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# Soil microbial-root and microbial-rhizosphere processes to increase nitrogen availability and retention in agroecosystems

Louise E Jackson, Timothy M Bowles,
Amanda K Hodson and Cristina Lazcano

Increased reliance on biological processes and root interactions with soil biota through 'ecological intensification' in agroecosystems generates environmental benefits and decreases reliance on fossil-fuel based fertilizers. Here we give some examples of recent progress in understanding how agricultural management affects soil microbial-root and microbial-rhizosphere processes and nitrogen cycling. These include processes within the root (arbuscular mycorrhizal symbiosis), in the rhizosphere (biological inhibition of nitrification), and with the soil organisms surrounding the root (soil food webs in cultivated vs. grassland ecology). More such information will lead to new management options and site-specific solutions to cycle and retain nitrogen in agroecosystems.

**Address**
Department of Land, Air and Water Resources, University of California Davis, Davis, CA 95616, USA

Corresponding author: Jackson, Louise E (lejackson@ucdavis.edu)

## Introduction: rationale for ecological intensification of N processes in agriculture

Policy-driven nitrogen (N) assessments are demonstrating the need for new approaches to N management in agroecosystems, mainly owing to the off-site effects of fertilizers and manure on degradation of water quality [from leaching of nitrate ($NO_3^-$)] and stratospheric ozone depletion [from nitrous oxide ($N_2O$) produced via nitrification and denitrification (e.g. [1])]. A wealth of scientific literature already exists on these problems and on solutions that involve conservation and precision in water and fertilizer use, as well as organic matter management to increase nutrient cycling. Less emphasis has been placed on management to enhance ecological processes in the root zone to cycle and retain N within agroecosystems [2].

Recent meta-analyses have shown that high N fertilizer inputs and inorganic N pools are strongly associated with a decrease in soil microbial biomass and total N storage, along with an increase in net N mineralization rates and substantially increased N losses [3,4]. Both high nutrient inputs and tillage disrupt the soil food web [5]. But moderate levels of N fertilizer can indirectly increase soil organic matter by increasing plant growth and the litter returned to the soil [3,6]. Synthetic fertilizers have been efficient in increasing food production over the past few decades, but concerns for environmental quality, fossil fuel use, and socioeconomic vulnerability suggest that increasing reliance on biological processes and root interactions with soil biota through 'ecological intensification' are needed to overcome these problems [7•,8,9].

This paper focuses on how understanding the complexity of root-microbial-soil food web N interactions could increase options for ecologically based interventions to improve N availability and retention in agriculture (Figure 1). The emphasis is on N cycling rather than biological N fixation by the legume–rhizobia symbiosis [9]. Three levels of ecological processes are described using examples of recent findings from within the root, the rhizosphere, and the soil food web surrounding the root.

## Within the root: N at the fungal-root cell interface

One example of recent progress in understanding root N uptake and assimilation is with respect to the arbuscular mycorrhizal (AM) symbiosis; it occurs in >80% of land plants and provides N and phosphorus (P) to plants in exchange for carbon [10•], especially under low N and P availability [11]. Detailed studies of plant gene expression and genomics have provided insights into the dynamic regulation of genes involved in direct, non-mycorrhizal root N uptake as well as AM-specific Pi transport [12,13], but until recently there was little understanding of the complex interplay between roots and AM fungi for plant N uptake. The discovery of putative plant AM-specific ammonium ($NH_4^+$) transporters in the roots of several plant species [14–17,18••] provides a molecular basis for how AM fungi can increase plant N nutrition and confirms previous models of symbiotic N transfer. These transporters apparently operate at the root-fungal interface, the periarbuscular membrane [17], based on $NH_4^+$ transporter activity [14]. The

