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Chapter 14

Nitrogen Transformations

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Chapter Contents

I INTRODUCTION

No other element essential for life takes as many forms in soil as nitrogen (N), and transformations among these forms are mostly mediated by microbes. Soil microbiology thus plays yet another crucial role in ecosystem function: in most terrestrial ecosystems N limits plant growth, and thus net primary production—the productive capacity of the ecosystem—can be regulated by the rates at which soil microbes transform N to plant-usable forms. Several forms of N are also pollutants, so soil microbial transformations of N also affect human and environmental health, sometimes far distant from the microbes that performed the transformation. Understanding N transformations and the soil microbes that perform them is thus essential for understanding and managing ecosystem health and productivity.

Nitrogen takes nine different chemical forms in soil corresponding to different oxidative states (Table 14.1). Dinitrogen gas (N_2) comprises 79% of our atmosphere and is by far the most abundant form of N in the biosphere, but it is unusable by most organisms, including plants. Biological N_2 fixation, whereby N_2 is transformed to organic N (described in Chapter 15), is the

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TABLE 14.1 Main Forms of Nitrogen in Soil and Their Oxidation States

Name	Chemical Formula	Oxidation State
Nitrate	NO_3^-	+5
Nitrogen dioxide (g)	NO_2	+4
Nitrite	NO_2^-	+3
Nitric oxide (g)	NO	+2
Nitrous oxide (g)	N_2O	+1
Dinitrogen (g)	N_2	0
Ammonia (g)	NH_3	-3
Ammonium	NH_4^+	-3
Organic N	R_{NH_3}	-3

Gases (g) occur both free in the soil atmosphere as well as dissolved in soil water.

dominant natural process by which N enters soil biological pools. All subsequent soil N transformations are covered in this chapter: (1) *N mineralization*, which is the conversion of organic-N to inorganic forms; (2) *N immobilization*, which is the uptake or assimilation of inorganic N forms by microbes and other soil organisms; (3) *nitrification*, which is the conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and then nitrate (NO_3^-); and (4) *denitrification*, which is the conversion of nitrate to nitrous oxide (N_2O) and to dinitrogen gas (N_2). Other forms of N (Table 14.1) are involved in these conversions primarily as intermediaries, and during conversion they can escape to the environment, where they can participate in chemical reactions or are transported elsewhere for further reactions.

Löhnis (1913) first formulated the concept of the N cycle, which formalizes the notion that N is converted from one form to another in an orderly and predictable fashion (Fig. 14.1), and that at global scale, the same amount of dinitrogen gas that is fixed each year by N_2 fixation must either be permanently stored in deep ocean sediments or converted back to N_2 gas via denitrification to maintain atmospheric equilibrium.

The fact that N_2 fixation—both biological and industrial—now far outpaces historical rates of denitrification is the principal reason N has become a major pollutant (Galloway et al., 2008). Making managed ecosystems more N conservative and removing N from wastewater streams, such as urban and industrial effluents, are major environmental challenges that require a fundamental knowledge of soil microbial N transformations (Robertson and Vitousek, 2009).

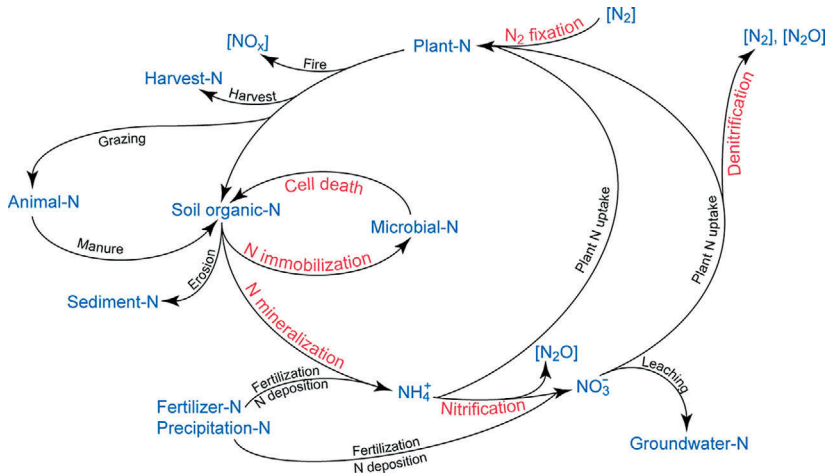

FIG. 14.1 Schematic representation of the major elements of the terrestrial nitrogen cycle. Those processes mediated by soil microbes appear in red. Gases appear in brackets.

Although the microbiology, physiology, and biochemistry of N cycle processes have been studied for over a century, much of our understanding of the N cycle has been derived from molecular and organismal scale studies in the laboratory. Laboratory observations and experiments have characterized the nature and regulation of the processes discussed in this chapter, but their reductionist nature has caused us to sometimes overlook the surprising possibilities for microbial activity in nature, thus impairing our ability to understand the ecological significance of these processes. The occurrence of denitrification (an anaerobic process) in dry and even desert soils is one example: theory and years of laboratory work suggest that denitrification ought to occur only in wetland and muck soils, but when new field-based methods became available in the 1970s, it became clear that almost all soils support active denitrifiers.

Key problems have also arisen from evaluating microbial N cycle processes in isolation from other biogeochemical processes (e.g., carbon (C) metabolism and plant nutrient uptake). This has resulted in an underestimation of the physiological flexibility of bacteria and archaea in nature (e.g., nitrifying denitrifiers, aerobic denitrifiers, *anaerobic ammonium oxidation* (anammox)). The disconnect between laboratory-derived knowledge and what actually occurs in the field is a problem throughout soil microbial ecology, but is perhaps most acute in the area of N cycling, which has great practical importance at field, landscape, regional, and global scales. When we attempt to increase information from the microbial scale to address important questions relating to plant growth, water pollution, and atmospheric chemistry at ecosystem, landscape, and regional scales, this problem becomes especially obvious and significant.

II NITROGEN MINERALIZATION AND IMMOBILIZATION

A critical process in any nutrient cycle is the conversion of organic forms of nutrients in dead biomass (detritus) into simpler, soluble forms that can be taken up again by plants and microbes. This conversion is carried out by microbes and other soil organisms that release, or mineralize, nutrients as a by-product of their consumption of detritus. Although microbes consume detritus primarily for a source of energy and C to support their growth, they also have a need for nutrients, especially N, to assemble proteins, nucleic acids, and other cellular components. If plant detritus is rich in N, microbial needs are easily met, and N release, or mineralization, proceeds. If plant detritus is low in N, microbes must scavenge inorganic N from their surroundings, leading to immobilization of N in their biomass.

