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Review

Nitrogen-loss and carbon-footprint reduction by plant-rhizosphere exudates

Yufang Lu,[1] Herbert J. Kronzucker,[2] Min Yu,[3] Sergey Shabala,[3,4] and Weiming Shi[1,5,*]

Low-carbon approaches to agriculture constitute a pivotal measure to address the challenge of global climate change. In agroecosystems, rhizosphere exudates are significantly involved in regulating the nitrogen (N) cycle and facilitating belowground chemical communication between plants and soil microbes to reduce direct and indirect emissions of greenhouse gases (GHGs) and control N runoff from cultivated sites into natural water bodies. Here, we discuss specific rhizosphere exudates from plants and microorganisms and the mechanisms by which they reduce N loss and subsequent N pollution in terrestrial and aquatic environments, including biological nitrification inhibitors (BNIs), biological denitrification inhibitors (BDIs), and biological denitrification promoters (BDPs). We also highlight promising application scenarios and challenges in relation to rhizosphere exudates in terrestrial and aquatic environments.

Highlights

Small rhizosphere exudates as chemical signals provide a green strategy to reduce nitrogen emissions and promote low-carbon agriculture.

Specific biological nitrification inhibitors (BNIs) and biological denitrification inhibitors (BDIs) could retard nitrification and denitrification, thus reducing N_2O emissions from terrestrial ecosystems.

Enhanced nitrogen removal rates and lower N_2O emissions are achieved by biological denitrification promoters (BDPs), such as root-derived fatty acid amides and sterols, and microbe-derived *N*-acyl-homoserine lactones (AHLs) in aquatic environments.

Cultivating BNI/BDI/BDP-enhanced plant varieties, intercropping and rotation with BNI/BDI plants, developing green nitrogen fertilizers, and designing water purification bioagents based on small rhizosphere exudates are promising application measures for supporting green low-carbon agriculture.

Nitrogen emissions and low-carbon agriculture

Since the Green Revolution in the 1960s, mineral N fertilizers have been a key factor in boosting crop yields and feeding a growing population. At the same time, the production and excessive use of mineral N fertilizers are associated with GHG emissions alongside other forms of N pollution [1], leading to eutrophication and the loss of terrestrial and aquatic biodiversity [2,3]. An estimated 108 Tg of N are exported from soil each year [4], with deleterious N loss in the form of **ammonia (NH_3) volatilization** (see Glossary) (~11% of N-fertilizer application, on average), **runoff** and **leaching** (~24% of N-fertilizer application), and **nitrous oxide (N_2O)** emission (~1% of N-fertilizer application) [5]. Globally, these N losses result (both directly and indirectly) in GHG emissions of ~2.29 Tg yr^{-1} of N_2O and 600 Tg yr^{-1} carbon-dioxide equivalents (CO_2e) [6]. In addition to terrestrial ecosystems [7], N_2O emissions from aquatic ecosystems, such as streams, rivers, and lakes, are also significant [8,9] (Table 1). Overall, global anthropogenic N_2O emissions, which are dominated by N-fertilizer additions to croplands, have increased by 30% over the past four decades [10]. This increase has had a significant role in the growth of emissions of carbon equivalents. Thus, lowering agricultural N emissions is a key target in achieving **low-carbon agriculture,** and cost-effective technologies must be developed in the context of **carbon neutrality** and environment sustainability.

In light of the above, strategies to reduce N emissions need to be deployed according to the different roles of N in different ecosystems (Figure 1). When N acts as a nutrient in a terrestrial ecosystem (e.g., on cropland or in a grassland), it can be maintained within a nutritional range through inhibiting a combination of **urea hydrolysis**, **nitrification**, and **denitrification**, thereby reducing adverse environmental impacts, such as NH_3 volatilization, N_2O emissions, and NO_3^- leaching. However, when substantial N is lost from terrestrial ecosystems and delivered to aquatic ecosystems (e.g., ponds, lakes, or rivers) and becomes a potential pollutant, the strategy should be the opposite: one needs to accelerate N removal by promoting microbial denitrification, especially the reduction of N_2O to N_2 [11,12], to alleviate water eutrophication and the emission of the potent GHG N_2O.

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[2]School of BioSciences, The University of Melbourne, Parkville, VIC 3010, Australia
[3]International Research Centre for Environmental Membrane Biology, Foshan University, Foshan 528000, China
[4]School of Biological Sciences, University of Western Australia, Crawley, WA 6009, Australia
[5]University of Chinese Academy of Sciences, Beijing 100049, China

*Correspondence:
wmshi@issas.ac.cn (W. Shi).

Table 1. CO_2e GHG emissions from direct N_2O emissions in terrestrial and aquatic ecosystems

Systems		N_2O emissions		Refs
		Tg N_2O yr^{-1}	Tg CO_2e yr^{-1}	
Terrestrial ecosystems	Cropland	1.53	405.45	[7]
	Forestland	3.68	975.20	
	Grassland	2.81	744.65	
	Total	8.02	2125.30	
Aquatic ecosystems	Lakes	0.52	137.80	[8]
	Rivers	0.49	129.85	
	Streams	0.36	95.40	
	Reservoirs	0.11	29.15	
	Total	1.48	392.20	

Both plants and microorganisms have important roles in reducing N emissions and consequent N pollution (Figure 1). In addition to direct N absorption, plant roots and microorganisms can secrete specific substances into the **rhizosphere** that alter N transformations in both soils and waterbodies [13–15]. There is emerging evidence that small-molecule substances secreted by organisms populating the rhizosphere, called rhizosphere exudates (including **root exudates** and microbial exudates), can act as **chemical signals**, and cause a cascade of intracellular reactions, even at very low concentrations [16,17]. Instead of simply acting nutritionally as carbon sources, these rhizospheric chemical signals act with precision in manipulating plant–bacteria–soil interactions. Here, we review recent evidence for the role of small-molecule rhizospheric exudates in reducing N emissions and pollution. We discuss chemical types, mechanisms of action, and application scenarios that can lead to reductions in N emissions in both terrestrial and aquatic ecosystems.

