

Roots, Nitrogen Transformations, and Ecosystem Services

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Key Words

ammonium, arbuscular mycorrhizae, elevated carbon dioxide concentration, nitrate, nitrogen cycle, rhizosphere

Abstract

This review considers some of the mechanistic processes that involve roots in the soil nitrogen (N) cycle, and their implications for the ecological functions that retain N within ecosystems: 1) root signaling pathways for N transport systems, and feedback inhibition, especially for NO_3^- uptake; 2) dependence on the mycorrhizal and *Rhizobium*/legume symbioses and their tradeoffs for N acquisition; 3) soil factors that influence the supply of NH_4^+ and NO_3^- to roots and soil microbes; and 4) rhizosphere processes that increase N cycling and retention, such as priming effects and interactions with the soil food web. By integrating information on these plant-microbe-soil N processes across scales and disciplinary boundaries, we propose ideas for better manipulating ecological functions and processes by which the environment provides for human needs, i.e., ecosystem services. Emphasis is placed on agricultural systems, effects of N deposition in natural ecosystems, and ecosystem responses to elevated CO_2 concentrations. This shows the need for multiscale approaches to increase human dependence on a biologically based N supply.

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Ecosystem service: ecological functions and processes by which the environment provides for human needs

Plant-microbe-soil N cycling: the exchange and transformation of N among plants, microbes, soil, water, and atmospheric N pools

High-affinity transport system (HATS): operates at low N concentrations

Low-affinity transport system (LATS): operates at high N concentrations

INTRODUCTION

Roots influence the complex set of nitrogen (N) transformations that regulate the production, flow, and loss of N in ecosystems. The linkages among root physiology, activity of soil biota, and N availability occur at various scales, affecting plant productivity, N use efficiency, and ecosystem N retention. These processes thereby contribute to the provision of ecosystem services, i.e., ecological functions and processes by which the environment provides for human needs, such as food and fiber, soil quality, reduction of greenhouse gas emissions, clean water, and aesthetic values (23). Roots and N transformations can potentially play a larger role in the provision of ecosystem services, particularly in agricultural ecosystems, although adapting to climate change may require new insights into how plant physiology and soil processes are altered by elevated CO₂. Insofar as possible in this short article, our objective is to review the physiological and ecological functions that contribute to plant-microbe-soil N cycling, to integrate information on N processes

across disciplinary boundaries, and to set this information in the context of increasing the ecosystem services provided by root-soil N interactions.

PLANT AND SOIL N TRANSFORMATIONS

Plant Nitrogen Uptake

Plant N acquisition relies on transport systems in the plasma membrane of root cells, root system architecture, and mechanisms that regulate the activity of N transport systems and root growth according to plants' growth requirements. External factors, such as soil ammonium (NH₄⁺), nitrate (NO₃⁻), organic N compounds, soil pH, light, and temperature, as well as internal factors such as carbon (C) and N metabolites, are mediated by plants to regulate N uptake.

High-affinity transport systems (HATS) in roots are able to scavenge NH₄⁺ and NO₃⁻ from the soil at concentrations between 1 μM and 1 mM, whereas the activity of low-affinity transport systems (LATS) becomes evident when these ions are plentiful (above ~0.5 mM, a concentration exceeded at least temporarily in virtually all cropping systems). Physiological studies using ¹³N revealed the presence of a constitutive low-capacity and inductive high-capacity HATS for NO₃⁻ (47), whereas the NH₄⁺ transporters in rice (*Oryza sativa*) are considered to be constitutive (71).

Studies involving putative NO₃⁻ transporters, belonging to either of two classes of proteins encoded by the *NRT2* and *NRT1* (nitrate transporter) gene families (86), have elucidated the regulation of NO₃⁻ acquisition at the level of transcription. Exposure of roots to NO₃⁻ causes induction of HATS (iHATS) and a concurrent increase of *NRT2* transcripts, whereas N metabolites, most likely glutamine, downregulate HATS (120) (**Figure 1**). The presence of NH₄⁺ inhibits NO₃⁻ uptake (44, 46, 104). Experiments using ion-selective microelectrode techniques showed that in both rice and maize

(*Zea mays*), NO_3^- absorption is suppressed from the apex to 60 mm behind the apex when both NH_4^+ and NO_3^- are supplied (113). The mechanism underlying the immediate inhibition of NO_3^- absorption by NH_4^+ (74) remains unclear. In the longer term, products of NH_4^+ assimilation (again glutamine) may have a role as feedback inhibitors of NO_3^- uptake.

Ammonium uptake is controlled by transporters that differ in their affinity for NH_4^+ , their regulation at the level of transcription, and their localization of gene expression (43, 44, 93, 109, 110). The putative NH_4^+ transporter proteins are encoded by the *AMT1* and *AMT2* (ammonium transporter) families of genes (76). In contrast to NO_3^- uptake regulation, which is governed by signals integrating the N status of the whole plant, the regulation of HATS for NH_4^+ depends mainly on the endogenous N status of the root section in contact with the external NH_4^+ ; in *Arabidopsis thaliana* plants little compensatory NH_4^+ absorption occurs when NH_4^+ supply to other parts of the root system is restricted (43). Increased cytosolic or high external concentrations of NH_4^+ lead to reductions in root NH_4^+ influx (43, 44, 93). Gene expression of *AtAMT1;1*, which is upregulated in response to N starvation (44), is negatively correlated with root glutamine concentrations (93).

The influx of inorganic N via LATS seems poorly regulated, with a potential considerable cost in energy to plants. In solution culture, efflux of NO_3^- and NH_4^+ increases relative to external concentrations and influx of these ions (13, 102, 121). Nitrate efflux represents a waste of energy because NO_3^- uptake is an energy dependent process (8). Yet NO_3^- uptake with LATS shows linear responses to external concentrations to as high as 50 mM (103). At high external NH_4^+ concentrations, the membrane electrochemical potentials allow for passive influx of NH_4^+ , but efflux of NH_4^+ , against the electrochemical gradient, is energy intensive (13). At 10 mM external concentration of NH_4^+ , efflux from barley (*Hordeum vulgare*) cells constitutes up to 80%

