

OPPORTUNITIES AND APPROACHES FOR MANIPULATING SOIL-PLANT MICROBIOMES FOR EFFECTIVE CROP NITROGEN USE IN AGROECOSYSTEMS

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KEYWORDS

nitrogen, microbiome, NUE, rhizosphere, phyllosphere, soil food web

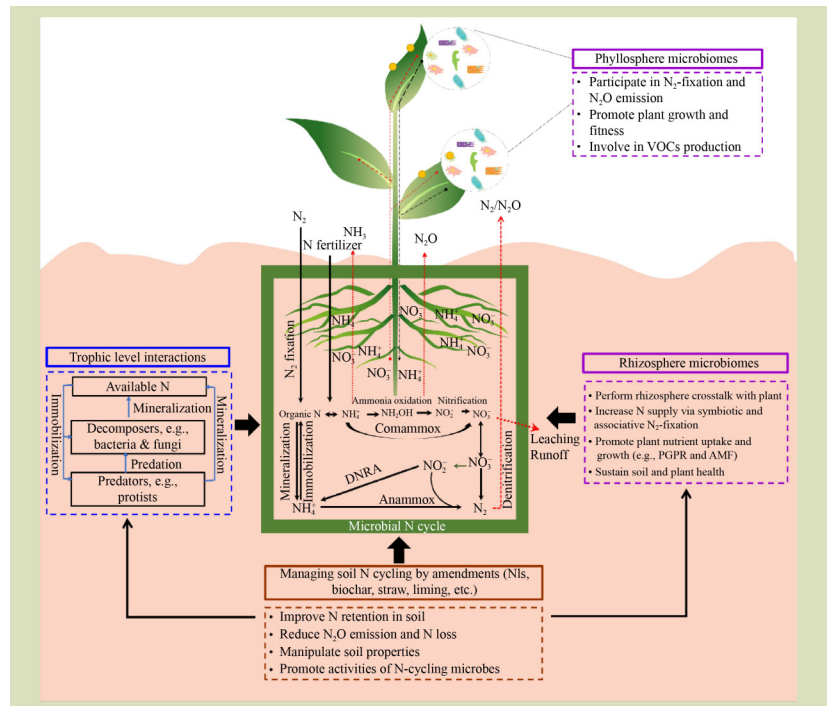
HIGHLIGHTS

- Matching nitrification inhibitors with soil properties and nitrifiers is vital to achieve a higher NUE.
- Enhancing BNF, DNRA and microbial N immobilization processes via soil amendments can greatly contribute to less chemical N fertilizer input.
- Plant-associated microbiomes are critical for plant nutrient uptake, growth and fitness.
- Coevolutionary trophic relationships among soil biota need to be considered for improving crop NUE.

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GRAPHICAL ABSTRACT



ABSTRACT

Soil microbiomes drive the biogeochemical cycling of nitrogen and regulate soil N supply and loss, thus, pivotal nitrogen use efficiency (NUE). Meanwhile,

there is an increasing awareness that plant associated microbiomes and soil food web interactions is vital for modulating crop productivity and N uptake. The rapid advances in modern omics-based techniques and biotechnologies make it possible to manipulate soil-plant microbiomes for improving NUE and reducing N environmental impacts. This paper summarizes current progress in research on regulating soil microbial N cycle processes for NUE improvement, plant-microbe interactions benefiting plant N uptake, and the importance of soil microbiomes in promoting soil health and crop productivity. We also proposes a potential holistic (rhizosphere-root-phylosphere) microbe-based approach to improve NUE and reduce dependence on mineral N fertilizer in agroecosystems, toward nature-based solution for nutrient management in intensive cropping systems.