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### Figure 1
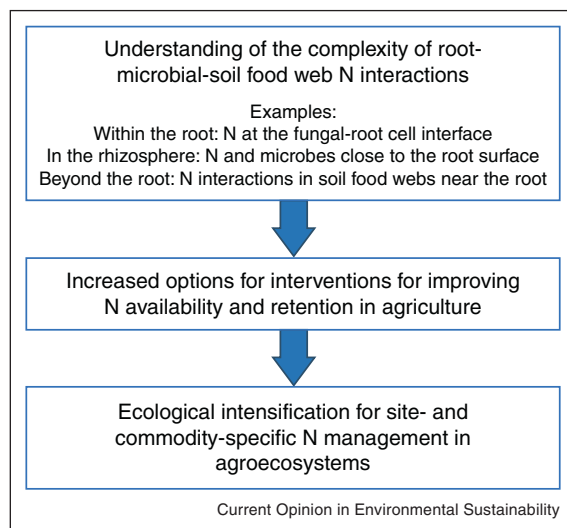


Framework for improving nitrogen management using ecological intensification, showing that understanding soil ecological interactions among biota and roots offers more options for managing nitrogen availability and retention, which can then be attuned to site-specific and commodity-specific aspects of actual agroecosystems.

currency of N exchange at this membrane is $NH_4^+$ (Figure 2); $NH_4^+$ is catabolized from arginine, the N form transported through fungal hyphae regardless of which form of N that AM fungi take up from soil [19].

Plants may increasingly rely on mycorrhizal-derived N rather than direct root N uptake when soil inorganic N ($NH_4^+$ and $NO_3^-$) is low, as suggested by patterns of gene expression at an organic farm using a mutant tomato with reduced mycorrhizal colonization [18••]. Compared to non-mycorrhizal tomato roots, mycorrhizal roots decreased their expression of genes involved in direct root uptake of inorganic N as well as $NO_3^-$ assimilation, but only at low levels of soil inorganic N. By contrast, genes for $NH_4^+$ assimilation had elevated expression levels in mycorrhizal roots under low soil inorganic N. Since AM-specific $NH_4^+$ transporters remained continually expressed, these transporters may have provided the additional plant N that stimulated $NH_4^+$ assimilation in mycorrhizal roots. Across three levels of N in this experiment, mycorrhizal plants had significantly higher shoot N assimilation and N concentrations compared to non-mycorrhizal plants. This experiment shows the importance of AM-supplied N for crop N nutrition in systems dominated by organic N mineralization compared to inorganic N fertilizer [11].

Mycorrhizal plants may better exploit situations with a variety of N sources than non-mycorrhizal plants, thus

### Figure 2
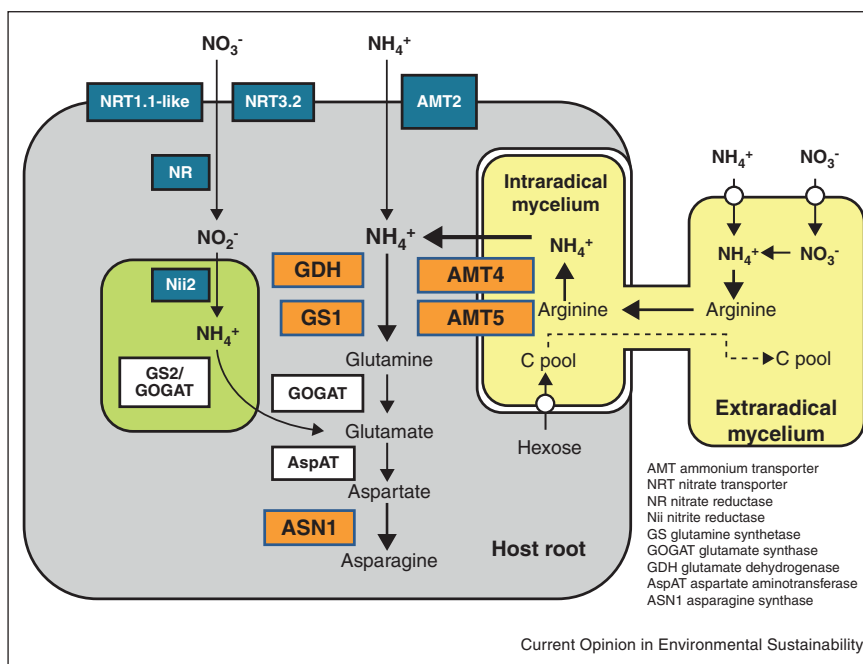


Model of plant root nitrogen uptake and assimilation in association with an active arbuscular mycorrhizal symbiosis under conditions of low inorganic N availability. From [18••]. Copyright permission obtained.

