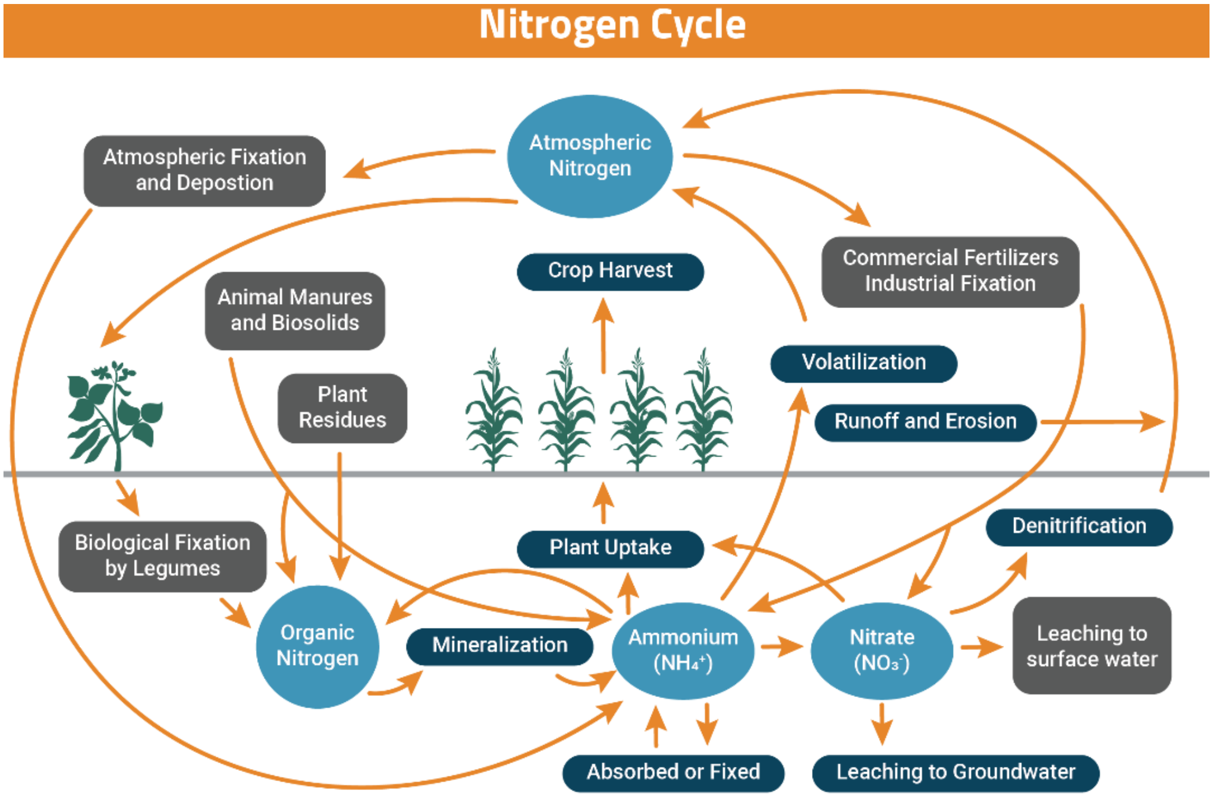

Figure 1. The N-cycle for agriculture, which includes natural and anthropogenic inputs of fixed-N. The production of N_2O occurs during NH_4^+ nitrification and NO_3^- denitrification. Adapted from the Minnesota Department of Agriculture.[1]

The questions posed in the following section are classified into the technical categories of interest. Illustrate the impact of the technology using metric units, rather than United States (U.S.) customary units, if possible. Information on metric units is available on the National Institute of Standards and Technology website.

Table 1. Synthetic N fertilizer application and N_2O emissions of bioenergy crops.[2]

Crop	Acreage grown in U.S. (Thousands of km^2)	Synthetic nitrogen application (tons/km^2/y)	N_2O emissions (g eCO_2/kg of biomass)
Corn	380.4	16.1	120
Soybean	323.7	0.6	195
Sorghum	24.2	7.4	140

[1] Minnesota Department of Agriculture. (2023, August 20). Nitrogen Cycle. https://www.mda.state.mn.us/chemicals/fertilizers/nutrient-mgmt/nitrogenplan/nitrogenmgmt.
[2] Wang, M. et al. (2022, October 10). Greenhouse gases, Regulated Emissions, and Energy use in Technologies Model®. Computer Software. U.S. DOE Office of Energy Efficiency and Renewable Energy (EERE).