The key to understanding mineralization-immobilization is to "think like a microbe," that is, attempt to make a living by obtaining energy and C from detritus. Sometimes the detritus has all the N that the microbe needs, so as C is consumed, any extra N is released (mineralized) to the soil solution. Sometimes the detritus does not have enough N to meet microbial needs, so as C is consumed, additional N must be immobilized from the soil solution. It has been shown that microbes invest more energy in the synthesis of enzymes (e.g., amidases to acquire N and phosphatases to acquire P) to obtain nutrients that they need when decomposing substrates of low quality. Microbial N uptake is also affected by organism growth efficiency. Fungi have wider C:N ratios in their tissues than bacteria and archaea and can grow more efficiently on low N substrates.

Mineralization results in an increase, whereas immobilization results in a decrease in plant available forms of N in the soil. Traditionally, ammonium has been viewed as the immediate product of mineralization, and in the older literature, mineralization is often referred to as ammonification. More recently, recognition of the fact that plants can take up simple, soluble organic forms of nutrients leads us to broaden our definition of mineralization products to include any simple, soluble forms of N that can be taken up by plants (see Schimel and Bennett, 2004). Plants from a variety of habitats have been shown to take up amino acids and other organic N forms; mycorrhizae can play a role in this uptake by absorbing amino acids, amino sugars, peptides, proteins, and chitin that are then used by their hosts as an N source.

Mineralization and immobilization occur at the same time within relatively small volumes of soil. Whereas one group of microbes might be consuming a protein-rich and therefore N-rich piece of organic matter (think seed or leguminous leaf tissue), another group, perhaps <100 μm away, might be consuming detritus rich in C, but low in N (think leaf stalk or wood). The first group is mineralizing N, while the second is immobilizing it, perhaps even immobilizing the same N that is being mineralized by the first. As a result of the simultaneous nature and small scale of these processes, it is important to make a distinction between gross and net mineralization and immobilization. Gross N mineralization is the total amount of soluble N produced by microorganisms, and gross N

immobilization is the total amount of soluble N consumed. Net N mineralization is the balance between the two. When gross mineralization exceeds gross immobilization, inorganic N in the soil increases (i.e., there is net mineralization). When gross immobilization exceeds gross mineralization, inorganic N in the soil decreases (i.e., there is net immobilization).

Soil fauna also play an important role in mineralization and immobilization processes. They are responsible for much of the preliminary decomposition of detritus, they feed on and can regulate populations of bacteria and fungi, and they can create or modify habitats for a wide array of organisms. For example, earthworms create burrows, isopods shred leaf litter, and termites macerate wood. All heterotrophic soil organisms consume organic materials for energy and C and, at the same time, immobilize and mineralize N.

The widely distributed nature of mineralization and immobilization processes means that the environmental regulation of these processes is relatively straightforward. Rates of activity increase with temperature and are optimal at intermediate soil water contents, similar to respiration, as seen in Fig. 14.2, although it is important to recognize that significant activity often occurs at extremes of both temperature and moisture. In most soils, the quantity and quality of detrital inputs are the main factors that control the rates and patterns of mineralization and immobilization. When moisture and temperature are favorable, large inputs of organic matter lead to high rates of microbial activity and the potential for high rates of mineralization and immobilization. However, in soils that are waterlogged or very cold (think wetlands or Arctic tundra),

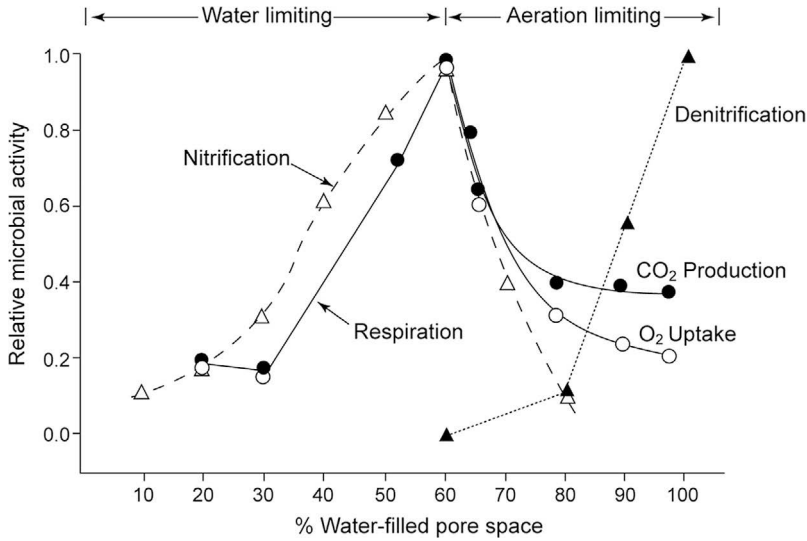

FIG. 14.2 The relationship between water-filled pore space (a measure of soil moisture availability) and relative amount of microbial activities. *Redrawn from Linn and Doran (1984).*

moisture and temperature can limit microbial activity, and soil organic matter and the organic N it contains will accumulate due to low rates of mineralization.

Water-filled pore space (WFPS) is a useful measure to examine moisture's influence on soil biological activity because it includes information about the impact of soil water on aeration in addition to information on water availability per se. The calculation of %WFPS is

$$\%\text{WFPS} = \frac{\text{soil water content} \times \text{bulk density} \times 100}{1\text{-}(\text{bulk density}/2.65)}. \qquad (14.1)$$

Soil water content is determined gravimetrically (g H_2O/g dry soil), bulk density (g cm^{-3}) is the oven dry weight of a given soil volume, and the value 2.65 is the density (g cm^{-3}) of sand grains and other soil mineral particles.

What controls the balance between N mineralization and immobilization? The answer is primarily organic matter quality—the availability of C in the material relative to its available N. Consider the effects of adding various organic materials with different C:N ratios to soil (Table 14.2). When one adds to soil manure with a relatively low C:N ratio (ca. 20:1), the microbes have no trouble obtaining N, and as a result, mineralization dominates over immobilization, and plant-available N increases in soil. This is why manure is frequently used as a fertilizer. On the other hand, were one to add sawdust to soil, a material with a high C:N ratio (625:1), the microbes would be keen to obtain the energy

TABLE 14.2 C:N Ratios in Various Organic Materials

Organic Material	C:N Ratio
Soil microorganisms	8:1
Soil organic matter	10:1
Sewage sludge	9:1
Alfalfa residues	16:1
Farmyard manure	20:1
Corn stover	60:1
Grain straw	80:1
Oak litter	200:1
Pine litter	300:1
Crude oil	400:1
Conifer wood	625:1

From Tisdale et al. (1993) and Hyvönen et al. (1996).

and C in the sawdust, but could not degrade this material without additional N because the sawdust does not have sufficient N to allow the microbes to build proteins. Thus, the microbes must immobilize N from their environment, resulting in a decrease in plant-available N in the soil. If there is no N to immobilize, microbial growth is slowed.