The role of rhizosphere exudates in reducing N emissions in terrestrial ecosystems

Microbial nitrification and denitrification processes are closely related to N emission and pollution. Several strategies have been proposed to achieve the goal of N-emission reduction, carbon neutrality, and sustainable crop production, including the use of enhanced-efficiency fertilizers, integrated biochar solutions, and other farming practices (e.g., optimal N-addition rates, depth, and time) [18–22]. Although such technologies have variable effects under different cropping systems, it is clear that more efficient and low-carbon measures need to be used to reduce N emissions. Exudates secreted from roots and rhizosphere-dwelling microbes offer a viable, natural, and potentially sustainable alternative approach (Table 2) [23,24].

Plant roots can exude specific substances that inhibit nitrification, known as **BNIs** [25]. Such natural inhibitors are more environmentally friendly compared with synthetic inhibitors and can be released continuously into the rhizosphere. They have the potential to act as alternatives to synthesized nitrification inhibitors (SNIs) [26,27]. Over the past two decades, several BNIs have been identified from the root exudates of pasture grasses and crops, including brachialactone from *Brachiaria humidicola* [28], methyl 3-(4-hydroxyphenyl) propionate (MHPP) [29], sorgoleone and sakuranetin from *Sorghum bicolor* [30], 1,9-decanediol and syringic acid from *Oryza sativa* [31,32], and zeazone, 2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one (HDMBOA), and 6-methoxy-2(3H)-benzoxazolone (MBOA) from maize [33,34]. In addition to root exudates, several BNIs have also been found from root and shoot extracts of pasture grasses and maize, such as methyl-p-coumarate, methyl ferulate, linoleic acid (LA), linolenic acid (LN), HMBOA, and HDMBOA-β-glucoside [33,35,36]. These BNIs include cyclic diterpenoids, flavonoids,

Glossary

Ammonia (NH_3) volatilization: gaseous ammonia escaping to the atmosphere from the soil surface, water surface, or plant surface.
Biological denitrification inhibitors (BDIs): compounds found in plants that inhibit denitrification.
Biological denitrification promoters (BDPs): compounds found in plants that stimulate denitrification.
Biological nitrification inhibitors (BNIs): compounds found in plants that inhibit nitrification.
Carbon neutrality: achievement of net-zero carbon dioxide emissions or elimination of carbon dioxide emissions altogether.
Chemical signals: chemicals released from plants or microorganisms that can trigger cellular physiological responses.
Denitrification: microbial reduction of NO_3^- to N_2O and, ultimately, N_2.
Leaching: drainage of water containing solutes away from the soil through the action of percolation.
Low-carbon agriculture: agriculture based on low-carbon power sources that therefore has a reduced output of GHGs into the atmosphere.
Nitrification: microbial oxidation of NH_4^+ to NO_3^-.
Nitrous oxide (N_2O): potent GHG.
Quorum sensing (QS): regulatory system that allows bacteria to regulate gene expression in response to cell density.
Rhizosphere: area of soil surrounding the root where chemical communication between root exudates and soil microbes occurs.
Root exudates: variety of primary and secondary metabolites released from the roots to the rhizosphere, establishing chemical communication between the plant and soil microbes.
Runoff: water, such as accrued as rainfall, that is not absorbed by the soil but instead is ported away from the agricultural site along the surface.
Urea hydrolysis: hydrolysis of urea into ammonia and carbon dioxide.

