

Manipulating the soil microbiome for improved nitrogen management



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The soil microbiome, including bacteria, archaea, fungi, viruses, and other microbial eukaryotes, has crucial roles in the biogeochemical cycling of nitrogen (N), the maintenance of soil fertility, and the plant N use efficiency (NUE) in agro-ecosystems¹. Recent advances in omics-based technologies (e.g. metagenomics, metatranscriptomics, and meta-proteomics) have expanded our understanding of the soil microbiome and their controls on specific N-cycling processes¹⁻³. Given the growing N-based fertiliser consumption and continuous land degradation, innovative technologies are needed to manipulate the soil microbiome to improve crop NUE, reduce N losses and increase N reservation in soil. This article discusses the research directions to facilitate the development of microbiome-manipulating technologies for sustainable management of N transformation processes.

The crop nitrogen use efficiency (NUE) in modern agro-ecosystems is notoriously low, as more than 50% of N fertiliser applied is lost to the environment through ammonia volatilisation, nitrate leaching, and emissions of nitrous oxide (N₂O), the third most important greenhouse gas^{4,5}. These losses are mostly driven by a myriad of N-cycling processes (in particular, nitrification and denitrification) that can be modulated by a broad range of soil microorganisms (Figure 1)^{6,7}. Conventional agricultural practices mainly rely on agronomic measures and chemical inputs to improve NUE, which could lead to soil degradation and loss of biodiversity, with detrimental consequences for soil health and ecosystem functioning⁸. For example, long-term use of synthetic fertilisers, herbicides, and pesticides can negatively influence bacteria and fungi that create organic matter essential to plants. To meet the increasing food

demand of a global population of more than 11 billion by 2100, there is an urgent need to discover new intervention points to manage N-cycling microorganisms for improved NUE and sustainable agricultural production.

Propelled by the evidence in manipulating gut microbiomes for improved human health, there are growing interests focused towards the manipulation of the soil microbiome to reduce soil erosion, to enhance plant growth and disease resistance in agro-ecosystems, and to promote the remediation of heavy metal-contaminated soils^{1,3}. In this article, we discuss the currently-used technologies and emerging microbial biotechnologies that can manipulate the soil microbiome *in situ* to mitigate the processes of agricultural N loss and improve crop NUE, leading to both enhanced crop yield and positive environmental and social outcomes.

Physicochemical approaches to manipulate the soil microbiome

Physicochemical approaches have been put forward to reduce agricultural N losses through manipulating the abundance, structure and activities of soil N-cycling microorganisms or controlling the amount of N resources available to microorganisms (Figure 2). Some practical tools utilised in agro-ecosystems to improve NUE include: (1) use of synthetic nitrification inhibitors (e.g. DMPP and DCD) to inhibit the activity of ammonia oxidisers and reduce the N loss through N₂O emission and nitrate leaching⁹; (2) use of urease inhibitors (e.g. N-(n-butyl) thiophosphoric triamide (NBPT)) to inhibit the expression of genes encoding ureases that catalyse urea hydrolysis¹⁰; (3) manipulation of soil properties (e.g. soil pH, C:N ratio, and moisture) by agrochemical amendments and agronomic

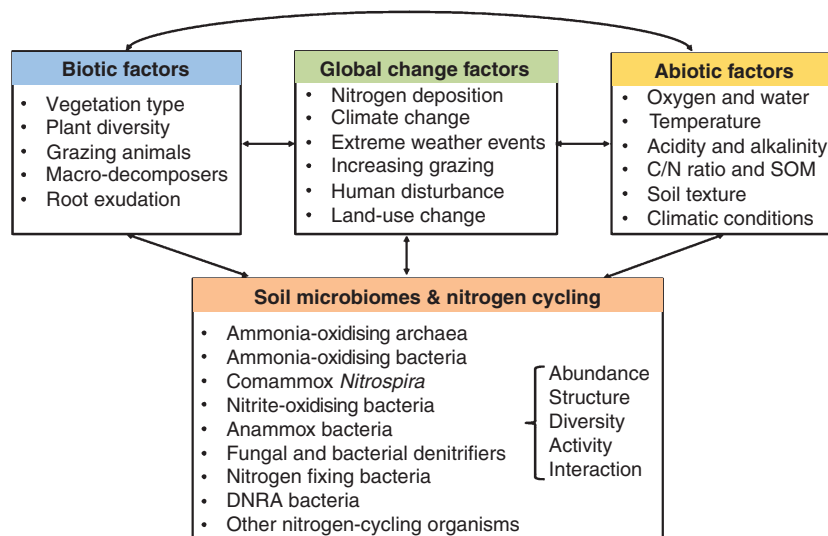


Figure 1. The soil microbiome components involved in nitrogen transformation processes are influenced by a wide range of abiotic, biotic, and emerging global change factors as well as their interactive effects.