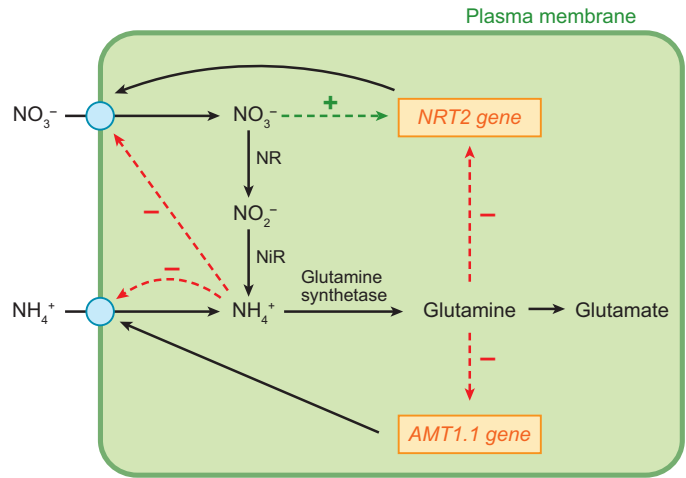


Figure 1

A model representing the proposed regulation of high-affinity transport systems (HATS) for nitrate (NO_3^-) and ammonium (NH_4^+). Glutamine represses the abundances of the putative *NRT2* and *AMT1* transcripts. Nitrate induces expression of *NRT2*. Cytosolic NH_4^+ suppresses NO_3^- and NH_4^+ uptake. Solid lines from *NRT2* and *AMT1.1* indicate transcription and translation leading to the increase of high-affinity transporters (circles) in the plasma membrane. Also shown are nitrate reductase (NR), nitrite (NO_2^-), nitrite reductase (NiR), glutamine synthetase, and glutamate. The diagram does not distinguish between plastidic and cytosolic nitrogen pools. Modified from Reference 48 with permission from Oxford University Press.

of primary influx. Root respiration attributed to efflux increases by 40% and is accompanied by a decline in growth. This inability to exclude NH_4^+ at the intake step seems to occur in other species that suffer NH_4^+ toxicity (83). In contrast, in rice, which is known to tolerate high levels of NH_4^+ (124), depolarization of the membrane occurs with increasing NH_4^+ provision, and the maximal NH_4^+ accumulation in the cytosol thereby decreases.

In addition to regulating uptake rates of nutrients, plants modify biomass allocation and root system architecture to optimize N capture efficiency with regard to C costs. Disproportionately more C must be allocated for the construction and maintenance of coarse roots, which grow rapidly and are relatively long-lived, than for the production of fine roots or root hairs, which are short-lived and must be replaced by new growth to maintain the same root length density (37).

Constitutive transport system: nutrient carrier system that operates regardless of prior presence of substrate

Induced transport system: nutrient carrier system that responds to exposure to specific substrate

Nutrient uptake models and their experimental validations show that varying root growth rate or root diameter affects plant capture of relatively immobile cations, such as potassium or NH_4^+ , more than the rate of uptake at the soil-root interface, whereas proportional changes of any of these three parameters affect the acquisition of the highly mobile NO_3^- similarly (105). In soil, NH_4^+ moves approximately 10 times more slowly than NO_3^- , and depletion zones for immobile cations typically form around the surface of roots (115). Thus, high root length density is more important for the capture of immobile ions than mobile ions (5).

Architectural plasticity in the form of localized root proliferation to nutrient availability and root biomass allocation from roots in nutrient-poor to those in nutrient-rich patches has been widely documented (29, 45, 51). However, for single plants or monocultures, it has been difficult to demonstrate that root proliferation in an N-rich volume of soil benefits plant growth and fitness because correlations between N capture from simple N sources, such as NO_3^- , and root length densities are often weak (36, 39, 61, 117). However, root proliferation may be important in capturing N from organic sources, in interspecific competition, and in competition with microbes (58). To maximize N capture when encountering a N-rich patch, an effectively competing root system would rapidly upregulate inflow (typical measured increases are 2–3 fold) and then increase root growth (98), which takes more time; e.g., for a range of grasses this would take approximately 35 days (60, 61).

The localized lateral root elongation of N-starved *Arabidopsis* plants in response to heterogeneous availability of NO_3^- is suppressed by high levels of N metabolites (125), and the expression of *ANRI*, a putative transcription factor of lateral root growth, decreases under conditions of N sufficiency and increases under N deprivation (42). The putative dual-affinity *Arabidopsis* NO_3^- transporter encoded by *NRT1.1* probably plays a role in the NO_3^-

signaling pathway for lateral root colonization of NO_3^- -rich patches, because neither glutamine nor NH_4^+ are able to restore lateral root growth in *NRT1.1* mutants (96). To date, no NH_4^+ sensory proteins have been discovered.

Much progress has been made in elucidating the regulation of plant biomass allocation and physiological responses. This knowledge must be combined with insight about the nature of the soil colonized by roots and other organisms to better understand N economy in the environment.

Microbial Symbioses

The colonization of roots by mycorrhizal fungi provides the plant with a well-distributed and extensive absorbing system in soil, and a greater chance of encountering fertile microsites not available to roots alone. The ability of mycorrhizal fungi to access small soil pores (28) and their morphologically plastic response to localized nutrient additions (19, 114) increase the availability of N to the plant. This is of particular significance in soils of low nutrient status and for immobile nutrients, such as NH_4^+ (3, 57).

Ectomycorrhizal fungal symbionts have NH_4^+ and NO_3^- transport systems, as well as NO_3^- and nitrite (NO_2^-) reductase genes (85). For arbuscular mycorrhizae (AM) fungi, which are endosymbionts, the N uptake mechanisms are largely unknown, but NH_4^+ is preferentially used. For example, corn plants colonized by *Glomus aggregatum* take up 10 times more N from a $^{15}\text{NH}_4^+$ patch than from a $^{15}\text{NO}_3^-$ patch (112).

Under high nutrient conditions, the formation of mycorrhizal associations may become a cost to the plant, because the plant is able to satisfy its own nutrient requirements (68). According to a meta-analysis of AM and ectomycorrhizal studies, colonization generally declines in response to N fertilization (116). Nevertheless, in an organic farming system, a mycorrhiza-defective tomato mutant has slightly lower N content than the

mycorrhizal wild-type (18), indicating that AM can increase N recovery in farming systems that do not use fungicides.

Ectomycorrhizal fungi can take up organic N compounds that often have slow diffusion, and thus uptake is facilitated by the greater soil access provided by hyphae (82). Mineralization of soil organic N by ectomycorrhizal fungi may be linked to the evolution of some species from saprophytes. Amino acid transporters have been identified in ectomycorrhizal fungi, and the K_m values are within the range of amino acid concentrations found in the soil (50). Genes encoding peptide and oligopeptide transporters and proteases have also been identified in ectomycorrhizae. Although AM fungi increase the recovery of N from ^{15}N -labeled decomposing plant residues in soil, it is unclear how much they rely on organic N, or the extent to which they accelerate decomposition of soil organic matter (59).