making them well-suited to systems that utilize organic matter for N availability. They can rely on both root and fungal encounters with highly mobile $NO_3^-$ and $NH_4^+$ as well as fungal exploitation of organic N. It is unknown if the diversity of AM fungi increases the exploitation of the various N forms resulting from microbial transformations of heterogeneous organic matter [20,21]. Increasing use of organic N additions and avoiding fungicide use [22] in agroecosystems are likely to maximize the benefits from AM fungi.

## In the rhizosphere: N and microbes close to the root surface

Intense interactions between roots and free-living microbes regulate much of the soil N cycle. Plants communicate with their most immediate environment, the rhizosphere, through root exudation of a diverse array of C-rich compounds [23]. Plant species (and even cultivars) have profoundly different patterns and rates of root exudation and as a result, different impacts on the composition and activity of their rhizosphere microbial communities [24•,25]. Plant species can increase biogeochemical cycling and N availability in the rhizosphere in different ways [25,26], but this has been little exploited in agriculture. In fact, for root exudates to increase the availability of inorganic N in the rhizosphere, microbial N mineralization depends on the availability of labile C and N, which is more abundant in soils managed with high organic matter inputs than with synthetic N

fertilizers [27]. Certain types of root exudates can also stimulate $N_2O$ reduction to $N_2$; future plant breeding may thus be able to manipulate the denitrification process for lower GHG production [28]. Retention of N may be enhanced when plants can compete well with nitrifying microorganisms (ammonia oxidizing bacteria and archaea) with traits such as rapid uptake, dense root systems, or regulation of oxygen availability in the rhizosphere [29,30] (Figure 3). Plant species effects on nitrification are particularly important because this is the microbial process that constitutes the main pathway of N loss in agroecosystems [28,31].