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1 INTRODUCTION

Soil is inhabited by an enormous diversity of microorganisms which consist mostly of bacteria, fungi, archaea, protists and viruses, collectively called the soil microbiomes. Soil microbiomes are essential to soil nitrogen supply and crop N uptake through their active involvement in driving the biogeochemical N cycle^[1] and plant N acquisition in agroecosystems^[2]. Plants take up N as ammonium and nitrate, and small molecules of organic N, the availability of which depends on multiple N transformation processes mediated by soil microbes. These transformations are often depicted as a sequence of six distinct processes that proceed in an orderly fashion: dinitrogen gas is first fixed to ammonia, which is assimilated into organic N (biomass) by plants and microorganisms. The degradation of organic N (ammonification) releases ammonium, which is subsequently oxidized to nitrate through nitrification ($\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) and eventually converted back to N_2 through denitrification ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$) or anaerobic ammonium oxidation (anammox; $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2$). In addition, nitrate and nitrite can be dissimilatory reduced to ammonium (DNRA; $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+$)^[3] (Fig. 1). These processes are closely related to plant N uptake by supplying available N substrates, but can also lead to massive N losses, such as nitrate leaching and runoff, N_2O emission, and NH_3 volatilization, which substantially result in the low crop N use efficiency (NUE) in agroecosystems and environmental pollution^[4,5].

Crops in the field live with a wide array of microorganisms (known as the phytobiome or plant microbiomes) which have coevolved with their hosts and are crucial for host performance and fitness to environment perturbations, and resistance to

pest and pathogens^[6]. The interactions between plants and their associated microbiomes in rhizosphere, root and phyllosphere have profound influence on N cycling and greatly benefit plant nutrient acquisition and growth. Also, microbiomes in the soil represent the largest nutrient pools and critical components of soil food web, being essential in sustaining soil fertility and soil health. Recent advances in modern multiomics technologies, including metagenomics, metatranscriptomics, metaproteomics, metabolomics and cultureomics, have greatly expanded understanding of soil and plant microbiomes and their roles in coordinating soil N supply and crop N uptake, shedding light on the potential of manipulating microbiomes to improve crop NUE and reduce global reliance on mineral fertilizers and their environmental impact. In this review, we aim to provide an overview of how soil and plant microbiomes drive and affect the N cycle and plant N uptake, and highlight the potential approaches of steering soil and plant microbiomes for crop NUE improvement and sustainable agricultural production.

2 RESEARCH TRENDS IN SOIL-PLANT MICROBIOMES MODULATING SOIL N CYCLE AND CROP N UPTAKE

To synthesize the research trends and hotspot in soil-plant microbiomes modulating soil N cycle and crop N uptake, bibliometric analysis was conducted by retrieving keywords from Web of Science Core Collection database with a time-span from January 2010 to August 2021 using the search terms “(nitrogen or N cycle or N_2 or ammon* or nitr?te or nitrous oxide or mineralization or nitrification or denitrification or commamox or anammox or DNRA or fertilization or nutrient\$) and (rhizosphere or phyllosphere or endophyte or