Biological N fixation, the conversion of N_2 to NH_4^+ , is accomplished by both free-living and endosymbiotic prokaryotes. For the *Rhizobium*/legume endosymbiosis, N uptake from the soil is less costly to the plant than maintaining the capacity to reduce atmospheric N_2 (75). Thus, under conditions of increased soil N, e.g., fertilization and atmospheric N deposition (34), dependence upon this symbiosis decreases. Nitrate generally has a greater inhibitory effect on N_2 fixation than NH_4^+ does (55). Under N limitation, higher rates of N_2 fixation occur in N_2 -fixing plants, and more N_2 -fixing plant species occur in natural ecosystems (55).

N_2 fixation is influenced by many environmental and edaphic factors, but temperature and available phosphorous (P) are two of the most important ones (55, 106). Under both low temperatures (e.g., in arctic and alpine regions) and high temperatures (e.g., due to nodulation failure) N_2 fixation and nodulation are increasingly hampered and N_2 fixation can be more affected than plant growth (55). Legumes have high requirements for P, and P acquisition is enhanced by AM, dense cluster roots, and organic acid secretion to

increase the availability and extraction of inorganic P, which in turn increases N-fixation capacity (73, 106). Legumes can increase the N status of neighboring plants and thus affect species distribution in grasslands (67), as well as improve the N status of subsequent crop plantings through decomposition and mineralization of N in plant material.

Soil Nitrogen Availability

The soil N cycle is driven by soil organic matter, which contains approximately 50% C and 5% N, of which typically <5% is in a labile form. Depolymerization of soil organic matter by extracellular enzymes, produced by C-limited fungi and bacteria, releases monomers, such as amino acids, which are recycled and reused through microbial metabolism, faunal grazing of microbes, and microbial death and damage that are caused by stress, such as from wet-dry or freeze-thaw cycles (99) (**Figure 2**). Root exudates, root turnover, and mycorrhizal turnover are other sources of compounds that increase the availability of labile C and N (4, 62). A key concept is that the C and N cycles are closely intertwined, and that soil C availability from root exudates and soil organic matter can drive the microbial processes that release soil N in plant-available forms.

Through mineralization, heterotrophic microbes break down organic monomers and release NH_4^+ , which can be used as an energy source by ammonia-oxidizing microbes to produce NO_2^- that is usually readily converted to NO_3^- (nitrification), and also to nitric oxide (NO) and nitrous oxide (N_2O) (49) (**Figure 2**). Nitric oxide reacts with volatile organic compounds to form ozone (O_3) in the troposphere, and it is also a precursor for nitric acid deposition via atmospheric transport. Nitrous oxide is a potent greenhouse gas, and it contributes to stratospheric O_3 destruction. Denitrification takes place when heterotrophic bacteria under oxygen limitation use NO_3^- as an alternative electron acceptor to produce N_2O and N_2 . The $\text{N}_2:\text{N}_2\text{O}$ ratio

Mineralization: the breakdown of organic matter into mineral forms, such as NH_4^+

Soil organic matter: living biomass, as well as dead and chemically transformed material of biological origin in soil

Nitrification: the microbial oxidation of NH_4^+ to form NO_3^-

Denitrification: the reduction of NO_3^- under anaerobic conditions to form N_2O and N_2

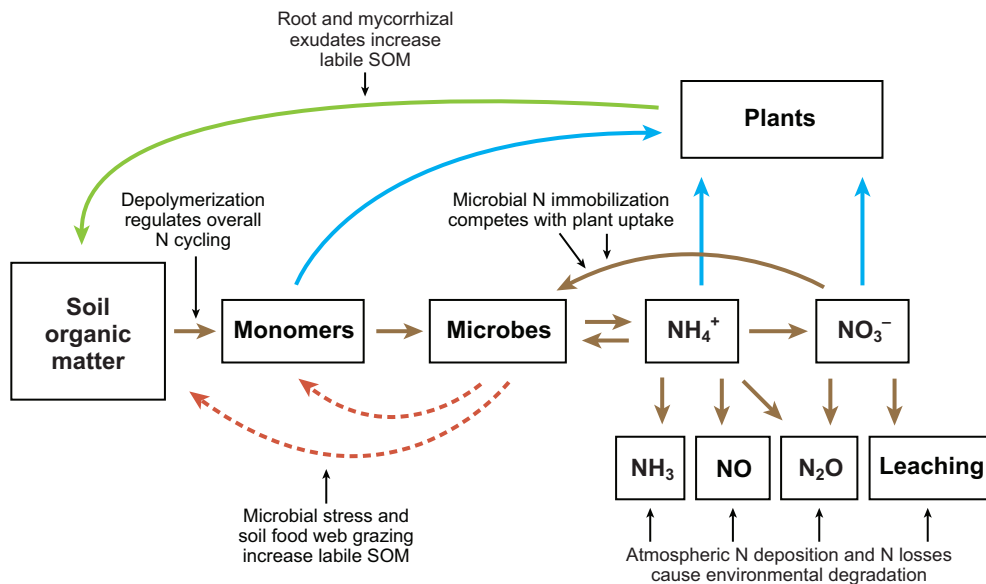


Figure 2

The soil nitrogen cycle, adapted from Schimel & Bennett (99) with permission. Mineralization refers to the microbial breakdown of organic N to form NH_4^+ . Only nonsymbiotic microbial N immobilization competes with plant N uptake. Nitrification is the microbial oxidation of NH_4^+ to form NO_3^- . Denitrification is the reduction of NO_3^- under anaerobic conditions to form N_2O and N_2 gas. SOM, soil organic matter.

increases with decreasing O_2 availability, and it often decreases under high NO_3^- availability (35). Emission of ammonia (NH_3) gas begins to increase at soil pH > 8, and ultimately contributes to N deposition elsewhere in the landscape. Leaching of NO_3^- , which contaminates groundwater, occurs in conditions of high water content, especially in coarse-textured soil. Runoff carries N in various forms to surface waters. The quantity of N in terrestrial ecosystems has more than doubled owing to industrial N fixation (Haber-Bosch process) in the last century, fossil fuel combustion, and cultivation of N-fixing crops. The yearly inputs of reactive forms of N through anthropogenic activities now exceed those from natural processes, and this has intensified N cycling and increased riverine exports of N (63) and atmospheric N emissions (41).

In almost all ecosystems, plants take up mainly NH_4^+ and NO_3^- , rather than amino acids or other monomers, which apparently

only play a role in extremely N-poor and cold ecosystems where N mineralization from soil organic matter is limited (100). Thus, the soil microbial processes of N mineralization and subsequent nitrification are important for the N supply to plants. Nitrification is inhibited at very low moisture, but increases with soil moisture up to -0.01 MPa, and then declines as the soil becomes saturated (107). Nitrification is more prevalent in tilled soils than in undisturbed soils (49). Mineralization is less sensitive to environmental conditions probably because so many different organisms in the soil food web are involved (see below). These processes also affect the fate of fertilizer N, some of which is taken up and metabolized by soil microbes (microbial N immobilization), and may be mineralized as the microbial biomass turns over.