Bioassays using a recombinant luminescent bacteria (*Nitrosomonas europaea*), have shown that several plant species ranging from temperate and tropical pastures to some field crops (sorghum, pearl millet, groundnut, and rice) release significant amounts of nitrification inhibitors from their roots [32,33]. A chemical compound responsible for the biological inhibition of nitrification (BIN) has been recently isolated from the root exudates of the tropical forage grass *Brachiaria humidicola* [32]. This compound, called 'brachialactone', was found to inhibit between 60 and 90% of the nitrification activity in *Nitrosomonas europea*. Its main mechanism of action is to block the ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) enzymatic pathways (Figure 3). A suggested third inhibition mechanism is the disruption of the electronic transfer pathway of the
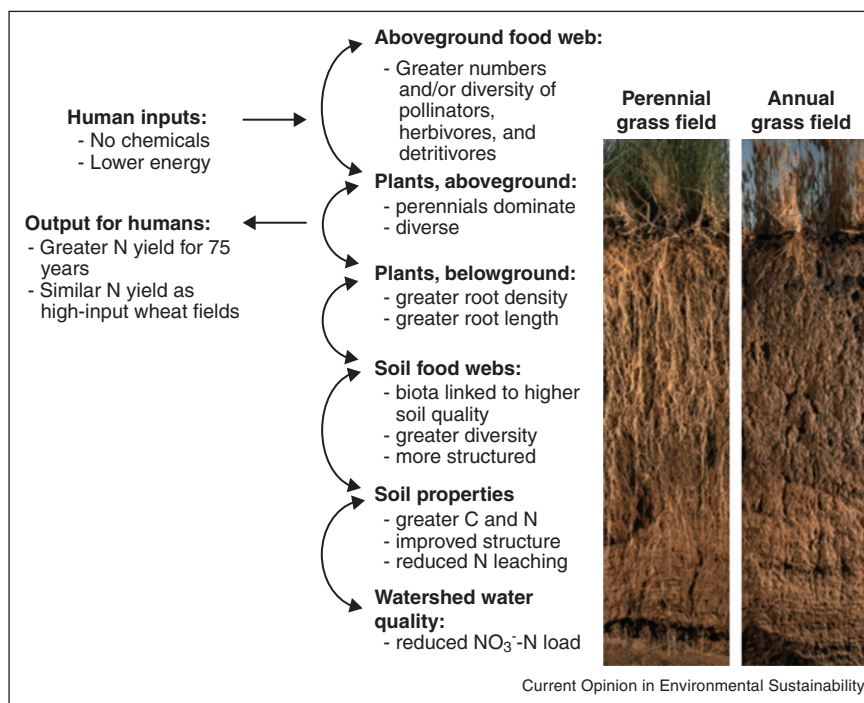
**Figure 3**
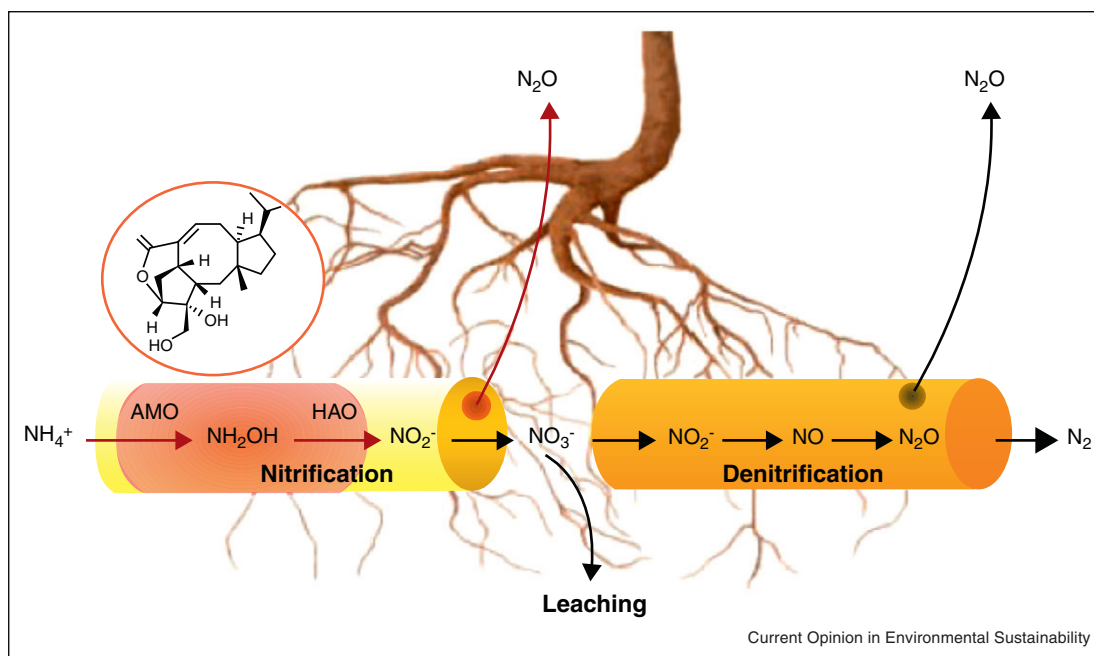


Overview of the enzymatic pathways (highlighted in red) inhibited by 'brachialactone' in the rhizosphere of *Brachiaria humidicola*. AMO: ammonia mono-oxygenase. HAO: hydroxylamine oxidoreductase. From [28]. Copyright permission obtained.

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**Figure 4**



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Ecosystem attributes of harvested perennial grass fields (in text on left side of figure) compared to annual wheat fields in the central United States. From [42**]. Copyright permission obtained.

cytochrome chain [34**]. By targeting three different enzymatic pathways, brachialactone is potentially more effective than single-action synthetic inhibitors and is also less vulnerable to any genetic changes in the population of soil nitrifying microorganisms [32].