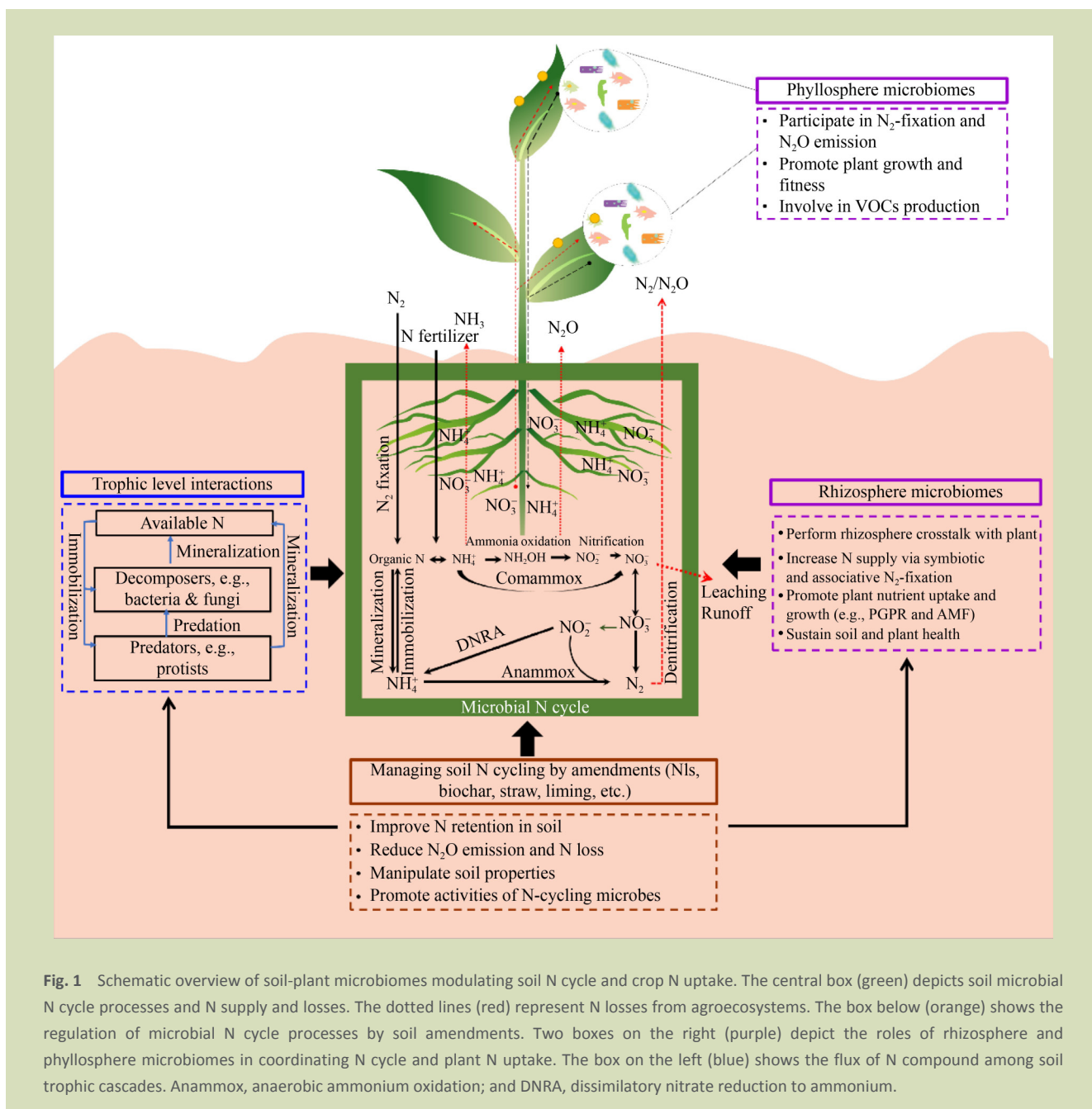


Fig. 1 Schematic overview of soil-plant microbiomes modulating soil N cycle and crop N uptake. The central box (green) depicts soil microbial N cycle processes and N supply and losses. The dotted lines (red) represent N losses from agroecosystems. The box below (orange) shows the regulation of microbial N cycle processes by soil amendments. Two boxes on the right (purple) depict the roles of rhizosphere and phyllosphere microbiomes in coordinating N cycle and plant N uptake. The box on the left (blue) shows the flux of N compound among soil trophic cascades. Anammox, anaerobic ammonium oxidation; and DNRA, dissimilatory nitrate reduction to ammonium.

root* or leaf or leaves or soil\$) and (fung* OR bacteria* or microb* or microorganism\$ or archaea* or virus or viral or protist* or mycorrhiza*) and (crop or maize or wheat or rice or barley or soybean or peanut or sorghum)”. A total of 17,379 research and review articles were obtained, and the co-occurrence analysis of keywords showed that recent research mainly focused on three themes (Fig. 2). (1) Effect of fertilization on N transformation processes and associated functional microorganisms. The theme focused more on the response of N transformation processes and N-cycling microbes to fertilization and soil management practices.

(2) Interactions between microbiota and plants, which focused on the function of soil/plant microbiomes in plant nutrient uptake, growth promotion and stress tolerance, and the applications of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculants in agriculture. It is noteworthy that phyllosphere microbiomes is an emerging research hotspot and attracting growing attention. (3) Microbial activity and soil fertility improvement, which emphasized on improving soil nutrient availability and microbial activity by fertilization, soil amendments and tillage, etc.