Soil N availability to plants is closely tied to the labile C in the soil organic matter. For example, when plant litter with a high C:N ratio

is mixed with soil, there is an initial increase in microbial N immobilization, which decreases NH_4^+ and NO_3^- availability to plants; as microbial processing proceeds, the C:N ratio decreases (11).

Plants and soil microbes compete for NH_4^+ and NO_3^- . In short-term studies (one to several days in length), microbes often take up more ^{15}N -labeled inorganic N than plants do, presumably because they have higher substrate affinities, larger surface area to volume ratios, and faster growth rates than plants (58, 99). But after a month, and for even longer time periods, plants contain an increasing proportion of the added ^{15}N , because the gradual release of microbial ^{15}N into the soil becomes available for root uptake and plants hold on to N longer than microbes (54).

The actual availability of NH_4^+ and NO_3^- is difficult to measure in soil. When rates of microbial N transformations and/or plant uptake are rapid, then NH_4^+ or NO_3^- levels can be undetectable in soil (14, 65). These pools can turn over one to several times per day. Ideally, measurements of microbial population sizes or transcripts may eventually be used as indicators of rates of N transformations in soil, and thus N availability to plants, but the current molecular methods to determine population sizes of nitrifiers and denitrifiers (24) are not yet robust enough to serve this purpose.

Rhizosphere Nitrogen Interactions

Carbon availability increases microbial and microfaunal activity in the rhizosphere, soil that exists near or is influenced by the root. Water-soluble exudates, mainly glucose, amino acids, organic acids (33), and water-insoluble materials, such as sloughed-off cells and mucilage, supply the energy for enhanced biological activity. In this region, the exchange of a diverse array of compounds takes place between roots and microflora (**Figure 3**). Some of these interactions affect N cycling among plants, rhizosphere organisms, and nonliving organic matter in soil.

Small amounts ($10 \mu\text{g C g}^{-1}$ soil) of root exudates can cause the activation of microbial biomass in a substrate-poor soil environment (**Figure 4**). Such trigger solutions lead to 2–5 times more C as CO_2 evolution than is contained as C in the exudates, and to accelerated rates of mineralization of added cellulose (27). This priming effect may occur because part of the soil microbial biomass maintains an elevated adenosine triphosphate (ATP) and adenylate energy charge ratio (AEC) to take advantage of unpredictable substrate availability, such as root exudates. Estimates of root C exudates range from 0.5–1.5% of total photosynthesis in hydroponics (33) to 5–10% in soil (69). Aboveground grazing of *Poa pratensis* promotes C root exudation, which stimulates microbial biomass activity and 7 days later results in higher soil inorganic N, plant N uptake, leaf N uptake, and photosynthesis (52). In short-term (3 h) ^{15}N isotope pool dilution experiments, the rate of average gross N mineralization is 10 times higher in rhizosphere soil of microcosms with *Avena barbata* plants compared with the rates in adjacent bulk soil (>15 mm away from the root surface) (56). The higher N production rates were attributed to enhanced N mineralization from the soil organic matter by microbes, rather than microbial biomass turnover.

Root efflux of N, mainly as amino acids, is well documented (33), and microbial products can enhance the net loss of C and N from roots (89, 90). In axenic culture, treating roots of several crop species with compounds that are released by *Pseudomonas* bacteria and *Fusarium* fungi increases the net efflux of 16 amino acids by 200% to 2600% in 3 h (89).

Rhizosphere foodweb interactions affect plant growth directly and indirectly. Root C and N efflux stimulates microbial growth. Protozoa and bacterivorous nematodes graze on microorganisms and release NH_4^+ for uptake by roots because these predators have a low C assimilation efficiency and a higher C:N ratio than the bacterial prey (20). Environmental factors, such as moisture fluctuations in the rhizosphere, may also play a role in

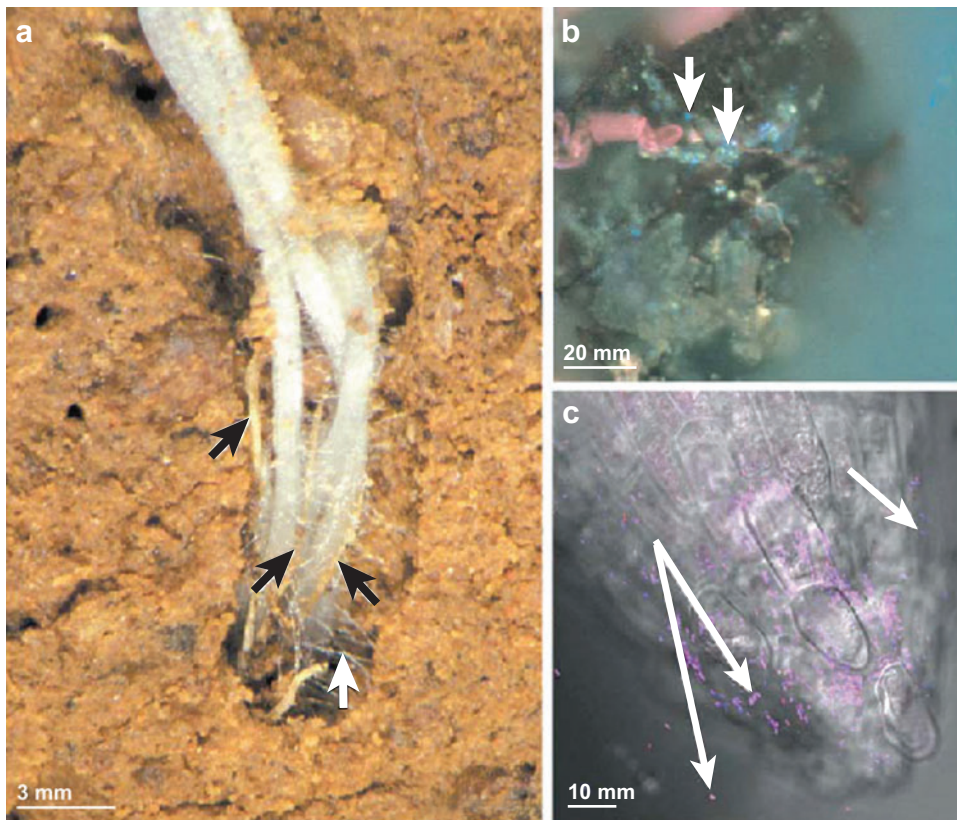


Figure 3

(a) Roots of canola growing into a soil pore, in close contact with each other and dead roots of wheat (black arrows). Root hairs (white arrow) extend from the canola roots to bind to soil and other living and dead remnant roots. Image credited to M.E. McCully. (b) Root hair of wheat associated with some dark soil organic matter, bacteria (bright blue spots, some indicated by arrows) and soil particles. (c) Tip of wheat seminal root growing on agar with *Pseudomonas* bacteria applied to the tip. Bacteria are hybridized to bacteria- and *Pseudomonas*-specific oligonucleotide probes that are conjugated to fluorescent dyes from Reference 122. Some bacteria are bound to the root cap, and others are retained in hydrated mucilage behind the tip (white arrows). From Reference 123 with permission from Oxford University Press.

stimulating N mineralization of soil organic matter (17). In addition to nutrient enhancement due to grazers, plant growth may also be promoted by complex mechanisms involving hormones released by rhizosphere organisms. For example, the presence of amoebae increased the size and branching of the root system of watercress seedlings (*Lepidium sativum*) and the proportion of bacteria that produced indolyl-3-acetic acid (IAA), which is known to influence root growth (9, 10) (Figure 5).