The balance between mineralization and immobilization is also affected by organism growth efficiency. For example, fungi have wider C:N ratios in their tissues than bacteria and therefore, have a lower need for N and will thus mineralize N more readily. As a general rule of thumb, materials with a C:N ratio > 25:1 stimulate immobilization, whereas those with a C:N ratio < 25:1 stimulate mineralization (Table 14.2). Highly decomposed substances such as soil organic matter, humus, and compost, in which labile C and N have been depleted, are the exception to this rule. Even though these substances may have a low C:N ratio, the undecomposed C is in complex forms inherently resistant to decomposition, so mineralization also proceeds slowly.

There are a wide variety of methods for measuring mineralization and immobilization (Hart et al., 1994; Robertson et al., 1999). Measurement of net mineralization and immobilization rates is much easier and more common than is the measurement of gross rates. Measurement of net rates usually involves measuring changes in inorganic N levels in some type of whole soil incubation. In most cases, these incubations are in containers, with no plant uptake or leaching losses, and changes in inorganic N levels are measured by periodic extractions of the soil. Incubation methods vary widely, from short (10-day) incubations of intact soil cores buried in the field to long (>52-week) incubations of sieved soils in the laboratory. Gross rates are measured using isotope dilution methods whereby small amounts of ^{15}N-labeled ammonium are added to the soil, and the subsequent dilution of the ^{15}N with natural ^{14}N from mineralized organic matter is used as a basis for calculating the gross production and consumption of ammonium.

III NITRIFICATION

Nitrification is the microbial oxidation of ammonia to less reduced forms, principally NO_2^- and NO_3^-. Autotrophic bacteria, first isolated in the late 1800s, gain as much as 440 kJ of energy per mole of NH_3 oxidized when NO_3^- is the end product. We know now that archaea and heterotrophic microbes can also nitrify, although autotrophic nitrification appears to be the dominant process in most soils.

The importance of nitrifiers to ecosystem function is substantial: although some nitrate enters ecosystems in acid rain or as fertilizer, in most ecosystems, nitrate is formed *in situ* via nitrification. Because nitrate is an anion, it is more mobile than ammonium, the ionized source of NH_3 in soil water:

$$NH_4^+(aq) \rightleftarrows NH_3(aq) + H^+(aq). \tag{14.2}$$

As a positively charged ion, ammonium can be held on cation-exchange sites associated with soil organic matter, clay surfaces, and variable-charge minerals. Nitrate, on the other hand, is mostly free in the soil solution and can be easily transported out of the rooting zone by water when precipitation exceeds evapotranspiration.

Nitrification in many soils is a major source of soil acidity, which can have multiple effects on ecosystem health, including the mobilization of toxic metals and the hydrologic loss of base cations as hydrogen ions displace other cations from exchange sites. In soils dominated by variable-charge minerals, which include most highly weathered tropical soils, soil acidity largely controls cation-exchange capacity (CEC), and nitrifier-generated acidity can drive CEC to very low levels. Further, some plants and microbes appear better able to take up ammonium than nitrate, and vice versa, implying a potential effect of nitrifiers on plant and microbial community composition. Finally, nitrifiers themselves can also be direct and important sources of the atmospheric gases NO_x and N_2O through nitrifier denitrification when O_2 is low (Zhu et al., 2013) or via by-product formation.

A The Biochemistry of Autotrophic Nitrification

Autotrophic nitrification is a two-step process, carried out by separate groups of bacteria and archaea—the ammonia and nitrite oxidizers, respectively. Autotrophic nitrifiers derive their C from CO_2 or carbonates, rather than from organic matter, and are obligate aerobes. The NH_3 oxidation is characterized as:

$$NH_3 + 1½O_2 \rightarrow NO_2^- + H^+ + H_2O. \quad (14.3)$$

The first step in this oxidation is mediated by the membrane-bound enzyme ammonia mono-oxygenase, which can also oxidize a wide variety of organic, nonpolar low-molecular-weight compounds, including phenol, methanol, methane, and halogenated aliphatic compounds, such as trichloroethylene:

$$NH_3^+ + 2H^+ + O_2 + 2e^- \xrightarrow{\text{ammonia mono-oxygenase}} NH_2OH + H_2O. \quad (14.4)$$

The reaction is irreversibly inhibited by small quantities of acetylene, which inhibits ammonia mono-oxygenase. This provides a means for experimentally differentiating autotrophic from heterotrophic nitrification in soil. Hydroxylamine is further oxidized to nitrite by the reaction

$$NH_2OH + H_2O \xrightarrow{NH_2OH\ \text{oxidoreductase}} NO_2^- + 4e^- + 5H^+. \quad (14.5)$$

Two of the four electrons released in this reaction are used in the prior NH_3 oxidation step; the remaining two are used in electron transport, generating energy for cell growth and metabolism:

$$2H^+ + ½O_2 + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O. \quad (14.6)$$

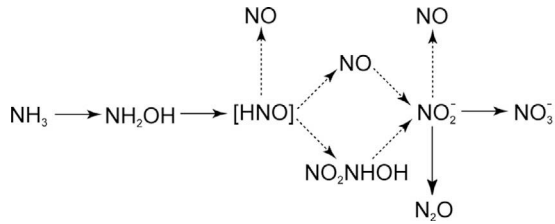

FIG. 14.3 Autotrophic nitrification pathways including pathways for gas loss. Broken lines indicate unconfirmed pathways. *From Firestone and Davidson (1989).*

Intermediary compounds formed during the oxidation of hydroxylamine to nitrite can result in the formation of NO (Fig. 14.3), which can escape to the atmosphere and influence the photochemical production of ozone (O_3) and the atmospheric abundance of hydroxyl (OH) radicals, primary oxidants for a number of tropospheric trace gases, including methane. Ammonia oxidizers are also able to produce NO via NO_2^- reduction, which results in the production of N_2O, an important greenhouse gas that can also escape to the atmosphere. Nitrite reduction occurs when ammonia oxidizers use NO_2^- as an electron acceptor when O_2 is limiting—effectively becoming denitrifying nitrifiers!

In most soils, the nitrite produced by ammonia oxidizers does not accumulate, but is quickly oxidized to nitrate by the nitrite-oxidizing bacteria when they perform nitrite oxidation:

$$NO_2^- + H_2O \xrightarrow{\text{nitrite oxidoreductase}} NO_3^- + 2H^+ + 2e^-. \qquad (14.7)$$

These reactions are membrane-associated, and because nitrite oxidoreductase is a reversible enzyme, the reaction can be reversed to result in nitrate reduction to nitrite. Up to 80% of the energy produced during nitrification is used to fix C; growth efficiencies of the nitrifiers are correspondingly low.