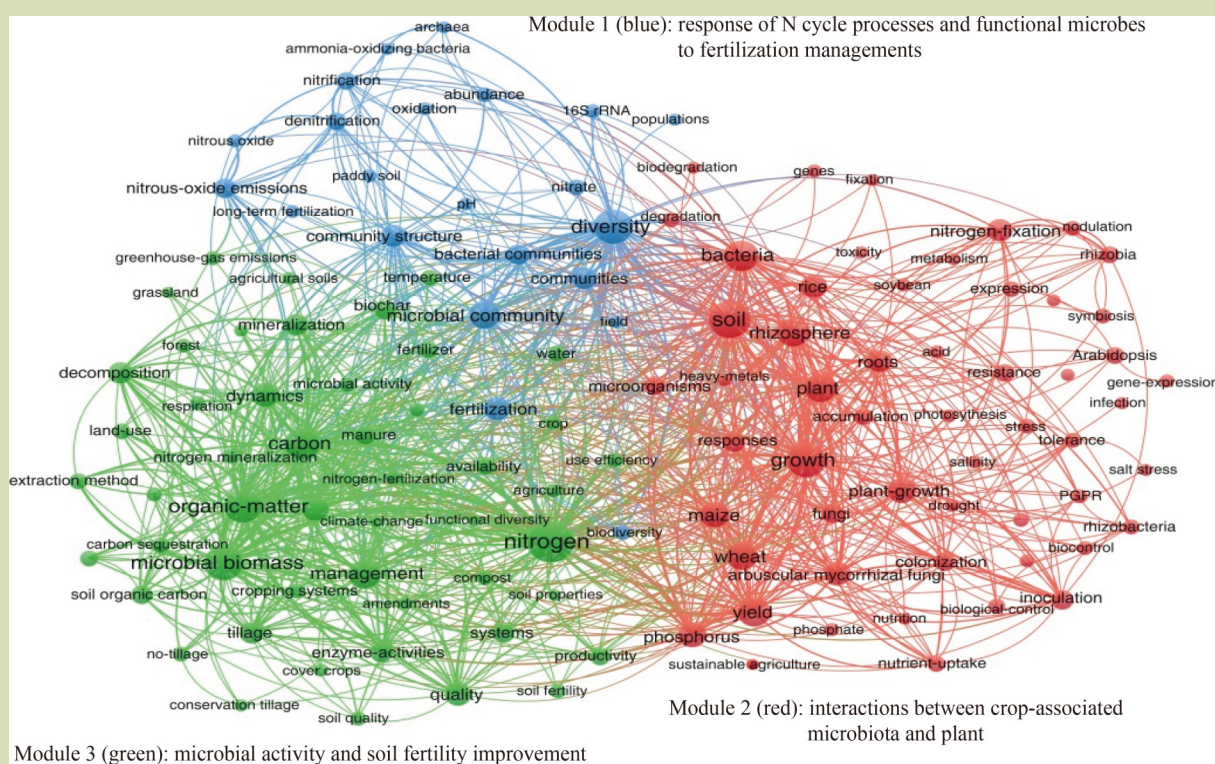


Fig. 2 Bibliometric analysis of research on soil-plant microbiomes modulating crop N uptake based on Web of Science Core Collection from January 2010 to August 2021. The co-occurrence pattern among keywords was constructed using the full counting method and visualized by bibliometric software VOSviewer (version 1.6.17)^[7]. The keywords with over 200 occurrences were selected, with synonymous keywords (such as, N_2O and nitrous oxide, corn and maize, or bacterial community and bacterial communities) merged. The final network contains 127 keywords (nodes), the node color represents different cluster modules and the node size is proportional to the link strength.

2.1 Manipulation of soil microbial N cycling processes for NUE improvement

Among the six N cycling processes, N fixation, immobilization and DNRA are responsible for N retention in soils, while ammonification and nitrification supply NH_4^+ and NO_3^- substrates to crops, respectively. In contrast, nitrification coupled with denitrification, anammox and denitrification processes largely contribute to N losses. Balance of N retention and loss determines N availability for crop uptake and thereby crop NUE^[1]. The increasing knowledge on microbial mechanisms of N cycle in recent years is paving the way for steering microbial N cycling processes for higher NUE. Particularly, managing the nitrification process in agricultural soils, has recently become the intensive research initiatives for reducing N losses^[8], as nitrification greatly contributes to groundwater and atmospheric pollution through nitrate leaching and N_2O emission^[9]. Specifically, ammonia oxidation, performed by canonical ammonia oxidizing bacteria (AOB), ammonia oxidizing archaea (AOA), and newly discovered complete ammonia oxidizers (comammox *Nitrospira*)^[10,11], is the first and a rate-limiting step in nitrification, which converts

immobile NH_4^+ to mobile anion NO_3^- , causing massive losses of N from agricultural system. Nitrification contributes to the production of N_2O as the intermediate products of ammonia oxidation or through fueling denitrification^[8]. A few strategies have been proposed to reduce N loss and pollution from nitrification, including use of nitrification inhibitors (NIs) or urease inhibitors to retard activity of ammonia oxidizers or urea hydrolyzers, and application of soil amendments (e.g., biochar, lime) to promote microbial N immobilization, and implementation of precision farming management (e.g., 4R optimal fertilizer application rate, source, timing and placement) to enhance synchronization between N supply and crop N demand^[12–22].