Some tropical pasture grasses directly influence soil N transformations through their release of nitrification inhibitors. Nitrification is suppressed by >90% in field soil under *Brachiaria humidicola* cultivation. The nitrification-inhibiting exudates, which blocked both the monooxygenase and hydroxylamino oxidoreductase pathways in *Nitrosomonas*, are more stable in a low pH (<4.5) environment and are released at three times higher rates when plants are grown with

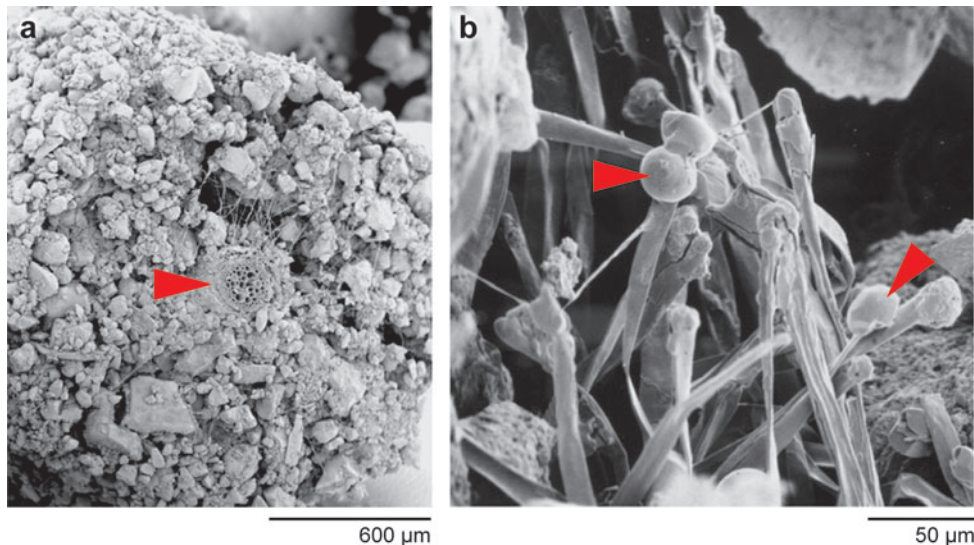


Figure 4

(a) The root/soil interface of a young root of buckwheat (*Fagopyron esculentum*). Root hairs extend into the rhizosphere and soil contact with the root varies. The arrowhead indicates the root epidermis. (b) Droplets of root exudate (arrowheads) on the tips of root hairs in the rhizosphere of broom corn (*Sorghum* spp.). The plant material was frozen in situ in the field with liquid N_2 and observed with a cryo-scanning electron microscope. From Reference 80, with permission.

NH_4^+ , rather than with NO_3^- (111). Several crop species show detectable amounts of these compounds in root exudates.

NITROGEN PROCESS INTEGRATION

Linking plant physiological and soil microbial N processes to ecosystem N cycling and N retention is a major challenge. By integrating across scales, the aggregate set of N processes controls ecological functions. Thus, scaling up from microsite processes ultimately determines ecosystem N flows, as suggested by the following examples that attempt to set plant-microbe-soil N cycling scenarios in an ecosystem context.

- 1) The regulation of plant N uptake systems and of root system architecture and biomass allocation reflect that plant communities evolved in nutrient-poor environments that were also colonized by bacteria, fungi, and fauna (92). Up-regulation of iHATS (48) and root

elongation when N-starved plants encounter NO_3^- may allow plants to effectively compete with other plant species and with microbes for spatially and temporally heterogeneously available N. To date, components of signaling pathways regulating root elongation in response to external signals were shown only for NO_3^- (38), although root proliferation also occurs when roots of N- or P-starved plants encounter NH_4^+ or phosphate, respectively (29). So, is the NO_3^- ion one among several cues signaling the presence of a nutrient-rich patch to the plant? Although NO_3^- can rapidly be absorbed because of its mobility in soil (98), the mineralization of organic N to NH_4^+ in a patch takes more time, as does the transfer of N between microbes, microfauna, and roots. Thus, NO_3^- may be an early and reliable signal that root proliferation will be a worthwhile investment by a plant. The