B The Diversity of Autotrophic Nitrifiers

Our taxonomic understanding of nitrifiers has been fundamentally transformed in the last few years by new molecular techniques that have revealed considerable taxonomic diversity, where before we thought there was little. The development and use of 16S rRNA gene primers and subsequent metagenomic techniques targeting genes for ammonia monooxygenase (*amoA*) have demonstrated both a greater diversity among bacterial nitrifiers as well as the presence of nitrifiers in a completely different kingdom, the Archaea. Remarkably, as first noted by Leininger et al. (2006), growing evidence suggests that archaeal nitrifiers may be as, or more, abundant than bacterial nitrifiers in many soils! Although, to date, we know little about the ecological

significance of this discovery, inference from nitrifiers successfully isolated from the marine environment suggests that archaeal soil nitrifiers may dominate in oligotrophic microenvironments where NH_3 concentrations are very low. The best-studied marine isolate has a vanishingly low substrate affinity for NH_4^+—over 200 times lower than that of the lowest bacterial isolate (Martens-Habbena et al., 2009). A soil isolate has only recently been cultured (Lehtovirta-Morley et al., 2011), and if its physiology is similar to the above nitrifiers, may be far more competitive for NH_4^+ in soil than is currently assumed. Such a substrate affinity may also give them access to NH_3 even in acid soils where high H^+ concentrations favor NH_4^+ over NH_3 (aq; He et al., 2012).

Prior to 2000, the bacterial nitrifiers were viewed as the single family Nitrobacteraceae, defined by their characteristic ability to oxidize ammonia or nitrite. Early work beginning with Winogradsky (1892) classified the ammonia-oxidizing genera of Nitrobacteraceae on the basis of cell shape and the arrangement of intracytoplasmic membranes. This yielded five genera: *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Nitrosolobus*, and *Nitrosovibrio*. Recent work with isolates, based principally on 16S rRNA oligonucleotide and gene sequence analysis, places terrestrial ammonia-oxidizing bacteria in the beta subclass of the Proteobacteria (Fig. 14.4); *Nitrosolobus* and *Nitrosovibrio* are no longer considered distinct from *Nitrosospira*, and *Nitrosococcus* is

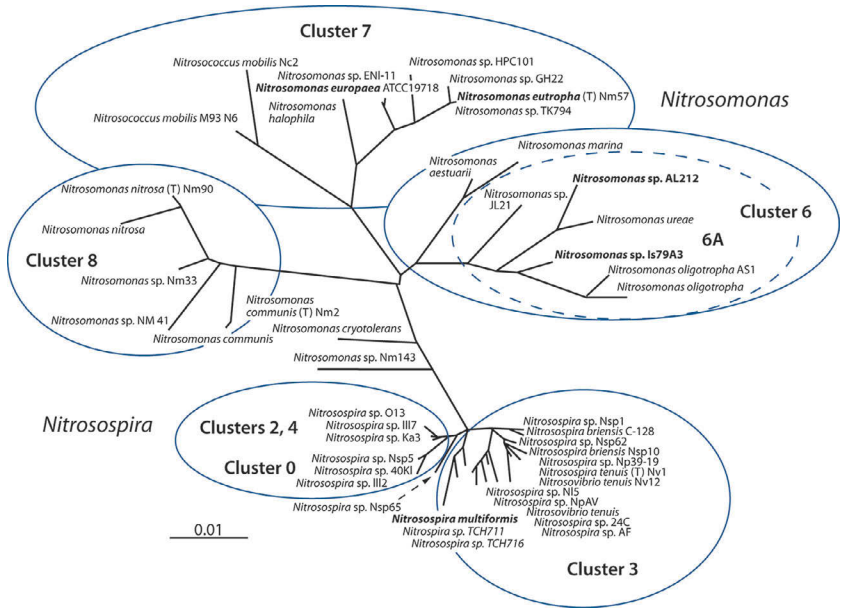

FIG. 14.4 A 16S ribosomal RNA guide tree for bacterial nitrifiers in the Betaproteobacteria based on isolates. The scale is substitutions per site. *Redrawn from Norton (2011).*

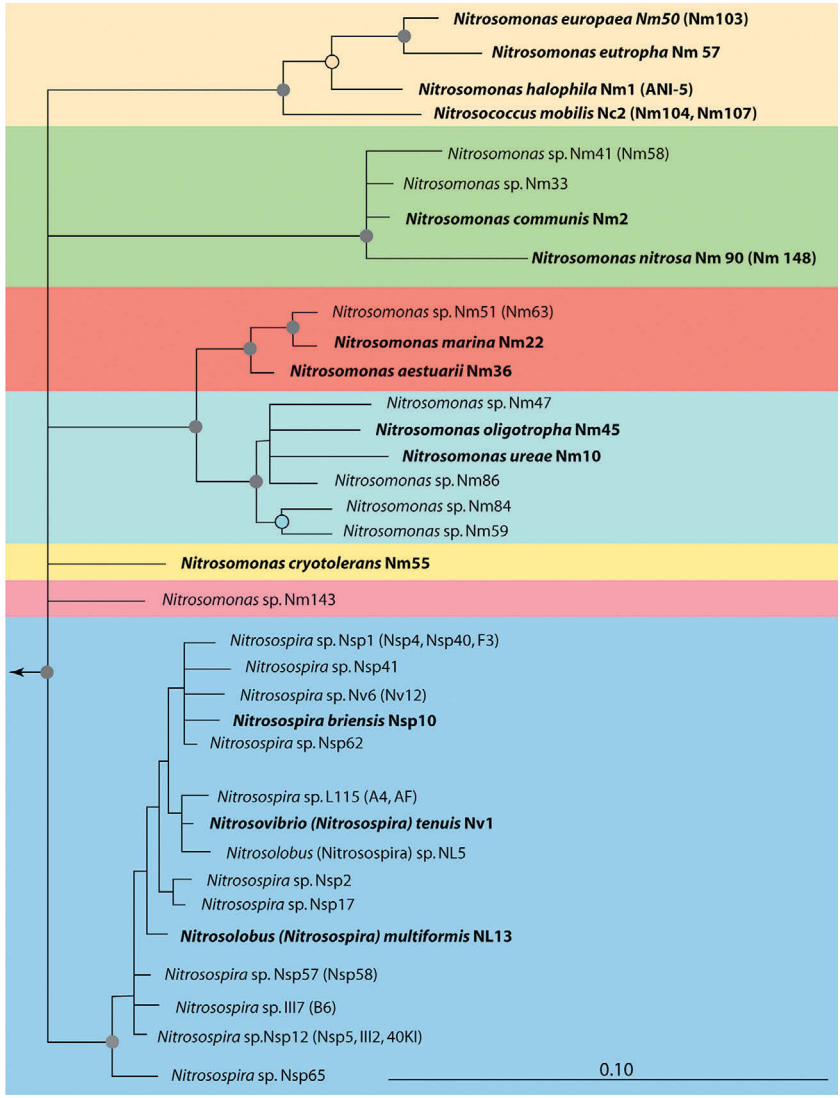

FIG. 14.5 16S rRNA-based phylogenetic tree of the betaproteobacterial ammonia oxidizers. The tree includes oxidizers of different genospecies (DNA-DNA similarity < 60%) with available 16S rRNA gene sequences longer than 1000 nucleotides. Strains with DNA-DNA similarity > 60% are in parentheses after the respective species name. Described species are depicted in bold. Scale bar represents 10% estimated sequence divergence. *From Koops et al. (2006).*

being reclassified to *Nitrosomonas* (Norton, 2011). Today, we have almost complete 16S rRNA gene sequences with >1000 nucleotides for the 14 described species of Betaproteobacteria ammonia oxidizers, which have a gene sequence similarity of 89% (Fig. 14.5; Koops et al., 2006).