Nitrification inhibition activity exists in some rice cultivars [33] and in a wild relative of wheat (*Leymus racemosus*) [35]. Genes conferring BIN capacity in *L. racemosus* have been located and were successfully introduced in cultivated wheat [34**,35]. However, subsequent field evaluation of BIN wheat showed higher susceptibility to rust disease [34**]. Not only must breeding avoid the introduction or expression of undesired genes that could negatively influence crop performance, but the influence of environmental factors on the expression and stability of nitrification inhibitors is still poorly understood [34**]. BIN activity is known to vary substantially with soil type [36,37]. BIN activity can be repressed in alkaline and heavy clay soils [34**] and it is speculated that nitrification inhibitors released by roots may be more easily degraded in soils with high organic matter and microbial activity [34**]. Understanding and exploiting BIN by plant roots offers an opportunity to shift to more $NH_4^+$-based systems, increase N use efficiency, and reduce environmental impacts associated with crop production [28].

## Beyond the root: N interactions in soil food webs near the root

Food web interactions increase N availability and retention in the soil surrounding plant roots. By decomposing plant material and gradually releasing N back to the soil, these interactions help synchronize nutrient supply with plant demand, causing, for example, increased plant growth without changes in soil inorganic N [38]. Grazing on rhizosphere bacteria by nematodes can enhance N availability and uptake by plants [39]. The structure of the soil community can also alter how litter composition influences N cycling, with larger macrofauna associated with greater N availability [40]. Overall, increased soil fauna biomass positively affects plant productivity both above and belowground [41•].

Studies on perennial cropping ecosystems show strong evidence of soil food webs maintaining N availability and increasing N retention. After 75 years, harvests of perennial hayed grasslands yielded similar levels of aboveground N compared to fertilized conventional wheat, while still maintaining significantly higher levels of total soil N [42**]. The perennial ecosystems had more microbial biomass, highly structured soil food webs, and significantly different communities of nematodes and bacteria [43] (Figure 4). Nematode communities, particularly, indicated higher levels of fungal

decomposition than in annual systems. The more complex fungal-based soil food webs were hypothesized to maintain soil fertility by synchronizing soil nutrients with plant demand, enabling consistent yields with few inputs and promoting high N retention. One possible mechanism is that higher levels of biological activity in the soil increased rates of N mineralization and N cycling [44]. The perennial ecosystems had lower N losses through denitrification and leaching, with $\approx 25\%$ less $NO_3^- $-N leached below rooting depths relative to annual agroecosystems [42$^{\bullet\bullet}$]. On a larger scale, an analysis of historical water quality and land use data showed that the fraction of the watershed in annual cropland vs. perennial grasslands significantly influenced riverine $NO_3^-$ concentrations [42$^{\bullet\bullet}$].

While soil food webs regulate the nutrients available to plants, they are also shaped by management decisions. For example, conversion from perennial grassland to wheat decreased the diversity of the soil community, even when management factors such as tillage were controlled [45]. After conversion, nematode communities were indicative of impoverished nutrient status and stressful conditions, perhaps owing to reduced root biomass, a readily accessible carbon source. These results suggest that the soil community responds negatively to factors like less root structure along with the pesticides, synthetic fertilizers, and tillage associated with annual cropping systems. Recent landscape-level surveys support these results, showing that increased agricultural intensification and high $NO_3^-$ is associated with decreases in soil biodiversity [46] and food web connectivity [47].

## Conclusions

To consistently supply and retain N, future farming strategies should aim to provide the soil ecosystem with sufficient resources to support N cycling, such as cover crops, incorporating more organic matter, or use of genetic resources to improve root responses. Multi-scale research will help explain the processes by which such management options are effective. This means greater emphasis on plant and microbial gene expression, ecophysiology, and rhizosphere ecology than at present. It is likely that intensification of these processes (i.e. ecological intensification) will require site-specific and commodity-specific interventions. There will not be a 'one size fits all' approach for increasing N cycling and retention, and thus innovation in both the research and agricultural sectors is needed to provide new options.

## Acknowledgements

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•• of outstanding interest

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