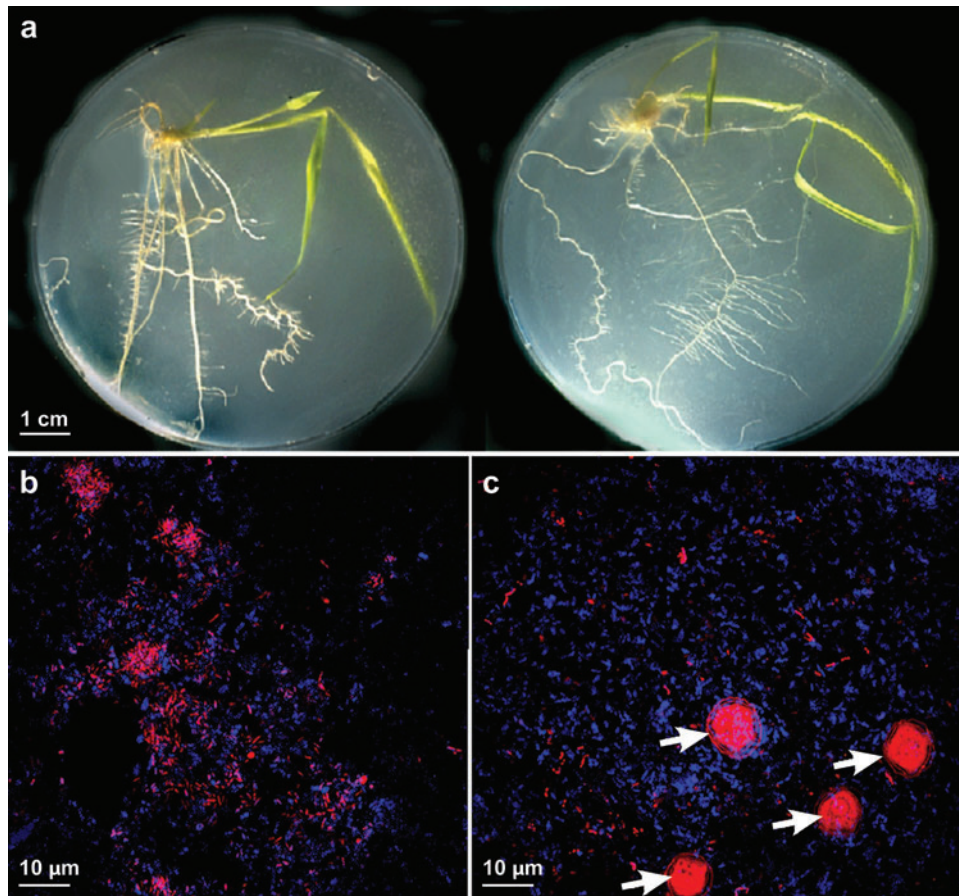


Figure 5

Protozoan effects on root architecture and bacterial communities in the rhizosphere of rice. (a) Root architecture of 16-day-old rice seedlings growing on agar inoculated with a diverse bacterial community in the absence (left) and presence (right) of amoebae. (b,c) Fluorescent in-situ hybridization of bacteria. Alpha-proteobacteria (red) and eubacteria (blue) on an agar surface near the lateral roots of plants in the absence (b) and presence (c) of amoebae. (c) Levels of alpha-proteobacteria decrease in the presence of amoebae. White arrows indicate red-fluorescent amoebal cysts. From Reference 9 with permission.

role of redundant signaling pathways for the activity of transport systems or root proliferation in agricultural soils is of special interest, because these soils can have high availability of NO_3^- , and yet even in fertilized crops, 40–80% of their N is derived from mineralization of N in soil organic matter (31).

- 2) In addition to rhizodeposition in the form of sloughed-off cells and root turnover, a substantial amount of C and N is released by roots in solu-

ble form (69). The exuded glucose and amino acids, which could alternatively be used for growth or seed production by plants, maintain a rhizosphere flora and fauna that in turn contributes to a consistent N supply for plants. Conversely, microorganisms benefit from high net primary productivity. Therefore, it is not surprising that in the rhizosphere, exchange of compounds that stimulate root C and N exudation or plant growth (88) has evolved. Other

Rhizodeposition:

the release of soluble and insoluble material from roots

symbioses, such as mycorrhizal associations or N₂ fixation, also have important roles in ecosystem N cycling. The recent awareness of exchanges of C, N, and hormones (10) in the rhizosphere will undoubtedly create greater interest in their ecological significance and their role in N retention at the ecosystem level.

- 3) Diel rhythms of plant and microbial N uptake may lead to increased N cycling in soil. Plant N uptake, and the genes encoding N uptake, show a diurnal periodicity increasing to a peak level at the end of the day, with highest amplitude on high irradiance days (47). This is probably related to tissue sugar rather than transpiration levels. At night, when transpiration stops, greater moisture availability could expose rhizosphere microbes to more substrate, stimulating microbial growth (17). The efflux of NO₃⁻ often exceeds influx in the dark (47). Higher rhizosphere N availability at night may coincide with higher microbial C availability. Furthermore, high microbial N demand at night might stimulate the secretion of long-lived exoenzymes (i.e., extracellular enzymes) that release N from soil organic matter. Some of this N could be usurped by the plant during its peak N demand during the day. Although diel transfers have not been proven, these hypothesized mechanisms illustrate a set of processes by which plants and rhizosphere microbes may have evolved to meet their N demands, and which simultaneously could retain N in the ecosystem by recycling N that might otherwise be susceptible to loss.

Understanding the coordination of plant-microbe-soil N cycling requires a greater emphasis on *in situ* studies in soil, and on framing experimental designs to determine ecosystem-level outcomes. In this way, physiological and ecological functions can be un-

derstood at different scales, and then serve as a basis for evaluating ecosystem services.

ECOSYSTEM SERVICES

Services and Tradeoffs

Ecosystem processes underpin ecosystem services: Plant-microbe-soil N cycling affects a diverse set of ecosystem services that meet human needs. These include provisioning services such as food and fiber production, regulating services such as waste decomposition and enhancement of water quality, longer-term supporting services such as soil formation and nutrient cycling, and cultural services such as aesthetic and spiritual fulfillment (81). The concept of ecosystem services allows us to evaluate multiple aspects of management decisions simultaneously. Although sometimes at odds (e.g., agricultural provisioning services have often outweighed regulating and supporting services), ecosystem services can be positively interlinked. If N management can balance ecosystem N budgets by maintaining ecosystem reservoirs of stored N, such that the sink capacity of the ecosystem contributes to plant nutrient supply and to plant-microbe-soil N cycling, then the need for surplus N additions that can cause environmental degradation decreases (30).

Provisioning Services

Fertilizer is not used in 40% of the world's agroecosystems (72), due either to lack of supply or lack of access by farmers (87). Application of fertilizers to infertile soils increases provisioning services (i.e., crop productivity on marginal lands), but it is likely to shift the dominant N cycling processes from dependence on root-microbe associations to higher nitrification and LATS pathways, and produces a somewhat greater potential for environmentally harmful N loss (**Figure 6**). In contrast, when N supply and access are high, N is typically overapplied, nitrification

Supply/access to fertilizer N

		Scarce	Adequate	Excess
N application	Underapplication to meet crop demand	<p>Dominant N processes: Low mineralization, but important for recycling N Low nitrification and denitrification BNF HATS (NH₄⁺ & NO₃⁻) Signaling pathways Recycling of rhizosphere N Mycorrhizal N uptake Root proliferation to scavenge N</p> <p>Ecosystem services: † Food and fiber † N loss, environmental degradation † C sequestration † Human nutrition</p>	<p>Ecosystem examples: Arid pastures or subsistence smallholder farms on poor soil such as sands</p>	<p>Under socio-political control: e.g., unfertilized pastures derived from forest or savanna that are abandoned when productivity declines</p>
	Adequate		<p>Dominant N processes: Moderate soil mineralization, nitrification & denitrification HATS & LATS (NH₄⁺ & NO₃⁻)</p> <p>Ecosystem services: † Food and fiber † N loss & environmental degradation † C sequestration † Human nutrition</p>	
	Overapplication to meet crop demand	<p>Under socio-political control: e.g., high N inputs applied to high value export crops but not subsistence crops</p>	<p>Ecosystem examples: Intensive cereal or vegetable production, on soils depleted in SOM due to tillage and low inputs of residues, manure, or compost</p>	<p>Dominant N processes: High soil mineralization, nitrification & denitrification LATS (NH₄⁺ & NO₃⁻)</p> <p>Ecosystem Services: †† Food and fiber †† N loss & environmental degradation † C sequestration †† Human nutrition</p>

Figure 6

Examples of the relationships between hypothesized dominant plant/soil N processes and ecosystem services in two types of agricultural scenarios affected by the supply and access to fertilizer N, and their decisions regarding the amount to apply. Adapted from Reference 87 with permission. The arrows show trajectories to attain optimal management. BNF, biological nitrogen fixation; HATS, high-affinity transport systems; LATS, low-affinity transport systems; SOM, soil organic matter.

is a dominant process, N cycles are open, and N losses are high. In this case, reduced fertilizer use can maintain high yields with lower N losses, and increase the provisioning services provided by plant-microbe-soil N transformations.