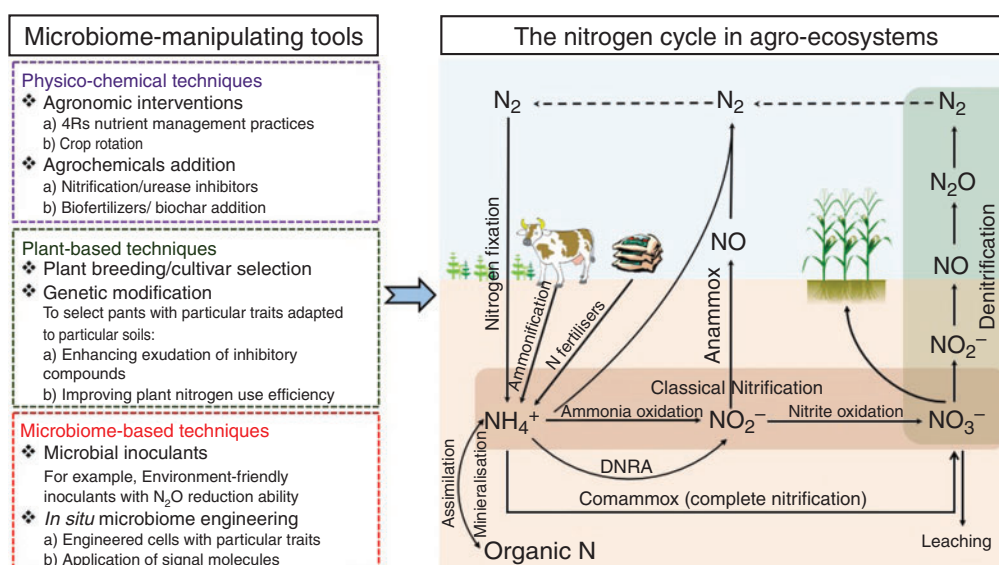


Figure 2. Schematic overview of the microbiome-manipulating tools that can be used for managing the nitrogen cycling processes in agro-ecosystems. DNRA, dissimilatory nitrate reduction to ammonium; Anammox, anaerobic ammonia oxidation.

practices to indirectly reshape the abundance, diversity and structure of soil microbiomes; (4) incorporation of plant residues to enhance microbial N immobilisation and reduce the amount of inorganic N available to soil microbes¹¹; and (5) use of precise nutrient management practices and high-efficiency fertilisers to better synchronise N supply and crop N demand and reduce N available to soil microorganisms. Tools (1) and (2) are direct practices that impact soil microorganisms while the other three tools are indirect practices.

The outcomes of these physicochemical technologies are variable across soils, primarily owing to their largely unknown impacts on soil microorganisms. For example, the nitrification inhibitor DMPP could effectively inhibit nitrification and N₂O emissions in alkaline

soils through influencing the abundance and metabolic activity of ammonia-oxidising bacteria, but had no significant effects in many acidic soils probably due to the fast degradation of DMPP^{9,10}. Other drawbacks of synthetic inhibitors include difficulties in application, rapid degradation, increased ammonia volatilisation, and migration into the food system^{5,12}. In addition, long-term use of chemicals has detrimental environmental impacts, resulting in the accumulation of residues in fields, loss of beneficial microorganisms, and disruption of the plant and soil microbiota association¹³. An improved understanding of the key functional genes, enzymes and regulatory mechanisms of N-cycling processes, and their responses to interactions between different climatic, soil and biotic properties (Figure 1), should be essential to improvement of physicochemical strategies.

Plant-based approaches to manipulate the soil microbiome

Plant physiological traits can be selected by plant breeding (cultivar selection) or genetic modification techniques to secrete specific compounds or signalling molecules for the direct manipulation of the soil microbiome *in situ*^{8,11}. Plants have developed intimate relationships with their interacting soil microbiomes and the environment (termed as the ‘phytobiome’)¹⁴. Some plant and crop roots (e.g. *Fallopia* spp. and *Brachiaria humidicola*) can exudate organic compounds to inhibit the ammonia monooxygenase (enzyme capable of oxidising NH_3 to NH_2OH) and hydroxylamine oxidoreductase (enzyme capable of oxidising NH_2OH to NO_2^-) of ammonia oxidisers¹⁵, or to inhibit the metabolic activity of denitrifiers¹⁶. Screening agricultural crops with similar traits may greatly enhance our ability to improve crop NUE by using them directly for *in situ* microbiome engineering. A conventional plant breeding programme, however, rarely takes into account the interactions within phytobiome¹⁴, which may result in loss of beneficial microbiota, disruption of symbiosis associations, and unknown consequences for other ecosystem processes¹³. Future plant-based strategies should integrate the knowledge of the phytobiome into the programme, by which specific N-cycling microorganisms are manipulated *in situ* without compromising beneficial microbiota and other ecosystem functions³.