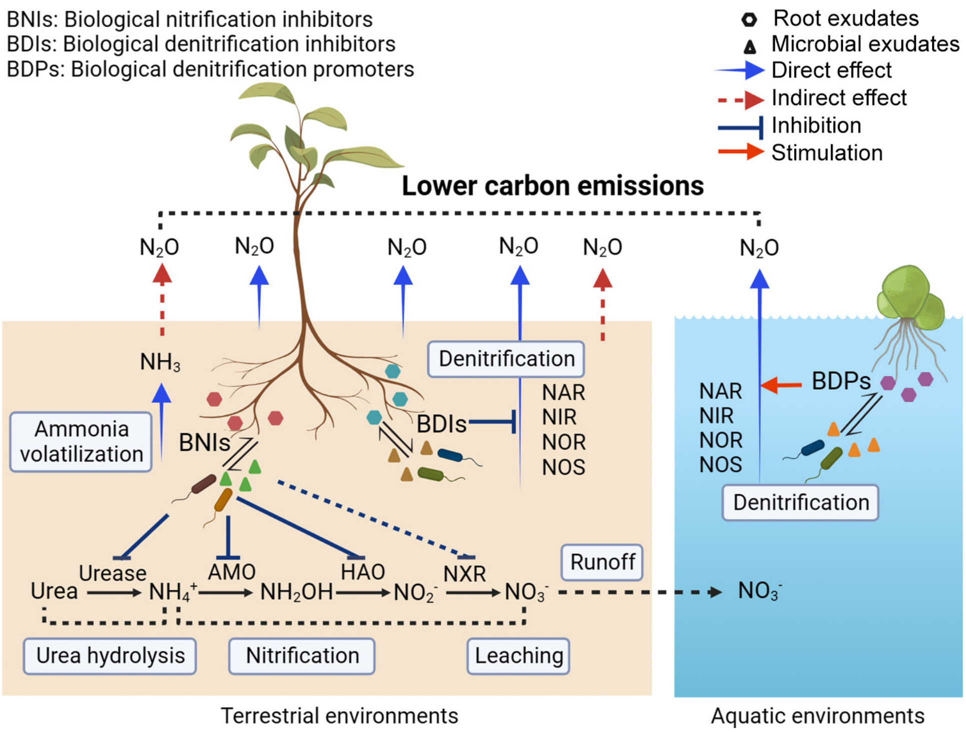

Figure 1. A conceptual figure of nitrogen (N) emission mitigation by small-molecule rhizosphere exudates in terrestrial and aquatic ecosystems. When N fertilizer, such as urea, enters soils, it is transformed through urea hydrolysis, nitrification, and denitrification, and the derived nitrate (NO_3^-) is then partially lost to aquatic ecosystems through leaching and runoff. Direct nitrogen oxide (N_2O) emissions are generated by microbial nitrification and denitrification (blue arrows), and indirect N_2O emissions can result from leaching, runoff, and ammonia volatilization (red-dashed arrows). Such N emissions can be mitigated by specific rhizosphere exudates from roots and microorganisms. In terrestrial ecosystems, plants release biological nitrification inhibitors (BNIs) and biological denitrification inhibitors (BDIs) to retard nitrification via ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), or nitrite oxidoreductase (NXR), and denitrification via nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS), and urea hydrolysis via urease; microorganisms also exude chemical signals to strengthen the crosstalk. In aquatic ecosystems, several biological denitrification promoters (BDPs) are exuded from roots and microbes to enhance N removal via denitrification, especially the reduction of N_2O to N_2. Lower N_2O and carbon emissions are achieved through precise regulation of the secretion of rhizosphere exudates.

benzoxazine, phenolic acids, fatty alcohols, and fatty acids. Due to different chemical structures, some BNIs have the ability to target both ammonia monooxygenase (AMO) and hydroxylamine reductase (HAO) in microorganisms [28,30], while others can only inhibit AMO [29,31,32]. The diversity of BNIs can provide a more lasting inhibitory efficacy compared with SNIs, because they may act on a wider range of ammonia-oxidizing microorganisms and, thus, are less likely to be afflicted by resistance development in nitrifying bacterial populations [26,27]. An increasing amount of data has established that most BNIs are effective against not only ammonia-oxidizing bacteria (AOB) in soils, but also ammonia-oxidizing archaea (AOA) [28,32,37,38], while SNIs can generally inhibit AOB [26,39]. Moreover, BNIs appear to target AOA preferentially over AOB [40]. Therefore, BNIs may retard nitrification in more soil types and cropping systems compared with SNIs, especially in acidic soils that are dominated by AOA [26,32,41].

The use of rhizospheric BNIs has been proposed as an effective mitigation strategy to tackle agricultural N_2O emissions [24,27]. However, the efficacy of BNIs to reduce N_2O emissions can be influenced by both soil type and BNI concentration. Soil-incubation experiments showed

Table 2. Types and mechanisms of small-molecule rhizosphere exudate involved in N emission in terrestrial ecosystems[a]

Compound	Source	Mechanism	N emission	Refs
Plant-derived BNIs				
Methyl 3-(4-hydroxyphenyl) propionate (MHPP)	Sorghum root exudates	Blocks AMO; inhibits AOA and AOB abundance and community	Reduces pot- and field-level N_2O emissions and N leaching; increases NH_3 volatilization; reduces N_2O and NH_3 emission and leaching with NBPT and/or biochar	[29,37,45,46,48,49]
Brachialactone	*Brachiaria humidicola* root exudates	Blocks AMO and HAO	Reduces N_2O emission in the field	[28]
Sorgoleone	Sorghum root exudates	Blocks AMO and HAO; inhibits AOA abundance; delays microbial network formation	Reduces N_2O emission in the greenhouse and field	[30,44,46,102]
1,9-Decanediol	Rice root exudates	Blocks AMO; inhibits AOA and AOB abundance and community	Reduces N_2O emission in incubation	[31,41]
Syringic acid	Rice root exudates	Blocks AMO; inhibits urease; inhibits AOA and AOB abundance	Reduces N_2O emission in incubation	[32,42]
Linoleic acid, linolenic acid	Shoot tissues of *Brachiaria humidicola*	Block AMO and HAO; inhibit urease	Increase N_2O emission in incubation	[36,47]
Plant-derived BDIs				
Procyanidins	*Fallopia* spp. extracts	Inhibit nitrate reductase and *nirS*- and *nirK*-type denitrifier abundance; stimulate growth of *nosZI* denitrifiers	Reduce N_2O emissions from denitrification; increase soil-available nitrate and plant productivity	[53–56]
Microbe-derived exudates				
Citrate, malate	AMF hyphal exudates	Trigger *nosZ* expression in *Pseudomonas fluorescens*	Reduce N_2O emission in bottle assay	[57]

[a]Abbreviations: AMF, arbuscular mycorrhizal fungi; AMO, ammonia monooxygenase; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; HAO, hydroxylamine oxidoreductase.