Most of the current agricultural emphasis on N is on fertilizer N use efficiency, largely in cereal grain production, either by optimal use of fertilizer form, rate, and

method, or by matching N supply with crop demand (84). Other approaches to increase fertilizer N use efficiency are agronomic practices that reduce crop stress and crop selection for greater yield stability, and thus result in higher and more consistent recovery of applied fertilizer N from year to year. None of these approaches focus on supplying N to crops via plant-microbe-soil N processes.

If there is societal interest in developing greater dependence on plant-microbe-soil N transformations for agricultural production (e.g., to reduce the fossil fuel used for production of N fertilizer by the Haber-Bosch process), then research approaches must better integrate plant physiology and soil microbial ecology. In improving crop N efficiency (yield of grain per unit available N), selection for genotypes that differ in their regulation of N uptake, N assimilation, and N recycling from vegetative to seed organs should be carried out under a variety of environmental conditions and N availability scenarios (53). Higher root biomass allocation (97), costs of symbiosis (108), and root exudates to fuel soil microbes may reduce crop productivity. Soil microbial community composition may be critical for rhizosphere N cycling, and it can depend on cultivar traits (31). For example, shifts in ammonia-oxidizing bacterial populations increase nitrification in the rhizoplane of a modern rice cultivar, and this cultivar benefits more from the coprovision of both NH_4^+ and NO_3^- than a traditional cultivar (12). Examples exist where selection eventually benefited crops owing to plant-microbe symbiosis, e.g., more efficient *Bradyrhizobium* strains that support higher levels of biological nitrogen fixation in soybeans (*Glycine max*) (2). Some forms of organic farming systems have soil N reservoirs and mycorrhizae that adequately support crop N demand (15, 18, 31). Clearly, the time is ripe to place greater emphasis on linking microbially mediated soil N transformations, plant-microbe interactions, and plant N uptake and utilization in agroecosystems.

Regulating and Supporting Services

N budgets exist for many ecosystems, and show the stocks of N in roots and soil, the amount of various N inputs, such as biological N fixation, and the net rates of N cycling through plants and soil (16, 22, 41, 66). However, a mechanistic understanding of plant-microbe-soil N cycling is often missing, but

is needed for the development of approaches that could increase regulating and supporting services, e.g., supply and mineralization of N from plant residues, C sequestration in woody plants and soil, reduced greenhouse gas emissions, and improved water quality.

One example is the deposition of N (i.e., the transfer of N from one ecosystem to another) that has affected many of the world's ecosystems and their services (81). Whereas NO_3^- pollution from agricultural runoff affects coastal, estuarine, and marine ecosystems (7), upland forests receive anthropogenic N inputs through wet and dry deposition (1). Atmospheric N deposition from urban areas now affects N cycling in natural ecosystems (34). The following examples show how an integrated understanding of plant-microbe-soil N transformations explains a decline in ecosystem services. In N-limited systems, such as evergreen and deciduous forests in the northeastern United States that have received excess N inputs (6–18-fold of background N deposition) for 15 years, most of the additional N was retained in the soil, whereas roots, foliage, and wood diminished as sinks for N, in spite of higher N concentrations in leaves and fine roots (78). Foliar N in pine (*Pinus resinosa*) needles accumulated as free amino acids, rather than as photosynthetic enzymes (6). Photosynthesis, tree growth, and fine root biomass declined, and mortality in pine stands after 15 years of N additions was 56%, but hardwood stands showed an increase in net primary productivity (6, 78). Nitrogen saturation altered chemical processes and microbially mediated processes in the soil. Mineralization of N in soil organic matter decreased, whereas gross nitrification and the production of NO increased (119) (Figure 7). These changes in soil N transformations were accompanied by acidification and probable cation losses, increases in NO_3^- leaching (1), and declines in soil fungal biomass and the levels of lignin-degrading enzymes (40). Microbial N immobilization did not occur to a great extent because of a lack of available C (1), and soil acidification diminished this

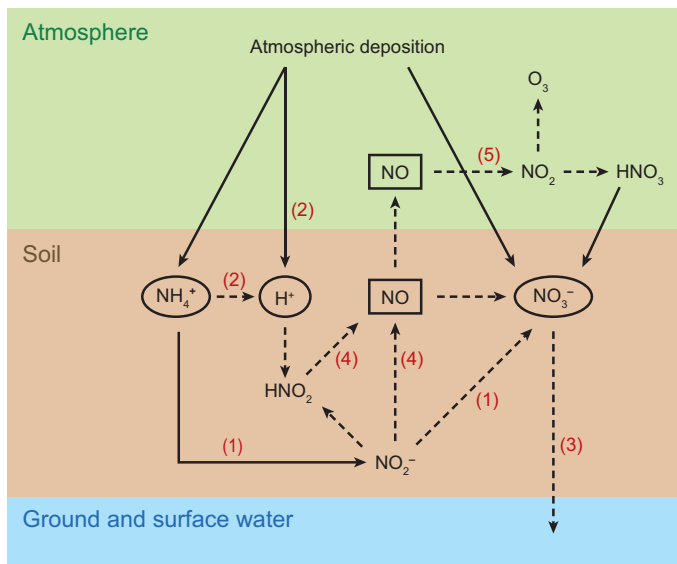


Figure 7

Illustration of the multiple influences of persistent atmospheric N deposition on pathways of N loss in forest soils, including increased (1) nitrification, (2) acidification, (3) NO_3^- availability and leaching, and (4) NO production via nitrous acid (HNO_2) decomposition and biological NO_2^- reduction. Also shown is (5) the role of NO emissions in contributing to local O_3 formation and downwind NO_3^- deposition. From Reference 118 with permission.

process further (119). This set of cascading, interrelated changes in N cycling illustrates how chronic N additions lead to ecosystem responses that depend on plant species composition, soil mineralogy, and precipitation patterns (79).