In arable soils, the *Nitrosomonas communis* lineage is numerically dominant among culturable strains. Unfertilized soils usually also contain strains of the *Nitrosomonas oligotropha* lineage and strains of *Nitrosospira* and *Nitrosovibrio* (Koops and Pommerening-Röser, 2001). The latter two tend to be dominant in acid soils, which contain few if any *Nitrosomonas*.

Molecular techniques, such as 16S rRNA sequencing and the retrieval of *amoA* clones, have been used to examine the diversity of ammonia oxidizers *in vivo*, which avoid the need for pure-culture cultivation and its bias toward those species that are cultivatable outside their native habitat. Although molecular techniques can themselves be biased because of their dependence on the extraction of nucleic acid from soil, PCR amplification, primer bias, and cloning methods, they nevertheless suggest that most soils are dominated by *Nitrosospira* and archaeal species—and not by *Nitrosomonas* (Prosser, 2011). The archaeal species are currently found in the new archaeal phylum Thaumarchaeota. Their ubiquity, numerical dominance in soils thus far examined, and unique physiology suggest surprises in store.

Worth noting in general is that neither classical nor molecular techniques normally provide quantitative information about the abundance and activity of different species *in situ*. Quantitative PCR and newer techniques based on membrane or *in situ* hybridization in concert with rRNA-targeted probes (e.g., fluorescence *in situ* hybridization or FISH, as used in aquatic and wastewater treatment studies; Juretschko et al., 1998) can directly relate community structure with activity and spatial distribution of targeted organisms. Prosser and Embley (2002) have shown how these techniques can be used to discover nitrifier community change in response to changes in ecosystem management and land use. Stable isotope probing can also demonstrate the activity and growth of particular groups; Zhang et al. (2010) used $^{13}CO_2$ stable isotope probing to show the incorporation of ^{13}C-enriched CO_2 into the *amoA* and *hcd* genes of Thaumarchaea in soil microcosms.

Nitrite-oxidizing bacteria appear in a broader array of phylogenetic groupings than do the ammonia oxidizers, but only the genus *Nitrobacter* and the candidate genus *Nitrotoga* have been cultured from soil (Daims et al., 2011). The 16S rRNA analysis shows the presence of *Nitrospira* in most soils, which appear to be more diverse than *Nitrobacter* (Freitag et al., 2005). Members of *Nitrobacter* form an exclusive and highly related cluster in the Alphaproteobacteria. Though widely distributed in nature, pairwise evolutionary distance estimates are less than 1%, indicating little genetic diversity within the group, a finding supported by 16S rRNA sequence comparisons (Orso et al., 1994). The other nitrite-oxidizing genera are in the delta (*Nitrospina* and *Nitrospira*), gamma (*Nitrosococcus*), and beta (*Candidatus* Nitrotoga) subclasses of the Proteobacteria.

C Heterotrophic Nitrification

A wide variety of heterotrophic bacteria and fungi have the capacity to oxidize NH_4^+. So-called heterotrophic nitrification is not linked to cellular growth, as it

is for autotrophic nitrification. There is evidence for two pathways for heterotrophic ammonia oxidation. The first pathway is similar to that of autotrophic oxidation, in that the nitrifying bacteria have similar ammonia- and hydroxylamine-oxidizing enzymes. These enzymes can oxidize a number of different substrates, and it may be that ammonia oxidation is only secondary to these enzymes' main purpose of oxidizing propene, benzene, cyclohexane, phenol, methanol, or any of a number of other nonpolar organic compounds.

The second heterotrophic pathway is organic and appears limited to fungi. It involves the oxidation of amines or amides to a substituted hydroxylamine followed by oxidation to a nitroso and then a nitro compound with the following oxidation states:

$$\underset{-3}{RNH_2} \rightarrow \underset{-1}{RNHOH} \rightarrow \underset{+1}{RNO} \rightarrow \underset{+3}{RNO_3} \rightarrow \underset{+5}{NO_3^-}. \tag{14.8}$$

These reactions are not coupled to ATP synthesis and thus produce no energy. Alternately, N compounds may react with hydroxyl radicals produced in the presence of hydrogen peroxide and superoxide, which may happen when fungi release oxidases and peroxidases during cell lysis and lignin degradation.

Heterotrophic bacteria such as *Arthrobacter globiformis*, *Aerobacter aerogenes*, *Thiosphaera pantotropha*, *Streptomyces grisens*, and various *Pseudomonas* spp. have been found to nitrify. The fungi *Aspergillus flavus* was first isolated as a nitrifier in 1954 and is the most widely studied of the nitrifying heterotrophs. Interest in heterotrophic nitrification increased substantially in the late 1980s when it became clear that accelerated inputs of atmospheric ammonium to acid forest soils were being nitrified to nitrate with alarming effects on soil acidity, forest health, and downstream drinking water quality. Until recently, it was assumed that most of this nitrification was heterotrophic; we know now that most nitrification in acid soils is autotrophic (De Boer and Kowalchuk, 2001), and as noted earlier, may be chiefly performed by archaeal nitrifiers able to scavenge NH_3 in low pH soils (He et al., 2012). Heterotrophic nitrification thus appears important in some soils and microenvironments, perhaps where autotrophic nitrifiers are chemically inhibited (see following section), but are thought now to rarely dominate the soil nitrifier community.

D Environmental Controls of Nitrification

The single most important factor regulating nitrification in the majority of soils is ammonium supply (Fig. 14.6). Where decomposition and thus N mineralization is low, or where NH_4^+ uptake and thus N immobilization by heterotrophs or plants is high, nitrification rates will be low. Conversely, any ecosystem disturbance that increases soil NH_4^+ availability will usually accelerate nitrification unless some other factor is limiting. Examples are tillage, fire, clear cutting, waste disposal, fertilization, and atmospheric N deposition—all of which have well-documented effects on nitrate production in soils, mostly due to their effects on soil NH_4^+ pools.