reduced N_2O emissions following 1,9-decanediol and syringic acid treatments in neutral paddy soil and acidic red soil, with recorded changes in soil NH_4^+ and dissolved organic carbon (DOC) content and altered AOA and AOB abundance [41,42]. In rice pot experiments, the application of MHPP eliminated ~60% of total N_2O emissions from a calcareous soil [43]. In the field, sorghum genetic stocks producing high amounts of sorgoleone suppressed soil N_2O emissions to a greater extent compared with low sorgoleone-producing genetic stocks [44], as well as high brachialactone-exuding *B. humidicola* [28] The direct application of MHPP along with root-zone fertilizers can inhibit 79% of N_2O emissions from rice fields [45]. The combination of MHPP and sorgoleone had a stronger effect on the reduction of N_2O emissions compared with MHPP or sorgoleone applied alone, and the effect manifested through a decrease in the abundance of AOA and AOB [46]. However, a potential risk of *de facto* increasing N_2O emissions through the addition of high concentrations of LN and LA has also been shown in a soil type with very high endogenous nitrification activity [47].

BNIs also have the potential to reduce N pollution that occurs as a consequence of leaching. Several studies examined MHPP, a phenylpropanoid that is widely available on the market. Application of MHPP decreased N leaching in pot experiments with rice and wheat [43,48]. The reduction of nitrate leaching by MHPP was stronger in calcareous soil than in acid soil [49]. When BNI MHPP was co-applied with the urease inhibitor *N*-(*n*-butyl), thiophosphoric triamide (NBPT) or with biochar, a synergistic effect was found on the reduction of N leaching in rice and wheat, accompanied by an enhancement of plant N uptake [43,48].

BNIs can also affect NH_3 volatilization, with large variation among BNI types. Two recent investigations showed that NH_3 volatilization was slightly enhanced by MHPP addition under a traditional surface-broadcasting regime in rice cropping [43,45], similar to the function of SNIs, which may increase the risk of NH_3 volatilization while reducing N_2O emissions [50]. However, more recent evidence suggests that BNIs, if applied judiciously, have the potential to simultaneously suppress NH_3 volatilization and N_2O emission. Some BNIs, such as syringic acid, LA, and LN, can inhibit urease activity, thereby delaying soil urea hydrolysis [32,36], the critical pathway leading to NH_3 volatilization. Furthermore, high BNI-exuding plant genotypes can promote N immobilization by maintaining higher microbial biomass and activity as well as denser root systems [51,52], which can also reduce NH_3 volatilization indirectly by increasing plant N acquisition, thus reducing substrate supply for microbial N transformation. ^{15}N-labeling experiments also showed that the BNI strength in root exudates was positively correlated with ammonium-use efficiency in 19 rice cultivars, indicating the potential of BNIs to increase N use efficiency (NUE) [31]. In addition to the direct regulation of BNIs, some agronomic measures can facilitate further reductions in NH_3 volatilization. For example, simultaneous inhibition of NH_3 volatilization and N_2O emission was observed when MHPP was applied to the root zone along with N fertilizer, the urease inhibitor NBPT, or biochar [43,45].

Plant roots can also release chemical substances that inhibit denitrification, known as **BDIs** [53]. Compared with our growing understanding of BNIs, information on specific BDI compounds remains sparse, possibly related to the biochemical and taxonomic diversity of denitrifying microorganisms. Only one BDI, a chemical belonging to the procyanidin class of flavonoid compounds, has been successfully identified, from root extracts of the invasive weed *Fallopia* spp. [54]. In a field experiment on lettuce vegetable crops, reductions in N_2O emission in the presence of procyanidin were accompanied by enhanced plant growth and plant N uptake [55]. This is possibly because procyanidins can inhibit nitrate reductase activity and the abundance of *nirS*- and *nirK*-type denitrifiers, as well as stimulate the growth of *nosZ*I-containing denitrifiers [56]. In addition to plant-derived exudates, carboxylates (such as citrate and malate) exuded by hyphae of arbuscular mycorrhizal fungi (AMF) have been shown to recruit denitrifying *Pseudomonas fluorescens* and trigger *nosZ* gene expression [57], thus reducing N_2O emission at rhizosphere soil microsites. This promising finding opens novel avenues to exploit cross-kingdom microbial interactions for sustainable low-carbon agriculture.

The role of rhizosphere exudates accelerating N removal from water

When excessive N from terrestrial ecosystems enters downstream waterbodies through leaching and runoff, inorganic N becomes one of the agricultural nonpoint-source pollutants causing eutrophication. Aquatic plants have been widely used in the biological purification of N-polluted waterbodies. There is clear evidence that interactions between aquatic plants and associated rhizosphere microorganisms can enhance the remediation of contaminants [58,59]. In addition to directly absorbing N, rhizosphere organisms can secrete specific chemical signals to enhance N removal rates through nitrification, denitrification, and anaerobic ammonia-oxidation processes (Table 3).