As ammonium and urea-based fertilizers are the most commonly used form of N in agriculture, NIs have been widely applied to inhibit the activity of ammonia oxidizers and reduce N loss from N_2O emission and nitrate leaching. Most frequently used NIs (DCD, nitrapyrin and DMPP) are suggested to act as metal chelators and hence to bind copper in the active site of the ammonia monooxygenase AmoB subunit,

although the precise mechanism of this inhibition has not yet been clarified. These NIs appear not to be effective toward AOA, possibly due to a structural difference of the AmoB subunit in these organisms^[12]. For example, DMPP effectively inhibits activity of AOB but has minor effect on AOA across a range of soil types^[13,14]. Comammox bacteria which coordinate the complete conversion of ammonia into nitrate was shown to be inhibited by allylthiourea, a copper chelator as well^[11]. In addition, the inhibitory effect of NIs on nitrification and N₂O emission is dependent on soil pH^[14]. Autotrophic nitrification rates and N₂O emissions in alkaline soils can be efficiently inhibited by the application of 1% DMPP^[15], but this inhibitory effect was not observed in acidic soils^[16]. The disparities in effectiveness of NIs has been ascribed to niche differentiation between ammonia oxidizers in soils with different soil pH and ammonia concentrations, of which AOA dominate ammonia oxidation in acidic and N-deficient soils while AOB dominate in alkaline and N-rich conditions^[17-19]. Thus, future application of NIs should consider the matching of NIs with soil properties and the dominant nitrifiers to achieve a high NUE with lower environmental costs.

Manipulating soil properties by amendments (e.g., biochar and lime) can improve NUE through indirectly regulating N-cycling functional microbes. For example, biochar amendment can stimulate both nitrification and denitrification processes, while reducing N₂O emissions by significantly enhancing the activity of N₂O reducers^[20,21]. Crop yield improved by biochar amendment has been suggested to be, in some cases, associated with promoted activities of N-cycling bacteria (especially AOB)^[22]. Liming also positively improves crop NUE by stimulating nitrification and ameliorating soil acidification^[23], but there is a time-dependent effect of liming on the activity of AOA and AOB^[24]. In addition, straw return is widely recommended to maintain or increase soil C and N storage in arable soils, but it may also affect soil microbial activities and result in reactive N losses^[25,26]. A global meta-analysis suggested that straw return have significantly positive effects on improving soil organic C content, crop yield, and crop N uptake, reducing N₂O emissions, N leaching and runoff from rice paddies, mainly due to enhanced microbial N immobilization^[27]. However, straw return can also increase N₂O and NH₃ emissions from upland fields due to the stimulation of nitrification/denitrification and soil urease activity. Biochar amendment was suggested to markedly reduce N₂O emissions induced by straw return via regulating functional microbes and soil physicochemical properties^[28]. Overall, when appropriately used for targeting soil and microbial attributes, soil amendments are able to improve available N substrates for crop growth, increase soil N

retention, and reduce N losses to achieve a higher crop yield and NUE.

In contrast to nitrification and denitrification causing N loss, biological N₂ fixation (BNF) and DNRA are beneficial to soil N supply and retention by assimilating N into microbial biomass and converting mobile NO₃⁻ to immobile NH₄⁺^[29]. It is estimated that NUE increases exponentially with increasing levels of BNF in soils but decreases drastically with increasing levels of applied synthetic N fertilizers^[30]. Fertilization exerts significant effects on activity of diazotrophs and DNRA-related microbes, and therefore affects the rates of the two processes. Long-term application of inorganic fertilizers strongly reduced (~50%) BNF^[13], and markedly suppressed diazotrophic abundance and nitrogenase activity^[31]. However, this can be largely alleviated by the combined application of organic manure due to improved soil properties, increased diazotrophic diversity and network stability^[32,33]. DNRA activity was shown to be suppressed by manure and peat-vermiculite application with decreased abundances of *napA* and *nrfA* genes (encoding enzymes of DNRA)^[34], while it can be improved by straw return in paddy soils despite the variation between soil types^[35]. Therefore, enhancing BNF and DNRA processes through optimizing fertilization management (e.g., growing legumes with manure application or straw return) will contribute greatly to increased NUE in agroecosystems.