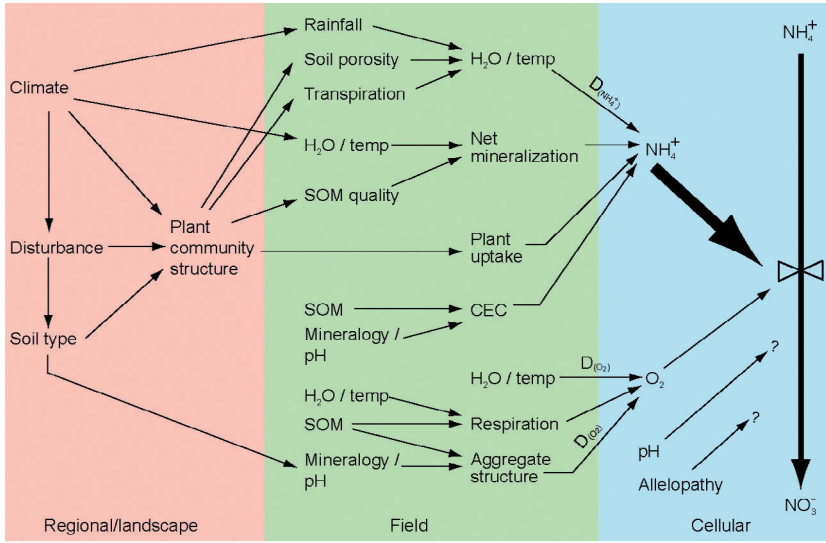

FIG. 14.6 Environmental controls on nitrification at different scales. *From Robertson (1989).*

Given that nitrification usually accelerates only when the NH_4^+ supply exceeds plant and heterotroph demand implies that nitrifiers are relatively poor competitors for NH_4^+ in the soil solution. In fact, this is the case: nitrification rates are typically low in midsuccessional communities and aggrading forests because of high plant demand for N. This also occurs following the addition of high C:N residues to agricultural soils because of high N demand by heterotrophic microbes (high immobilization; Fig. 14.1). In old-growth forests and mature grasslands, plant N demand has diminished and consequently, nitrification is usually higher than in midsuccessional communities where plant biomass is still accumulating, but not usually as high as in early successional and agricultural ecosystems, where N-supply often greatly exceeds demand (Robertson and Vitousek, 1981).

Oxygen is another important regulator of nitrification in soil. All known nitrifiers are obligate aerobes, and nitrification proceeds very slowly, if at all, in submerged soils. In flooded environments, such as wetlands and lowland rice, nitrifiers are active only in the oxidized zone around plant roots and at the water-sediment interface, which is usually only a few millimeters thick. In addition, even though some nitrifiers have the capacity to use nitrite rather than O_2 as an electron acceptor during respiration, O_2 is still required for ammonia oxidation.

Nitrifiers are little different from other aerobic microbes with respect to their response to temperature, moisture, and other environmental variables (see Fig. 14.2). Nitrification occurs slowly but readily under snow and in refrigerated soils, and soil transplant experiments (Mahendrappa et al., 1966) have

demonstrated an apparent capacity for nitrifiers to adapt to different temperature and moisture regimes. For many decades, nitrifiers were thought to be inhibited in acid soils, probably because in many cases, especially in soils from cultivated fields, raising soil pH with calcium or magnesium carbonate stimulates nitrification, and culturable nitrifiers exhibit a pH optimum of 7.5-8 (Prosser, 2011). We now recognize that nitrification can be high even in very acid forest soils (pH < 4.5; Robertson, 1989), although the physiological basis for this is still not well understood (De Boer and Kowalchuk, 2001).

IV INHIBITION OF NITRIFICATION

Nitrification is unaccountably slow in some soils, and in some circumstances, it may be inhibited by natural or manufactured compounds. A wide variety of plant extracts can inhibit culturable nitrifiers *in vitro*, even though their importance *in situ* is questionable. Likewise, commercial products, such as nitrapyrin and diocyandimide, can be used to inhibit nitrification in soil with varying degrees of success. Most commercial compounds are pyridines, pyrimidines, amino triazoles, and sulfur compounds, such as ammonium thiosulfate. Another innovation is paraffin-coated calcium carbide (CaC_2; Freney et al., 2000). Calcium carbide reacts with water to form acetylene (C_2H_2), which inhibits nitrifiers at very low partial pressures, ca. 10 Pa. As the paraffin wears off, CaC_2 is exposed to soil moisture, and the C_2H_2 formed inhibits nitrification. Likewise, neem oil, extracted from the Indian neem tree (*Azadirachta indica*), has been used commercially to coat urea fertilizer pellets to slow its nitrification to NO_3^-.

The potential value of managing nitrifiers in ecosystems can be easily seen from the position of nitrification in the overall N cycle (Fig. 14.1). Nitrogen is lost from ecosystems mainly after its conversion to NO_3^- and prior to plant uptake, so keeping N in the NH_4^+ form keeps it from being lost via nitrate leaching and denitrification, the two principal pathways of unintentional N loss and subsequent atmospheric and water contamination in most ecosystems. Because many plants prefer to take up N as NO_3^-, it is not desirable to completely inhibit nitrification, even in intensively managed ecosystems such as fertilized row crops, but slowing nitrifiers or restricting their activity to periods of active plant growth is an attractive—if still elusive—management option.

V DENITRIFICATION

Denitrification is the reduction of soil nitrate to the N gases NO, N_2O, and N_2. A wide variety of mostly heterotrophic bacteria can denitrify, whereby they use NO_3^- rather than oxygen (O_2) as a terminal electron acceptor during respiration. Because nitrate is a less-efficient electron acceptor than O_2, most denitrifiers undertake denitrification only when O_2 is unavailable. In most soils, this

mainly occurs following rainfall as soil pores become water saturated, and the diffusion of O_2 to microsites is drastically slowed. Typically denitrification starts to occur at water-filled pore space concentrations of 60% and higher (Fig. 14.2). In wetlands and lowland rice, soil diffusion may be restricted most of the time. Oxygen demand can also exceed supply inside soil aggregates and in rapidly decomposing litter.

Denitrification is the only point in the N cycle where fixed N reenters the atmosphere as N_2; it thus serves to close the global N cycle. In the absence of denitrification, N_2 fixers (see Chapter 15) would eventually draw atmospheric N_2 to nil, and the biosphere would be awash in nitrate. Denitrification is also significant as the major source of atmospheric N_2O, an important greenhouse gas that also consumes stratospheric ozone.