Duckweed is a small floating aquatic plant widely distributed in farmland ditches, ponds, and rivers. In a eutrophic water with high N content, two duckweed species, *Spirodela polyrrhiza* and *Lemna minor*, were shown to secrete the root exudates erucamide (a fatty acid amide) and *cis*-7-hexaenoic acid methyl ester (a fatty acid methyl ester) [60]. These two **BDPs** stimulated the N-removal efficacy of the denitrifier *P. fluorescens* by targeting both bacterial nitrate reductase (NAR) and nitrite reductase (NIR) [61]. When *Pseudomonas* spp. were attracted to the duckweed rhizosphere, stigmasterol secretion was induced from duckweed roots. This sterol further

Table 3. Types and mechanisms of small-molecule rhizosphere exudate involved in N removal in aquatic ecosystems[a]

Compound	Source	Mechanism	N pollution effect	Refs
Plant derived				
Erucamide	Duckweed root exudates	Stimulates NAR and NIR in bacteria	Stimulates N-removal efficacy of *Pseudomonas fluorescens*	[60,61]
Stigmasterol	Duckweed root exudates	Stimulates NIR and rhizosphere community composition of *nirS*- and *nirK*-type denitrifiers	Stimulates N-removal efficacy of duckweed systems	[62]
Microbe-derived				
C_4-HSL, C_6-HSL	Batch experiment	Induce gene expression in anammox bacteria	Improve N-removal rate	[72]
C_6-HSL	Biofermentor	Increases anammox bacteria activity	Increases ammonium removal	[73]
C_6-HSL, C_8-HSL	Moving bed biofilm reactor	Increase denitrogenation-related enzyme activities and gene function and abundance of QS bacteria; enhance biofilm formation	Simultaneously improve biofilm formation and N transformation	[74]
3-oxo-C_6-HSL, C_6-HSL, C_8-HSL, C_8-oxo-HSL	Sequencing batch reactor (SBR)	Regulate electron transport carriers and lysophosphatidylcholine metabolism; promote exopolysaccharides	Improve N removal rate and biomass aggregation	[75]
3-oxo-C_6-HSL	Granular sludge	Accelerates sludge aggregation by increasing growth, microbial activity, and extracellular protein	Facilitates sludge granulation process for nitritation during initial startup stage	[76]
C_{14}-HSL, 3-oxo-C_{14}-HSL	Activated sludge	Mediate AOA and AOB composition	Increase ammonium oxidation rate	[78]
Diffusion signal factor (DSF)	SBRs	Changes EPS and amino acid levels and community structure dynamics in anammox consortia	Enhances anammox activity; increases N-removal rate	[79]
C_6-HSL	*Paracoccus denitrificans*	Affects transcription of nitrite reductase and nitric oxide reductase genes in *Paracoccus denitrificans*	Suppresses N_2O accumulation in aerobic conditions; stimulates N_2O production in anaerobic conditions	[80]
2-Heptyl-3-hydroxy-4-quinolone	*Pseudomonas aeruginosa*	Increases NIR activity; inhibits NOR and nitrate respiratory chain activity	Suppresses NO_3^- reduction and N_2O production	[81]

[a]Abbreviations: EPS, extracellular polymeric substances; NAR, nitrate reductase; NIR, nitrite reductase; NOR, nitric oxide reductase.

strengthens the N-removal efficacy of duckweed wastewater treatment systems by a combination of effects on bacterial enzyme activity and the composition of the microbial community responsible for nitrite reduction [62]. Given that these plant-derived denitrification stimulators mostly act at low, micromolar doses, they are likely to act as chemical signals rather than as traditional carbon sources (e.g., unlike glucose or methanol) when participating in the purification of N-polluted water.

N-cycling microorganisms in aquatic ecosystems can also secrete chemical signals that enhance N removal from waterbodies. *N*-acyl-homoserine lactones (AHLs) are well-known signaling molecules involved in mediating **quorum sensing (QS)**, which has a key role in N metabolism, including nitrification, denitrification, and anaerobic ammonia/ammonium oxidation (anammox) [63,64]. The chemical structures of AHLs produced by nitrifiers and denitrifiers are diverse due to the presence of different AHL synthetases. For example, C_6-HSL, C_8-HSL, C_{10}-HSL, C_{10}-1-HSL, C_{14}-HSL, and 3-oxo-C_{14}-HSL were discovered in microbial exudates from nitrifying AOB and NOB [65–68], while denitrifying bacteria appear to secrete AHLs with shorter or longer side chains (C_4-HSL and C_{16}-HSL) [69,70]. Anammox bacteria were associated with the synthesis of C_6-HSL, 3-oxo-C_6-HSL, C_8-HSL, and 3-oxo-C_8-HSL [71].

In wastewater treatment systems, AHLs can accelerate biological N removal through different mechanisms. The nitrification and anammox activity could be enhanced by the addition of AHLs (C_4-HSL, C_6-HSL, and C_8-HSL) by affecting the enzyme activity and expression of key genes [72–74]. A second line of evidence from sequencing batch reactors and granular sludge systems showed that short- and medium-chain AHLs (C_6-HSL, C_8-HSL, 3-oxo-C_6-HSL, and 3-oxo-C_8-HSL) accelerated N-removal rates by inducing extracellular polysaccharide, biofilm formation, and sludge aggregation [74–76]. Furthermore, the functions of granular sludges or biofilms are strongly associated with microbial community structure, and there is increasing evidence that QS signals also have an important role in community structure assembly [77]. Long-chain C_{14}-HSL and 3-oxo-C_{14}-HSL improved the ammonia oxidation rate of activated sludge by changing the composition of the AOA rather than AOB microbial community [78]. Despite powerful intra-signals, the exogenous addition of an intersignal diffusion signal factor (DSF) could lead to a change in extracellular polymeric substances (EPS) and amino acid levels as well as community structure dynamics in anammox consortia and improve the N-removal rate of anammox reactors [79].