Emerging microbial biotechnology approaches to manipulate the soil microbiome

Microbial biotechnologies have shown enormous potential in reducing N losses via N_2O emissions in soybean root systems where denitrifiers harbouring N_2O reductase, enzyme capable of reducing N_2O to N_2 , were amended¹⁷. There is evidence that the application of organic fertilisers inoculated with N_2O -reducing denitrifiers decreased N_2O emissions in agricultural soils at field scales¹⁸. However, the persistence and functionality of these inoculated microbiota are uncertain, as most of them are unlikely to persist in soil due to the strong competition from indigenous microbiota. When using specific bacterial or mycorrhizal inocula as a strategy to manipulate the soil microbiome, there is an urgent need to modify the mode of delivery to increase their colonisation potential. Some approaches⁸ include: (1) use of consortia of multiple compatible microbes, rather than single-strain formulations, to better compete with indigenous microbiota; (2) use of symbiotics to provide support for colonisation of the inoculated strains; (3) use of slow release systems for inocula to provide continual inoculation under field conditions; and (4) using chemical pesticides or predators for the indigenous microbiota to create new niches for the introduced

microbiota. The combination of these approaches might help to achieve maximum benefits and improved crop NUE.

Emerging microbial biotechnology tools are proposed to precisely manipulate the soil microbiome *in situ*, by adding or withdrawing chemicals¹⁹, to regulate N transformation processes under various conditions. Multidisciplinary approaches, especially genome engineering and synthetic biology, by fully taking advantages of microbiome knowledge, are needed for maximising the contribution of microbiome-based biotechnologies to sustainable management of the N cycle. Here, we highlight the key opportunities and research priorities to harness the soil microbiome to manage N transformation processes:

- (1) Exploration of the core soil microbiome components involved in N cycling processes and their signalling compounds (or their inhibitors) for chemical conversations, and how they are impacted by plants, climate, soil properties, and agronomic practices (Figure 1). These efforts will lead to the identification of a set of functional taxa that should be prioritised for further research, and provide new ways through direct manipulation of the microbiome activities or via genetically engineering the native microbiomes *in situ*. Microbiome-based approaches targeting at reducing rates of nitrification and denitrification (pathways leading to N losses) and increasing rates of dissimilatory nitrate reduction to ammonium (DNRA, the pathway capable of reserving N in soil), would have multiple benefits such as reduced N_2O emissions, increased farm productivity, reduced water contamination, and higher farm profitability through reduced use of fertilisers.
- (2) Technological improvements are needed to decipher the ‘dark matter’ of microbial chemistries, as current metabolomics studies can only match a small fraction of data to known chemical compounds and biochemical pathways²⁰. Quorum sensing signals have been found to regulate the communication between ammonia oxidisers and nitrite oxidisers, and to regulate the production and consumption of N oxide gases in a model nitrite oxidiser²¹. We are just beginning to recognise the diversity and specificity of signalling molecules, with the advancement of integrated metabolome and proteome technologies¹⁴, and thus becoming more reliable to develop microbiome-engineering strategies that could utilise the natural signalling channels of the N-cycling microorganisms.
- (3) Harnessing the emerging synthetic biology and genome editing tools to directly engineer the genomes and metabolic pathways of indigenous soil microbiome mediating N-cycling processes *in situ* with high specificity and efficacy¹⁹. We need a comprehensive knowledge of the gene regulation frameworks and modelling tools (through integrating various components of microbiome datasets, soil parameters, weather data and new computational methods) to predict the effects of microbiome manipulations *in situ* and reliably monitor the engineering outcomes. Precision tools such as sequence-specific gene editing using CRISPR/Cas9 delivered by phage or conjugative elements²², and synthetic microbial consortia engineered to disrupt or replace existing communities, are needed for modifying microbiota and their genes *in situ*.
- (4) The emerging *in situ* microbiome-manipulation tools (in particular, use of genetically modified organisms) in the natural environment are subject to regulatory requirements and societal concerns¹³. Coordinated efforts and multidisciplinary networks of policy makers, industry stakeholders, engineers, public and private partners, and agricultural communities will

consolidate and translate new microbiome-related innovations into practical solutions for farmers and ensure that risks associated with microbiome research are properly addressed. In addition to traditional agency-specific requests for proposals, strategic funding investments by national-level interdisciplinary initiatives (e.g. USA National Microbiome Initiative) could ensure availability of sufficient resources for developing broadly applicable microbiome-based tools^{2,19,23}.

Concluding remarks and future perspectives

Although there are a range of ways in which crop NUE and agricultural productivity could be improved by the management of the soil microbiome, this is an area of great challenge which requires advances in multi-omics technologies, systems biology, synthetic biology, data analytics, standardised protocols, and modelling, as well as new collaborative efforts among scientists, engineers, agribusiness professionals and agricultural communities. Therefore, utilisation of existing physicochemical technologies will be the major approaches to manipulate the soil microbiome in short or medium terms. Over a longer term, we envision the innovation in *in situ* genome engineering technology will offer precise microbiome management approaches to sustainably increase agriculture productivity. These technologies will show enormous potential in managing N transformation processes and can be integrated into next-generation precision agriculture for site-specific management. Under a context of global change and a growing human population, harnessing the capabilities of Earth's microbiomes will potentially lead to reduced chemical inputs, improved soil and water health, and increased productivity and sustainability of global agro-ecosystems.

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