From a management perspective, denitrification is advantageous when it is desirable to remove excess NO_3^- from soil prior to its movement to ground or surface waters. Sewage treatment often aims to remove N from wastewater streams by managing nitrification and denitrification. Typically, wastewater is directed through sedimentation tanks, filters, and sand beds designed to remove particulates and encourage decomposition and the mineralization of organic N to NH_4^+, which is then nitrified under aerobic conditions to NO_3^-. The stream is then directed to anaerobic tanks, where denitrifiers convert the NO_3^- to N_2O and N_2, which is then released to the atmosphere. Part of the nitrification/denitrification management challenge is ensuring that the stream is exposed to aerobic conditions long enough to allow nitrifiers to convert most NH_4^+ to NO_3^-, but not so long as to remove all dissolved organic C (known as biological oxygen demand or BOD to wastewater engineers), which the denitrifiers need for substrate. Recently, anammox (described later in the chapter) has been utilized for wastewater N removal.

Denitrification can also remove nitrate from groundwater prior to its movement to streams and rivers. In most wetlands and riparian areas, nitrate-rich groundwater must move across a groundwater-sediment interface that is typically anaerobic and C-rich. As nitrate moves across this interface, it can be denitrified to N_2O and N_2, keeping it from polluting downstream surface waters.

It is usually desirable to minimize denitrification in managed ecosystems to conserve N for plant uptake. In regions with ample rainfall, N losses due to denitrification can rival or exceed losses by nitrate leaching. There are no technologies designed to inhibit denitrification *per se*; usually denitrifiers are best managed indirectly by manipulating water levels (e.g., in rice cultivation) or nitrate supply (e.g., nitrification inhibitors).

A Denitrifier Diversity

Denitrification is carried out by a broad array of soil bacteria, including organotrophs, chemo- and photolithotrophs, N_2 fixers, thermophiles, halophiles, and various pathogens. Over 50 genera with over 125 denitrifying species have been

identified (Zumft, 1992). In soil, most culturable denitrifiers are facultative anaerobes from only 3-6 genera, principally *Pseudomonas* and *Alcaligenes*, and to a lesser extent, *Bacillus*, *Agribacterium*, and *Flavibacterium*. Typically, denitrifiers constitute 0.1-5% of the total culturable soil population and up to 20% of total microbial biomass (Tiedje, 1988).

Organisms denitrify to generate energy (ATP) by electron transport phosphorylation via the cytochrome system. The general pathway is

$$2NO_3^- \xrightarrow[nar]{} 2NO_2^- \xrightarrow[nir]{} 2\uparrow NO \xrightarrow[nor]{} \uparrow N_2O \xrightarrow[nos]{} \uparrow N_2. \qquad (14.9)$$

Each step is enacted by individual enzymes: nitrate reductase (*nar*), nitrite reductase (*nir*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*). Each is inhibited by O_2, and the organization of these enzymes in the cell membrane for G^- bacteria is described in Fig. 14.7. At any step in this process, intermediate products can be exchanged with the soil environment, making denitrifiers a significant source of NO_2^- in soil solution and important sources of the atmospheric gases NO and N_2O.

Each denitrification enzyme is inducible, primarily in response to the partial pressure of O_2 and substrate (C) availability. Because enzyme induction is sequential and substrate dependent, there is usually a lag between the production of an intermediate substrate and its consumption by the next enzyme. In pure culture, these lags can be on the order of hours (Fig. 14.8); *in situ* lags in soil can be substantially longer, and differences in lags among different microbial taxa may significantly affect the contribution of denitrifiers to fluxes of NO and N_2O to the atmosphere. That induced enzymes degrade at different rates, and more slowly than they are induced, also leads to a complex response to the environmental conditions that induce denitrification; whether a soil has denitrified recently (whether denitrifying enzymes are present) may largely

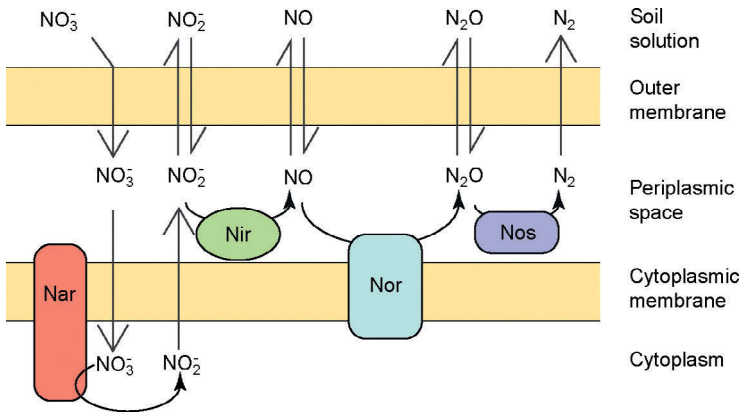

FIG. 14.7 The organization of denitrification enzymes in gram-negative bacteria. *Adapted from Ye et al. (1994).*

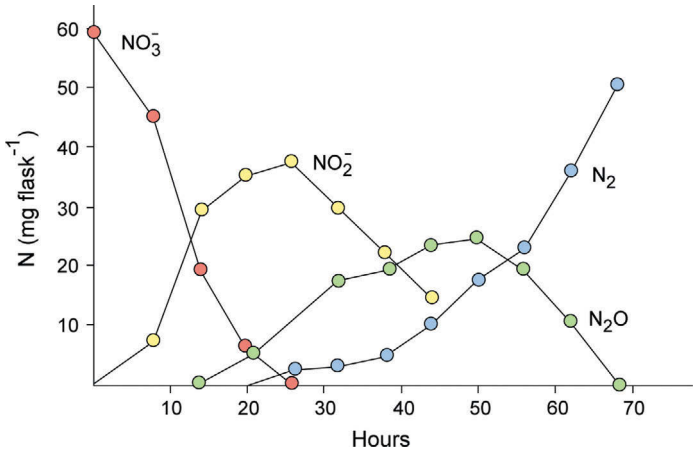

FIG. 14.8 The sequence of products formed during denitrification *in vitro* as different enzymes are sequentially induced. *Adapted from Cooper and Smith (1963).*

determine its response to newly favorable conditions for denitrification. Rainfall onto soil that is moist, for example, will likely lead to a faster and perhaps stronger denitrification response than will rainfall onto the same soil when it is dry (Groffman and Tiedje, 1988) and will affect the proportion of N product that is N_2O vs. N_2 because of the presence of *nos* in recently wet soil (Bergsma et al., 2002).

B Environmental Controls of Denitrification

For decades after its discovery as an important microbial process, denitrification was assumed to be important only in aquatic and wetland ecosystems. It was not until the advent of whole-ecosystem N budgets and the use of ^{15}N to trace the fate of fertilizer N in the 1950s that denitrification was found to be important in unsaturated soils. These studies suggested the importance of denitrification in fertilized agricultural soils, and with the development of the acetylene block technique in the 1970s, the importance of denitrification in well-drained forest and grassland soils was also confirmed. Acetylene selectively inhibits nitrous oxide reductase (*nos*; see Fig. 14.7), allowing the assessment of N_2 production by following N_2O accumulation in a soil core or monolith treated with acetylene. Unlike N_2, small changes in N_2O concentration are easily detected in air.