In addition to stimulating N removal, the presence of QS signals may also mitigate N_2O emission. For example, C_6-HSL suppressed N_2O accumulation under aerobic conditions, while it stimulated the production of N_2O under anaerobic conditions, by affecting the transcription of nitrite reductase and NO reductase genes of the denitrifying bacterium *Paracoccus denitrificans* [80]. Apart from AHLs, the quinolone QS signal 2-heptyl-3-hydroxy-4-quinolone was found to inhibit N_2O production of *P. aeruginosa* by inhibiting NO reductase activity via iron chelation [81]. Nevertheless, evidence for the regulation and mechanism of the QS signal in the context of N_2O production is limited thus far to incubation experiments and, therefore, mitigation in wastewater treatment systems warrants further study.

Application scenarios for rhizosphere exudates

Direct breeding and cultivation of BNI/BDI-enabled plants

Small-molecule rhizospheric exudates have an important role in reducing N emissions from croplands and in accelerating N removal from downstream waterbodies. If exploited properly, such chemical signals can serve as a nature-based green solution in efforts to reduce N footprints while maintaining crop yields. One application scenario is the deployment of already BNI/BDI-enabled species by co-cultivating them in croplands, grasslands, and forested areas (Figure 2, Key figure). The prerequisite for this application is the selection of suitable varieties from numerous germplasm accessions. This approach has been proposed as an N_2O-emission-mitigation strategy in intensively agricultural and grazed livestock systems [82]. Soils containing a *Brachiaria* cultivar with high BNI capacity emitted 60% less N_2O from urine patches compared with soils with low BNI-capacity cultivars [83]. A 90% suppression of N_2O emission was shown in field plots of *B. humidicola* (CIAT 16888) [28], although a more recent field trial reported an increase in grassland N_2O emissions under *B. humidicola* cultivation [84]. Key varieties of Guinea grass with high N_2O-reduction potential were identified under greenhouse conditions [85]. In crop systems, lower N_2O emissions from soils were found in sorghum genetic stocks with high levels of sorgoleone synthesis compared with those genetic stocks producing low sorgoleone amounts [44]. By contrast, field trials in temperate forests showed that *in situ* N_2O emissions were decoupled from the BNI-production capacity of the forest tree species [86]. Such differences in outcomes may be because, in addition to simple nitrification and denitrification rates, factors such as the ratio of (*nirS+nirK)/nosZ* genes in the soil ecosystem are also responsible for N_2O emissions from soils. In addition, the organisms responsible for nitrification and denitrification in forest soils can be distinct from those in grasslands and croplands, including, for instance, fungal communities not involved to a significant extent elsewhere [87,88]. Therefore, the conditions and mechanisms underlying the efficacy of BNI-enabled varieties will require detailed examination in different types of terrestrial ecosystem, with special regard to soil type, soil pH, porosity, oxygen availability, and the nature of soil-microbial communities.

Key figure

Application scenarios of small-molecule rhizosphere exudates to achieve low-carbon agriculture