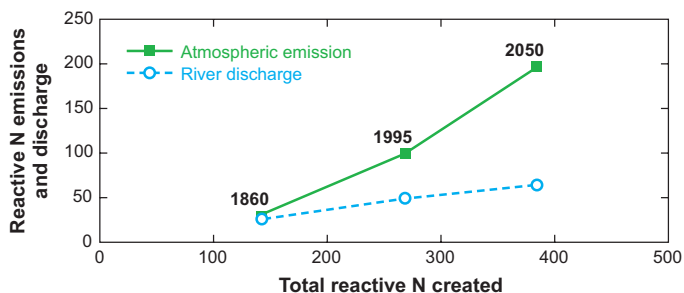


Figure 8

Reactive N creation in 1860, early 1990s, and projected in 2050 as a function of atmospheric emissions of NO_x + NH_3 and riverine reactive N discharge to the coastal zone (Tg N yr^{-1}). From Reference 41 with permission.

Land use change is another factor that reduces the regulating and supporting services provided by plant-microbe-soil N transformations in natural ecosystems. For example, between 1860 and the early 1990s, the amount of reactive N produced by natural terrestrial processes, such as biological N fixation, decreased by 15% (120 to $\sim 107 \text{ Tg N yr}^{-1}$), while anthropogenic production increased ~ 10 fold (~ 15 to 156 Tg N yr^{-1}) (41) (**Figure 8**). Agricultural intensification, i.e., use of high levels of nonrenewable inputs such as inorganic fertilizers, has greatly increased productivity as well as the leakiness of N cycling. Global estimates are that $\sim 50\%$ of applied N fertilizer is removed by the crop, 2–5% is stored as soil N, $\sim 25\%$ is emitted to the atmosphere, and $\sim 20\%$ is discharged to aquatic systems (41). Thus, nearly 50% of N applied as fertilizer has a negative effect on regulating and supporting services that support environmental quality.

Elevated Atmospheric CO_2

Nitrogen availability will become increasingly important for ecosystem services as atmospheric CO_2 increases. Several long-term free air CO_2 enrichment (FACE) experiments in grasslands with and without N additions showed a declining stimulation of plant biomass production with time when no N was added (32, 94, 101). Across a number of types of studies, the absence of N additions, gross N mineralization, and available N are either unchanged or decline under elevated compared with ambient CO_2 conditions (26, 64, 95). These results imply that over the long term, growth stimulation of non-legumes by higher levels of CO_2 cannot be sustained without N additions.

Responses to elevated CO_2 depend on plant functional types and the form of available N. Most plants that employ the C_3 photosynthetic pathway respond to elevated CO_2 with increased rates of photosynthesis and growth, mainly because photorespiration is reduced, whereas C_4 plants show

little stimulation. Biomass tissue N concentrations decline on average by 16% and 7% for C₃ and C₄ or N-fixing plants, respectively, when CO₂ concentrations are doubled (21). The decline in N, soluble protein, and Rubisco (ribulose 1,5-bisphosphate carboxylase/oxidase) concentrations, which is sometimes interpreted as a dilution effect, may also be due to a decrease in shoot NO₃⁻ assimilation by C₃ plants that results from suppression of photorespiration under elevated CO₂ (91). Nitrogen rhizodeposition under elevated CO₂ may also differ between C₃ and C₄ plants. More ¹⁵N fed to leaves of wheat plants as ¹⁵NO₃⁻ ends up in the soil, microbial biomass, and in unlabeled N receiver plants than under ambient CO₂ conditions, whereas no differences in N release are observed for corn (25). Nitrogen fixation increases under elevated CO₂, although this response is also dependent on the availability of other nutrients (26). Higher yields of effectively nodulating soybean cultivars under elevated CO₂ demonstrate the advantage of NH₄⁺ availability through N₂ fixation because near-isogenic ineffectively nodulating lines have lower yields even with high N fertilization (77). Thus, N availability, as well as the proportion of soil NH₄⁺ versus NO₃⁻, can be expected to influence plant responses to elevated CO₂.

As atmospheric CO₂ concentrations increase, N fertilization in cropping systems will tend to increase yields and maintain grain protein levels (70), with the inherent risk of also increasing environmentally harmful N losses. Cropping systems that receive organic matter, rather than only fertilizer N inputs, and thus, show high microbial activity and continuous N mineralization-immobilization dynamics, may have fewer detrimental side-effects while still supplying adequate N in a mixture of plant-available forms.

CONCLUSIONS

Our long-range challenge is to increase the utilization of plant-microbe-soil N transformations to increase a range of ecosystem services, including provisioning, regulating, and supporting services. Optimizing food and fiber production must be balanced with concerns about N pollution that can affect public health and the preservation of natural resources. It is in the public interest to increase awareness of the need for a biologically based N supply. There is a need to foster the collaborations among plant biologists, microbiologists, ecologists, and soil scientists that will link advances in plant N physiology and soil microbial N processes with N flows and fates at the ecosystem level.

SUMMARY POINTS

1. Nitrate uptake is regulated by the whole plant N status, with glutamine acting as a putative feedback signal for the high-affinity transport system, whereas uptake of NH₄⁺ via the high-affinity transport system is under localized root control. High influx of NO₃⁻ and NH₄⁺ via low-affinity transport systems is accompanied by high efflux.
2. Higher root length density is more important for the capture of immobile (NH₄⁺) than mobile (NO₃⁻) ions in soil, but may improve competition for N with other plants and with soil microbes for both ions.
3. Mycorrhizal fungi increase the availability of N to the plant owing to their access to small soil pores, plastic response to localized nutrient addition, and for ectomycorrhizae, the ability to increase N mineralization from soil organic matter.

4. Labile C is an important resource in the soil because it drives microbial processes that control rates of N transformations, and thus, availability of N to plant roots. One source of labile C is root exudates and sloughed-off cells; another source is the labile C in soil organic matter.
5. Food web interactions in the rhizosphere, which increase N availability and plant growth, are controlled by plant C and N efflux or exudates that inhibit nitrification and by microbial products that promote plant growth or the release of C and N from roots.
6. The integration of plant-microbe-soil N processes requires that plant physiological processes are linked to soil microbial N processes, and are studied within the context of ecosystem N cycling and N retention. As a result, ecological functions and processes by which the environment provides for human needs (i.e., ecosystem services) can be assessed.
7. Better utilization of plant-microbe-soil N transformations can potentially increase a range of ecosystem services, including provisioning (related to food and fiber production) and regulating and supporting services (e.g., decomposition and nutrient cycling that supports water and soil quality), but will require increased public interest in the need for a biologically based N supply, especially in relation to elevated atmospheric CO₂, which will likely require greater N inputs to sustain food production.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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