The above practices all work closely associated with regulations of N transformation and functional microorganisms, which can effectively improve NUE under optimum management strategies and should be taken into consideration in future N management in agroecosystems. As N-cycling microbes are widely involved in production and consumption of greenhouse gases, understanding how they affect climate change and how they respond to climate change are vital for achieving a sustainable N management with sound NUE and lower climate change effects^[36].

2.2 Plant-microbe interaction benefiting plant N uptake

Rhizosphere is a hotspot of plant-microbe interactions and plant root exudates can directly coordinate N transformation and crop N uptake^[2]. Some chemicals in root exudates have been identified as the signaling molecules mediating the crosstalk between plant and N-cycling microbes. In symbiotic N₂-fixing system of legume and rhizobia, flavonoids from legume root exudates have been demonstrated to stimulate the expression of nod genes in rhizobia and synthesize nodulation

factors which in turn activate a Ca^{2+} -dependent signaling cascade for nodule formations in legume^[37]. More importantly, N_2 fixation of faba bean can be promoted by intercropped maize, the root exudates of which effectively promote flavonoid synthesis in faba bean^[38]. Root exudates are thus supposed to be more important in explaining yield-improving effects in the polyculture than in the monocultures, rather than niche complementarity effect of different crops^[39].

Root exudates are also important mediators controlling soil nitrification as biological nitrification inhibitors (BNIs). A series of BNIs such as methyl 3-(4-hydroxyphenyl) propionate, brachialactone, syringic acid and 1,9-decanediol have been identified in root exudates from sorghum^[40], *Brachiaria humidicola*^[41], rice^[42], wheat^[43] and maize^[44], and demonstrated effective inhibition effect on ammonia oxidation process. Recent screening on a number of wheat and rice varieties and cultivars indicated strong BNI potential in considerable genotypes, and the strength of BNI inhibition are positively correlated with crop NUE^[42,43]. Similarly, biological denitrification inhibitor (BDI) activity has been found in root extracts of *Fallopia* spp., invasive weeds with low denitrification potential in soil^[45], while the universality and the chemical compounds of BDI in root exudates from crops remain elusive. Nevertheless, the discovery of BNI and BDI showed the considerable promise in breeding biological nitrification/denitrification inhibition into modern crop cultivars^[8]. However, rhizosphere was supposed to be a favorable niche for anammox bacteria which can lead to N loss in rice paddy soil^[46]. It is therefore necessary to characterize microbial N transformation processes and the pathways of N loss in different agroecosystems to facilitate crop screening and plantation optimization.

In addition to modulating N transformation processes, plant root exudates can profoundly modify microbial communities in rhizosphere and exert considerable influence on soil N supply and crop N uptake, with large variations across different plant species and genotypes^[8,47]. The root-accelerated mineralization and priming can account for up to one-third of the total C and N mineralized in temperate forest soils^[47]. Strong coupling between crop N uptake and protease activity in maize rhizosphere was also observed in agriculture soil, which was attributed to phylogenetically distinct microbial community shaped by plant phenotypes^[48]. More importantly, it was found that the *indica* varieties of rice possessing nitrate transporter and sensor NRT1.1B gene can enrich more N-cycling microbes in the rice root to acquire more N from soil under field condition^[49]. In addition, root anatomical phenotypes largely influence crop nutrient acquisition by

regulating the transport of water, interactions with soil biota like mycorrhizal fungi, pathogens and rhizosphere microbiomes^[50]. With more unknown intersection between the root microbiota, soil N cycle, gene regulation and host plant traits unraveled, future technologies will be able to integrate this information into crop breeding and screening for modulating root microbiota for improved crop NUE and agricultural sustainability.