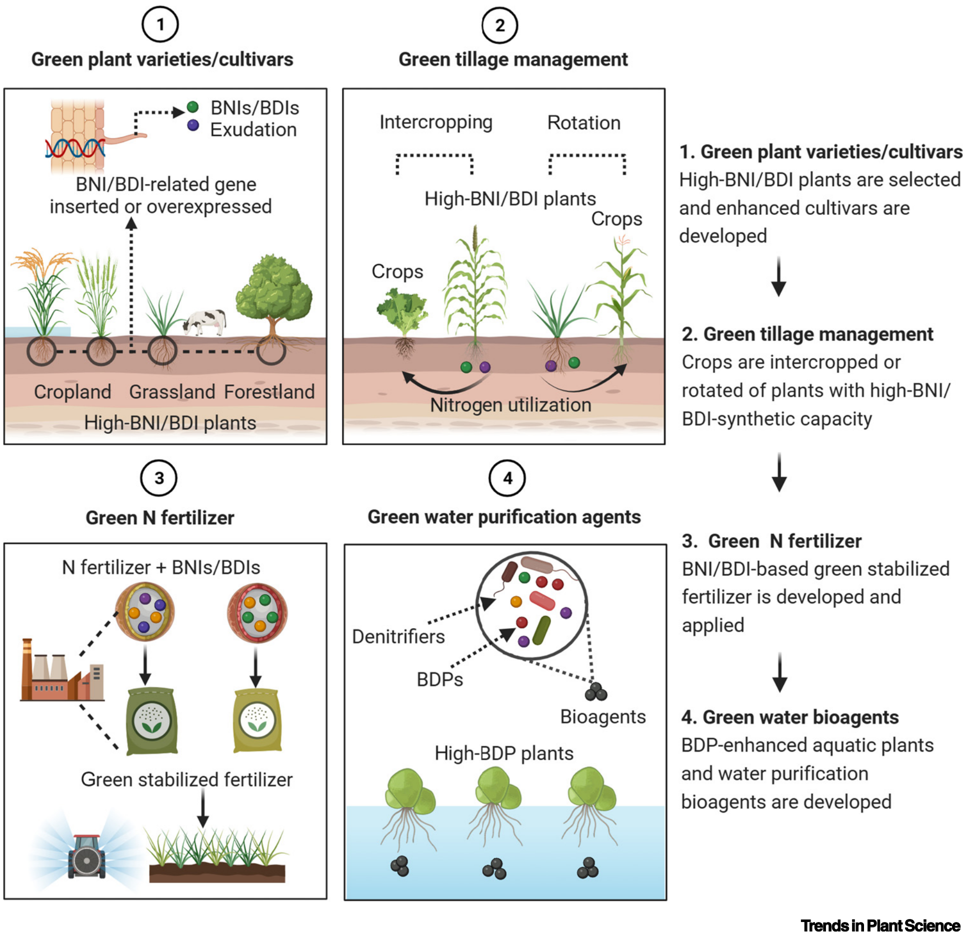

Figure 2. (1) High-biological nitrification inhibition (BNI)/biological denitrification inhibition (BDI) plant varieties are selected, and BNI/BDI-enhanced green crop cultivars can be developed via genetic engineering to optimize the synthesis and secretion of rhizosphere exudates [24]; (2) green tillage management involving intercropping or rotation of crops with high-BNI/BDI-synthetic capacity [46,91]; (3) addition of specific rhizosphere exudates (BNIs and BDIs) as green nitrogen (N)-fertilizer synergists to N fertilizer [32,46]; (4) when excessive N fertilizer is lost to aquatic environments, rhizosphere secretions can be applied as green water purification agents to remove N from eutrophic water [62], or biological denitrification promotion (BDP)-enhanced aquatic plants can be deployed. These technologies can be applied together to achieve lower carbon emissions.

Another application scenario is to breed and cultivate BNI/BDI-enhanced ‘green’ cultivars by optimizing the synthesis and secretion of rhizosphere exudates via genetic engineering (Figure 2). However, direct evidence for key candidate genes that control BNI activity or BNI secretion remains scarce. A recent transcriptomics and metabolomics study highlighted several key genes that may influence the synthesis of two newly identified BNIs (oxalic acid and protocatechuic aldehyde) in *Suaeda salsa* [89]. Subbarao *et al.* successfully transferred the 3NsbS chromosome arm that controls root nitrification-inhibitor production from a wild grass (*Leymus racemosus*) into an elite wheat cultivar, without disrupting its key agronomic features; the BNI-enabled wheat reduced N_2O emissions and improved grain yield and N uptake [24]. Compared with external additions of SNIs or BNIs, such green BNI-enabled or -enhanced plants have the potential to potentially lower agricultural operation costs without requiring synthetic production, shipping, and the complications arising from mechanical application [82]. Additionally, such varieties will have a longer lasting effect due to their direct and ongoing release of BNIs from roots [27,42], although the environmental factors influencing rates of synthesis and release need to be better understood. Given that the release of BNIs from plants typically occurs in deeper soil layers, it is possible that the use of green BNI-enhanced plant cultivars would have a dual role in suppressing NH_3 volatilization and N_2O emission.

Intercropping or rotation with high-BNI/BDI-enabled plants

Intercropping or rotation with high-BNI/BDI-enabled plants is a green tillage management strategy to lower N emissions and enhance crop yields. The turnover of rhizosphere exudates can be used in such a crop-rotation strategy. Sorghum (*Sorghum bicolor*), *B. humidicola,* and *Fallopia* spp. are good candidates as cover or intercrops, due to their sufficient release of BNIs or BDIs. When interplanting high-BNI sorghum, field assessments showed lower annual N_2O emission as well as benefits to yields of neighboring vegetable crops and maize [46,90]. In a pasture–maize rotation cropping system, significant residual effects of BNIs exuded by *B. humidicola* on N recovery and grain yield of subsequently cultivated maize were observed, although these were evident for less than 1 year [91]. By contrast, a sorghum–wheat rotation showed an increase of 77% in cumulative N_2O emission compared with monoculture wheat, probably due to a greater abundance of microbial heterotrophic-denitrification genes under soil with 45–60% water-filled pore space (WFPS) conditions [92]. Therefore, appropriate crop varieties and rotation type need to be considered carefully in the field.

Green N fertilizer based on BNIs and BDIs

Specific rhizosphere exudates can be directly delivered along with N fertilizer as effective synergists (Figure 2). This approach provides a more convenient and practical solution, while the above-mentioned plant screening and breeding programs should be viewed from a longer-term perspective. Urease and nitrification inhibitors are considered core components of recent stabilized fertilizer technologies. Compared with chemical SNIs, plant-derived BNIs have several advantages. The first is the dual inhibition of AOA and AOB by BNIs [28,32,37,38,41], that is, targeting a wider range of soil microorganisms and, therefore, being suitable for deployment in a greater variety of soil types. A second advantage is that some BNIs are able to suppress both nitrification and urea hydrolysis [32,36], which reduce both N_2O emission and NH_3 volatilization. Third, combinations of diverse BNIs may have a synergistic effect, which would enhance N utilization and emissions reduction while reducing the likelihood of the development of soil-microbial resistance [32,46]. Finally, the reported signaling role of low-dose BNIs suggests that they are involved in regulating plant root growth and development via the auxin and abscisic acid pathways [93,94]. These BNI characteristics render them promising substitutes or supplements for currently used nitrification or urease inhibitors. Their addition to fertilizer formulations is expected to add greatly to the arsenal available to the global stable fertilizer industry and to reduce the number of necessary N-fertilizer applications [27,41]. N fertilizers fortified with BNIs have shown a superior reduction in N_2O emission, especially in acidic soils dominated by AOA [41,42,49], where SNIs often have no effect [39]. Therefore, the development of new BNI/BDI-based N fertilizers is promising for marginal soils on which many traditional approaches have failed. However, currently known biological inhibitors also have several drawbacks, including a propensity for pyrolysis during production and easy decomposition in soils [37,41], but these are partially overcome by continuous secretion into the rhizosphere. Nevertheless, it will be necessary to develop technologies to slow the degradation of inhibitors, such as using green coating materials, nanomaterials, and designing equipment for large-scale inhibitor production.