RFI Questions:

The questions posed in this section are classified into several different groups as appropriate. Provide responses and information about any of the following. **ARPA-E does not expect any one respondent to answer all, or even many, of the prompts in this RFI.** In your response, indicate the group and question number in your response. Appropriate citations are highly encouraged. Respondents are also welcome to address other relevant avenues or technologies that are not outlined below, except for those that fall under the "Areas Not of Interest" described above.

I. Biological Approaches Enabling N-Inputs

ARPA-E anticipates that reducing the utilization of synthetic N fertilizer will require addressing both plant and microbial N uptake and metabolism and the plant-microbe interactions that drive the agricultural N-cycle. Strategies have been developed to isolate and engineer diazotrophic (N_2-fixing) rhizosphere bacteria to deliver fixed-N to corn plant roots in the presence of high levels of N fertilizer application.[3] ARPA-E is seeking information on developing engineered isolates that function under low- or no- applied N and show enhanced N uptake by crops while sustaining yields. Approaches that aim to solve the challenges associated with the fitness, persistence, and on-seed application of engineered strains are of particular interest.[3] A complementary approach to improving microbial N delivery to plants is to harness native microbiomes that develop on or within plant roots.[4] ARPA-E is seeking information on methods that would allow for the determination of community dynamics of native nitrogen fixing microbiomes, along with the enhancement of nitrogen fixation either through the integration of engineered strains or delivering designed consortia of microbiomes to improve nitrogen fixation.

For plants, methods to improve architectural or physiological traits related to N uptake capacity (NuPC), uptake efficiency (NuPE), and use efficiency (NUE) as defined by Ciampitti and Lemaire (2022) are of interest.[5] Another area of interest for ARPA-E is to enhance plant-mediated nitrification inhibition to reduce N_2O emissions.[6] Enhancing or engineering plant microbe interactions with diazotrophic bacteria—both native species found in root microbiomes and engineered isolates introduced into the rhizosphere—are of interest. ARPA-E is also interested in approaches to express the microbial nitrogenase system in plants to generate self-fertilizing plants, and in approaches to develop nodulation in corn and sorghum that mimics leguminous plants.[7]

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[3] Bloch, S.E. et al. (2020). Biological nitrogen fixation in maize: optimizing nitrogenase expression in a root-associated diazotroph, *Journal of Experimental Botany*, 71(15): 4591–4603.
[4] Van Deynze, A. et al. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PloS Biol* 16(8): e2006352.
[5] Ciampitti, I.A. and Lemaire, G. (2022). From use efficiency to effective use of nitrogen: A dilemma for maize breeding improvement. *Science of the Total Environment* 826:154125.
[6] Saud, S., Wang, D., and Fahad, S. (2022). Improved nitrogen use efficiency and greenhouse gas emissions in agricultural soils as producers of biological nitrification inhibitors. *Front. Plant Sci*. 13:854195.
[7] Buren, S., Lopez-Torrejon, G., and Rubio, L.M. (2018). Extreme bioengineering to meet the nitrogen challenge. *PNAS* 11(36): 8849-8851.

I.A. Microorganisms

I.A.1. Engineering rhizosphere diazotrophs and other root microbes for N-fixation

a. What is the current state-of-the-art (SoA) for engineering rhizosphere diazotrophs for N_2 fixation in biofuel crops and other crop species? Which parameters (e.g., N-fixation rate, total N delivery, percent increase in yield, reduction in fertilizer usage) are the most critical to define the SoA? How much improvement can be further achieved?
b. Beside the SoA, which rhizosphere diazotrophs can also be candidates for N_2 fixation in biofuel crops? What tools/approaches can be used to isolate and engineer these promising species? What are the anticipated technical challenges and risks involved?
c. How can engineered diazotrophs be introduced and established on biofuel crops? Which parameters and metrics are critical to define successful introduction and establishment of the engineered isolates?
d. To maintain the current crop yield while significantly reducing synthetic N application, what rates and total N delivery/crop or per unit land are required? Have those rates been achieved in the lab or small-scale field trials? What are the technical barriers for transferring these applications to the field?
e. How can we increase the stability and persistence of engineered microbial isolates on crops, and maintain their benefits over 2-5 seasons? What methods can be used to measure the stability and persistence of these isolates?
f. How can we monitor and quantify the effects of engineered microbes in active farming conditions?