Also, an increasing evidence has shown that phyllosphere microbiomes are critical for multiple aspects, including plant growth promotion, plant fitness improvement and pathogen resistance^[51]. Phyllosphere microbiomes also participate in N_2 fixation, N_2O production, metabolizing of plant metabolites and producing volatile organic compounds^[52]. Studies based on acetylene reduction activity assay, $^{15}\text{N}_2$ stable isotope labeling and N-free mannitol agar medium cultivation, consistently demonstrated active N_2 -fixation in the phylloplane of wheat^[53], grasses^[54] and other higher plants^[55]. Particularly, bacterial taxa within the genus *Methylobacterium* were frequently recognized as keystone taxa in the phyllosphere of various plants including maize, wheat and barley^[56,57], and were demonstrated to be able to fix N_2 and promote host biomass^[52]. Plants have also been proposed as a source of N_2O in grazed grassland in which AOB present on leaves emit N_2O via ammonia oxidation^[58]. Metagenomic analysis suggested that maize phyllosphere host a wide range of microbiota harboring N-cycling genes, for example, *nifH* and *nifK* (N_2 -fixation), *narG* and *narH* (nitrate reduction), *nosZ* (nitrous oxide reductase gene), *nasA* and *nasB* (N assimilation gene)^[57]. Also, recent studies have suggested that plant phyllosphere is a hotspot of plant-microbes-environment interactions, and phyllosphere microbiomes assembly is mainly determined by host and the surrounding environment such as air and rainwater^[57,59]. Seeds can transmit a large part of microbes to the phyllosphere and roots^[60], and are possible to transfer beneficial microbiota to phyllosphere and benefit plant growth. Uncovering microbial function, transmission, and plant-microbiome-environment interactions in the phyllosphere will provide essential information for the future microbiomes manipulation toward high NUE and agriculture sustainability.

2.3 Importance of soil microbiomes in maintaining soil fertility and crop growth

As essential components of soil biota, entire soil microbiomes, comprised of bacteria, fungi, archaea, protists and viruses, are vital for maintaining soil fertility as indispensable decomposers of plant and animal residues, macromolecular compound, and catalyzers of soil organic matter formation. As most N in the

soil (> 95%) is contained in dead organic matter as complex insoluble polymers such as proteins, nucleic acids and chitin, considerable amounts of N taken up by plants are derived from mineralization of soil organic matter^[61]. Conversely, mineral N fertilizer applied to the soil and soluble nutrients mineralized by decomposition are liable to be converted to organic form as microbial biomass. Soil microbiota therefore represent a large N and P nutrient pool in agricultural soils, but meanwhile they compete for available N with plant, depending on the soil C:N stoichiometric ratio^[62]. The net flow of N compounds in the soil are thus interactively regulated by microbial N use efficiency, plant N uptake and assimilation efficiencies, and other N transformation processes such as nitrification and denitrification^[63]. Therefore, the interaction between plant and microbial N use, besides the rates of main N transformation processes, need to be taken into account when developing N management strategies to match nutrient supply with plant demand for high NUE^[64].

Soil microbiomes are vital for sustaining soil health and plant performance in agroecosystems^[17,34,65]. For example, in addition to symbiotic N₂-fixing rhizobia (e.g., *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*), soil can contain free-living or associative diazotrophs (e.g., *Azoarcus*, *Azospirillum*, *Azotobacter*, *Burkholderia* and *Herbaspirillum*), which greatly benefit crop growth and yield in cereals like maize rice and wheat^[66]. These microbes, collectively known as PGPR, can facilitate plant growth by increasing N supply, solubilizing minerals, and secreting phytohormones^[67]. In addition, N cycling in agroecosystems is heavily dependent on AMF present in the soil^[68]. AMF can develop symbiotic relationship with 70%–90% of plants, including cereals and legumes^[69], thus increasing their ability to acquire N and influence the availability of soil mineral N through modifying N-cycling microbes^[70]. PGPR and AMF are therefore the most widely applied microbial inoculants in agriculture. However, their efficiencies vary under different soil conditions and are influenced by multiple factors such as fertilization, tillage and competition from other soil microorganisms. When using PGPR and AFM as bioaugmentation, there is an urgent need to optimize the delivery mode to increase their colonization and activity in light of a specific soil-crop system, possibly by designing multiple compatible microbes, using synbiotics and staggering application with N fertilization^[71]. Meanwhile, it is also promising and necessary to further ascertain which agricultural practices are favorable to maximize the benefits of PGPR and AFM in situ in the soil to improve crop NUE.