Development of green water purification agents based on BDPs

When excess N from cropland, grassland, and forested areas is lost to aquatic environments, rhizosphere secretions can be applied as water purification agents to remove N from eutrophic water (Figure 2). The secretions serve as chemical signals at low dosage to facilitate the activity and community structure of N-cycling microorganisms. Such secretions can either be used alone or in combination with rhizospheric growth-promoting bacteria/denitrifying bacteria. For example, the combination of the root exudate stigmasterol and the rhizosphere denitrifying bacterium RWX31 had a synergistic effect on N removal in duckweed purification systems [62]. The application of the microbial QS signal C_6-HSL was shown to promote the granulation of granular sludge in wastewater treatment systems and to accelerate the start-up of bioreactors [95]. Compared with traditional purification agents (e.g., short-chain sugars and alcohols), low-dose rhizosphere exudates are considered promising as novel denitrification biostimulants that can avoid the clogging problems encountered in permeable reactive barriers [96], and may overcome the bottlenecks encountered with the often-long start-up times of bioreactors. Similar to high BNI-enabled plants, high-BDP aquatic plants can also be selected and deployed. The examples listed highlight the importance of rhizosphere exudates in enhancing the purification process of N-polluted wastewater.

Concluding remarks and future perspectives

The role of rhizosphere exudates in reducing N emissions and pollution from terrestrial and aquatic ecosystems is critical for plant productivity and environmental sustainability. These hidden small-molecule players in the rhizosphere target key components of microbial N-cycling processes and, therefore, can make substantial contributions to low-N and low-carbon practices. It is encouraging that plant BNI/BDI/BDP activity can be improved by altering the synthesis and secretion of rhizosphere exudates, and the possibility of generating environmentally friendly plants with enhanced BNI-BDI-BDP capacity through breeding or genetic engineering offers exciting prospects for tackling the issue. Progress in this area could be enhanced by identifying the candidate genes and mechanisms involved in BNI/BDI/BDP synthesis and release enabled by PANOMICS technology [97], such as genome-wide association analysis (GWAS), metatranscriptomics, and metabolomics. However, the desirable effects and concentrations of BNIs/BDIs/BDPs must be considered, because excessive exudate application can also inhibit plant root growth or threaten the diversity of beneficial microorganisms in soil [98], as well as increase plant carbon costs. A life-cycle assessment suggests positive impacts from BNI wheat with 40% nitrification inhibition by 2050. Planting such BNI wheat could allow for a 15% reduction in N fertilizer application, a 15.9% reduction in life-cycle-GHG emissions, and a 16.7% improvement in NUE at the farm level [99]. Thus, an optimum goal could be achieved with low input and environmental load while maintaining soil health through genetic engineering of BNI/BDI/BDP capacity.

Chemical signaling of rhizosphere organisms is not limited to a single direction, but extends to the bilateral interactions between roots and microorganisms (i.e., crosses interspecies and interkingdom boundaries). A recent study showed that the release of the BNI sorgoleone from sorghum, while inhibiting nitrifying bacteria, was associated with the establishment of a more intense mycorrhizal and fungal network [100]. In addition to the direct impacts on N-cycling microbes, downstream impacts of rhizosphere exudates on the composition and function of the entire microbial community warrant further investigation. It is important to better understand the nature of the feedback loops that rhizosphere exudates participate in in the rhizosphere. An

Outstanding questions

What are the main types of rhizosphere exudate that mitigate N emissions in different ecosystems? What are the underlying mechanisms and related control genes?

How do small-molecule rhizosphere exudates drive the N cycle and microbial community succession in different ecosystems? How do they create low N-emission environments?

What is the relationship between rhizosphere exudates and the environment? How do the synthesis and release of exudates adapt and change in different habitats? How do they reshape the rhizosphere structure of roots and rhizosphere microbes to achieve the dual goal of low carbon intensity and high yield?

How can the application of low-carbon technologies based on rhizosphere exudates be expanded?

understanding of the mechanisms of rhizosphere signal exchange and transmission will lay the foundation for a precise regulation of N emissions by rhizosphere signals in the future.

While a series of rhizosphere exudates have been identified as key synergists for the development of stabilized, 'green' fertilizers and water purification agents, the evaluation of their environmental impact has to date been based on limited soil types or plant species. There is a lack of robust evidence for the efficacy of rhizosphere exudates and the ability to predict N-emission reductions under a range of temporal and spatially variable conditions, especially in the field. Such studies are needed to produce a more realistic picture of where and how rhizosphere exudates influence N emissions in terrestrial and aquatic ecosystems. In addition, the cost and stability of rhizosphere exudates needs to be considered if they are to be market-friendly in the future. Green biosynthesis technologies of rhizosphere exudates are expected to reduce their production and application costs [101]. In addition to reducing N_2O emissions, carbon dioxide and methane removal from the atmosphere could also be enhanced by root exudates through soil carbon sequestration [45,90]. Thus, rhizosphere exudates are expected to contribute to carbon neutrality in a variety of ways, while preserving crop yield and crop quality. Overall, a deeper understanding of the underlying mechanisms by which rhizosphere exudates reduce N emissions and pollution as well as regional suitability and application measures, will be advantageous for both future crop productivity and development of low-carbon strategies that ensure environmental sustainability in a nature-based manner (see Outstanding questions).

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Declaration of interests
None declared by authors.

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