I.A.2. Engineering microbiomes and synthetic microbial consortia

a. What are the methods to promote the growth of native diazotrophic microbiomes on plant roots? What methods can identify active diazotrophs in these microbiomes and measure their N_2 fixation and N export activities?
b. How do community dynamics influence the ability of root-associated microbiomes to deliver fixed-N to plant roots?
c. Can engineered isolates be introduced that augment the ability of native microbiomes to deliver fixed-N to plant roots? If yes, explain.
d. Can the native microbiomes be replaced by synthetic microbiomes with defined isolates or transplanted native microbiomes that display high N_2 fixation activity? If yes, explain.

I.A.3. Symbiotic plant-microbe interactions

a. What specific interactions can be engineered between plant roots and diazotrophic bacteria?
b. Can plants roots be programmed to identify a specific species or strain and exclude interactions with other competing bacteria? If yes, explain.
c. How can nodule-like structures be engineered into cereal crops such as corn and sorghum?
d. Can a physiochemical model of the root-microbe interface be constructed to predict the specific interactions?

I.B. Plants

I.B.1. Improving NUE traits through genetic engineering or crop breeding

a. Will increasing the NUE, NuPC, or NuPE in plants reduce nitric oxide (NO) and N_2O emissions from a field? If so, how much N_2O can be avoided if corn or sorghum were to be bred for maximum nitrogen uptake efficiency? Explain your answer.
b. What is the SoA for NUE, NuPC, and NuPE regarding corn, soybean, and sorghum? What are reasonable genetic targets for improving NUE of these crops based on current SoA?
c. How much nitrogen fertilizer can be reduced if crops are functioning at the highest NUE, NuPC, or NuPE?
d. What traits or mechanisms would be relevant to consider that can contribute to increasing NUE, NuPC, or NuPE in crops? Is there an overlap in the genes or root architecture that contribute to these traits?
e. What approaches will accelerate the identification of new genes, pathways, or architecture that contribute to NUE, NupC, and NuPE in crops?
f. Of the traits NUE, NuPC, and NuPE, which of these traits could more greatly reduce the formation of N_2O?
g. Can we use genome-wide association studies to identify genes responsible for improving NuPC, NuPE, and NuE? Describe any relevant limitations or technology development that may be needed.
h. Are there metabolic models that account for NuPC, NuPE, and NuE that can predict N uptake rates and distribution of N in the plant?

I.B.2. Engineering of autogenic nitrogen fixation

a. What is the current SoA for engineering autogenic N_2 fixation in corn, sorghum, and other non-legume crops?
b. What are the anticipated technical challenges for engineering autogenic N_2 fixation in corn or sorghum? What tools are needed to address these challenges?
c. If autogenic N_2 fixation were feasible, how long would it take to successfully engineer autogenic nitrogen fixation in corn or sorghum? What percentage of N can be replaced with this approach? What will be the effects on biomass yield if autogenic N_2 fixation is achieved in corn or sorghum?

II. Biological Approaches Enabling Significant Reduction in N_2O Emissions

ARPA-E is interested in biotechnological solutions to mitigate N_2O emissions from corn, soybean, and sorghum cultivation. Microbial mitigation of N_2O emissions may include microbiome engineering strategies to suppress nitrification of NH_4^+ in soils and promote N_2O-reducing bacteria in the soybean nodules or the soil.[8] Isolates with the capability for N_2O reduction, either present natively or engineered through synthetic biology, may be introduced into soybean nodules or the rhizosphere to prevent N_2O build-up.[9]

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[8] Hu, H.W., He, J.Z., and Singh, B.K. (2017). Harnessing microbiome-based biotechnologies for sustainable mitigation of nitrous oxide emissions. *Microbial Biotechnology*, *10*(5), 1226-1231.
[9] Itakura, M. et al. (2013). Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. *Nature Climate Change* 3.3: 208-212.