Soil microbiomes represent the largest biodiversity in terrestrial ecosystems, and are essential components of the soil

food web, which profoundly influence ecosystem functions including nutrient cycling and plant productivity^[34,65,72]. Numerous studies have shown that the diversity of soil bacterial taxa, keystone phylotypes, and even bacterial and fungal community composition, are predictors for crop yield in various agricultural ecosystems^[57,73,74]. Abundance of kinless hubs within soil microbial networks are positively and significantly correlated with the abundance of functional genes involving in C fixation, C degradation, C methanol, N cycling, P cycling and S cycling, which highlighted the importance of soil biodiversity in maintaining ecosystem function in croplands^[75]. It has been reported that ultrahigh yield paddy soil contains more taxa with N metabolism functions and more abundant genes involved in the nitrification process, which promotes effective transformation of ammonium to nitrate in rice fields and stimulates high expression of nitrate transporters in rice roots^[22]. The interactions between bottom-up (primary producers) and top-down (decomposers) in soil food web also control N cycling. Under agricultural intensification, distinctive change-response patterns have been identified between bacterial- and fungal-based food^[76]. Notably, fungal-dominated food web systems are linked with reduced N loss especially under drought conditions^[77]. Manure fertilization can enhance the jackfruit yield via reshaping the soil food web of microbes and nematodes^[78]. Also, predatory organisms (i.e., protists, microparasites and viruses), which can structure food webs, influence energy flow, and alter rates and pathways of nutrient cycling, are abundant in agricultural ecosystems^[79]. Importantly, predation on primary producers largely determines microbial functions, which can also lead to modification of metabolic preference between C and N due to nutrient limitation, thereby affecting mineralization rates^[80]. Notably, the predatory activities of protists on bacteria could lead to increased N mineralization and subsequent N uptake by plants^[81]. Protists are highly responsive to fertilization^[82] and their selective phagotrophic activities on bacterial and fungal communities can be a potential predictive tool for plant health especially during the early growth stages of tomato^[74]. These findings indicate the importance of utilizing coevolutionary trophic relationships among soil microbiomes to enhance biocontrol and plant growth promotion.

3 CONCLUDING REMARKS

Overall, the rapid advances in modern molecular techniques open up powerful avenues for deciphering mechanisms underlying soil microbiome-mediated N cycling and its agricultural significance. Soil microbiomes can strongly influence soil N supply and crop N uptake with multiple ways,

including: (1) directly participating in N transformation process and controlling N availability and losses; (2) closely associating with plant and facilitating plant nutrient uptake and growth promotion; and (3) serving as the large N and P nutrient pool and the mediator of soil food web and energy flux in soil. Although a range of practices, such as NIs, soil amendments, and bioaugmentation of PGPR and AMF, have been adopted to improve crop NUE and yield in agroecosystems, future research needs to further elucidate the mechanisms by which genomic diversity drives variation in metabolic traits of N cycling at the systematic level and to fully characterize the types of beneficial microbes in association with soil N cycling and plant N uptake. Although, we have mainly highlighted the significance of soil and plant microbiomes in coordinating soil N cycling and crop N uptake in this review,

the activity, function of soil microorganisms, plant-microbe interactions and crop N uptake are interactively influenced by multiple climatic, edaphic factors and field management practices (e.g., fertilization, irrigation, tillage, straw return and soil amendments). Comprehensively considering the interactions between microbes, and these factors to optimize field management practices, is also important for improving crop NUE in agroecosystems. Nevertheless, reinforcing the function of soil microbial community for host nutrient uptake and breeding for microbiome-associated phenotype can help to reduce the use of mineral fertilizers or pesticides and lead to higher yields and more resilient crops. This will ultimately guide the development of microbe-based solutions toward a holistic (rhizosphere-root-phylosphere) crop management.

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Compliance with ethics guidelines

Jingjing Peng, Olatunde Oladele, Xiaotong Song, Xiaotang Ju, Zhongjun Jia, Hangwei Hu, Xuejun Liu, Shuikuan Bei, Anhui Ge, Limei Zhang, and Zhenling Cui declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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