For plants, improved NUE will aid in mitigating nitrification of NH_4^+ in the soil. Additionally, the plant production of biological nitrification inhibitors, already demonstrated for sorghum, may lower N_2O emissions.[10]

ARPA-E is interested in information about gene editing technologies that will select for both plant and microbial phenotypes favorable for growing in low applied N and mitigating N_2O emissions. ARPA-E is also interested in high-throughput plant breeding and phenotyping technologies, like technologies developed in the ARPA-E Transportation Energy Resources from Renewable Agriculture (TERRA) and Rhizosphere Observations Optimizing Terrestrial Sequestration (ROOTS) programs, that are focused on NUE. On-field N_2O measurement technologies, as developed in the ARPA-E Systems for Monitoring and Analytics for Renewable Transportation Fuels from Agricultural Resources and Management (SMARTFARM) program, may provide validation of the N_2O mitigation technologies developed in a future program.

II.A. Altering N-cycling in soils

a. What contributes to N_2O emissions more: nitrification or denitrification pathways? What are the factors that can impact the formation of N_2O the most?
b. If the microbiome can be altered at a field scale, what genera would be most critical to target or what genera should be introduced to contribute to the reduction of N_2O?
c. How can the denitrification pathway be modified to reduce the formation of N_2O?
d. What will be the anticipated challenges with developing a microbial-based technology that inhibits the formation of N_2O emissions either via nitrification or denitrification pathways?
e. What approaches can be used to alter the denitrification rates, so that less N_2O gasses were produced relative to N_2 gases evolved? How much N_2O gas would be reduced if the selected approach were to scale?
f. How can changes in microbial N_2O production be measured in fields? How would biological strategies to reduce N_2O be validated?
g. Can models that incorporate microbial N-cycling account for the observation of N_2O hotspots on fields? Describe the SoA along with its limitations.
h. Can plants produce exudates that inhibit nitrification, improving NUE and reducing N_2O production?
i. What key factors or approaches are we not considering in our line of questioning that will be impactful for this end goal of N_2O emissions avoidance/reduction from the field?
j. If plants were used to inhibit nitrification, what genetic mechanisms and traits would contribute to nitrification inhibition?

III. Enabling Conditions and Production Practices

ARPA-E seeks to identify technical approaches that are feasible, scalable, and marketable. In addition to technical input, we welcome responses related to the potential or challenges of the application of technologies at scale. ARPA-E seeks the perspectives from agricultural producers and technical

[10] Coskun, D. et al. (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nature Plants* 3.6: 1-10.

consultants about how these technologies could be designed to be commercially and agronomically attractive to the end users (i.e., seed distributors, fertilizer distributor companies, and growers).

III.A. Enabling end-user uptake of new technologies

a. What market conditions would enable widespread uptake of low-synthetic fertilizer or N_2O emissions-reduction approaches?
b. How will these solutions fit into existing crop marketing approaches (i.e., conventional commodity sales, voluntary labeling and/or certification utilizing climate friendly, non-genetically modified, organic, sustainable farming frameworks)?
c. How can these technologies integrate into modern farming practices (e.g., sustainable, no-till farming, cover crop, remote sensing, and automation)?
d. Considering availability and access to production solutions, what would most rapidly accelerate utilization of these methods across farms, acres, states, growing regions, crop types, and crop varietals?
e. What pricing and points of access to the seed/microbial solution would meet farmer needs?

III.B. Research demonstration and data for end users

a. What methods of research demonstration would provide the information and data necessary for agronomists and others to evaluate the merits of these new production approaches?
b. Which factors are essential to include in any demonstration of the technology, so that it may inform end-user decision making, risk management assessments, and determination of suitability for lending, especially for operating loans on a seasonal basis, and farm viability on a long-term basis?

III.C. Expected impacts from change in synthetic fertilizer use

a. What is the likely impact of these tools on the agricultural economy at a macroeconomic scale, as well as at an on-farm microeconomic scale?
b. What would be expected changes in ground and surface waters, crop productivity, soil carrying capacity for water, soil capacity for carbon sequestration, resilience to extreme weather, and other related systems?
c. Characterize the tools available to value these and other ancillary impacts, including using economic impact reports and models.

III.D. Frameworks for measurement, reporting, and verification

a. Noting the complexity of agricultural systems across geographies, growing seasons, and growing condition trends, what measures should be utilized to analyze the impact of proposed interventions?
b. What standards would enable crops produced using these solutions to be marketed in traditional commodity markets?
c. What ancillary data is important to establish a high-quality dataset on technologies developed and piloted under this research (e.g., soil, rainfall, soil moisture, soil carrying capacity of field and adjacent fields, historic utilization of biome-suppressing inputs)?