Today, denitrification is known to be an important N cycle process wherever O_2 is limiting. In unsaturated soils, this frequently occurs within soil aggregates, in decomposing plant litter, and in rhizospheres. Soil aggregates vary widely in size, but in general are composed of small mineral particles and pieces of organic matter <2 mm in diameter that are glued to one another with biologically derived polysaccharides. Like most particles in soil, aggregates are

surrounded by a thin water film that impedes gas exchange. Modeling efforts in the 1970s (Smith, 1980) suggested that the centers of these aggregates ought to be anaerobic owing to a higher respiratory demand in the aggregate center than could be satisfied by O_2 diffusion from the bulk soil atmosphere. This was confirmed experimentally in 1985 (Sexstone et al., 1985), providing a logical explanation for active denitrification in soils that appeared otherwise to be aerobic, and an explanation for the almost universal presence of denitrifiers and denitrification enzymes in soils worldwide.

In addition to O_2, denitrification is also regulated by soil C and NO_3^-. Carbon is important because most denitrifiers are heterotrophs and require reduced C as the electron donor, although as noted earlier, denitrifiers can also be chemo- and photolithotrophs. Nitrate serves as the electron acceptor and must be provided via nitrification, rainfall, or fertilizer. However, O_2 is the preferred electron acceptor because of its high energy yield and thus must be largely depleted before denitrification occurs. In most soils, the majority of denitrifiers are facultative anaerobes that will simply avoid synthesizing denitrification enzymes until O_2 drops below some critical threshold.

In the field, O_2 is by far the dominant control on denitrification rates. Denitrification can be easily stimulated in an otherwise aerobic soil by removing O_2, and can be inhibited in saturated soil by drying or otherwise aerating it. The relative importance of C and NO_3^-, the other major controls, will vary by ecosystem. Under saturated conditions, such as those found in wetlands and lowland rice paddies, NO_3^- limits denitrification because the nitrifiers that provide NO_3^- are inhibited at low O_2 concentrations. Consequently, denitrification occurs only in the slightly oxygenated rhizosphere and at the sediment-water interface, places where there is sufficient O_2 for nitrifiers to oxidize NH_4^+ to NO_3^-, which can then diffuse to denitrifiers in the increasingly anaerobic zones away from the root surface or sediment-water interface. It is often difficult to find NO_3^- in persistently saturated soils, not only because of low nitrification, but also because of the tight coupling between nitrifiers and denitrifiers. In wetlands with fluctuating water tables or with significant inputs of NO_3^- from groundwater, NO_3^- may be more available.

On the other hand, the availability of soil C in unsaturated soils more often limits denitrification. In these soils, C supports denitrification both directly, by providing donor electrons to denitrifiers, and indirectly, by stimulating O_2 consumption by heterotrophs. It can be difficult to experimentally distinguish between these two effects; from a management perspective, there probably is no need to.

VI OTHER NITROGEN TRANSFORMATIONS IN SOIL

Several additional microbial processes transform N in soil, although none are thought to be as quantitatively important as mineralization, immobilization,

nitrification, and denitrification. *Dissimilatory nitrate reduction to ammonium* (DNRA) refers to the anaerobic transformation of nitrate to nitrite and then to ammonium. Like denitrification, this process allows for respiration to go on in the absence of O_2 and is thought to be favored in environments where the ratio of C to nitrate is high because the process consumes more electrons than denitrification. A capacity for DNRA has been found in facultative and obligately fermentative bacteria and has long been thought to be restricted to high C, highly anaerobic environments, such as anaerobic sewage sludge bioreactors, anoxic sediments, and the bovine rumen. However, DNRA has been found to be common and important in some tropical forest soils (Silver et al., 2001) and in a variety of freshwater sediments (Burgin and Hamilton, 2007). In these soils, the flow of inorganic N through DNRA is as large as or larger than the flow through denitrification and nitrification and may help to conserve N in these ecosystems by shunting nitrate into ammonium rather than to N_2O or N_2.

Nonrespiratory denitrification, like respiratory denitrification, also results in the production of N gas (mainly N_2O), but the reduction does not enhance growth and can occur in aerobic environments. A variety of nitrate-assimilating bacteria, fungi, and yeast can carry out nonrespiratory denitrification, which may be responsible for some of the N_2O now attributed to nitrifiers in well-aerated soils (Robertson and Tiedje, 1987).

Anammox, in which ammonium and nitrite are converted to N_2 (Mulder et al., 1995; Jetten, 2001) is known to occur in sewage treatment plants and oceanic systems (Kuypers et al., 2005), where they can be the dominant source of N_2 flux. Anammox bacteria grow very slowly in enrichment culture and only under strict anaerobic conditions, and are thus likely to be part of a significant soil process only in periodically or permanently submerged soils (Strous, 2011).

Bacteria capable of performing anammox occur within the single order Brocadiales in the phylum Planctomycete. In these bacteria, anammox catabolism occurs in a specialized organelle called the anammoxosome, wherein

$$NH_4^+ + NO_2^- \rightarrow N_2H_2 \rightarrow N_2, \tag{14.10}$$

although much remains to be learned about the biochemistry and bioenergetics of the process, including intermediate compounds (Kartal et al., 2011).

Chemodenitrification occurs when NO_2^- in soil reacts to form N_2 or NO_x. This can occur by several aerobic pathways. In the Van Slyke reaction, amino groups in the α position to carboxyls yield N_2:

$$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2. \tag{14.11}$$

In a similar reaction, NO_2^- reacts with NH_3, urea, methylamine, purines, and pyrimidines to yield N_2:

$$HNO_2 + NH_3 \rightarrow N_2 + 2H_2O. \tag{14.12}$$

Chemical decomposition of HNO_2 may also occur spontaneously:

$$3HNO_2 \rightarrow HNO_3 + H_2O + 2NO. \tag{14.13}$$

In general, chemodenitrification is thought to be a minor pathway for N loss in most ecosystems. It is not easily evaluated *in situ*, however, and in the lab requires a sterilization procedure that does not itself significantly disrupt soil N chemistry.

VII NITROGEN MOVEMENT IN THE LANDSCAPE

Microbial transformations of reactive N (Table 14.3) have great importance for soil fertility, water quality, and atmospheric chemistry at ecosystem, landscape, and regional scales. It is at these scales that differences between what we have learned in the laboratory and what we observe in the environment (see Introduction) become most obvious.

TABLE 14.3 Forms of N of Concern in the Environment

N Form	Sources	Dominant Transport Vectors	Environmental Effects
Nitrate (NO_3^-)	Nitrification	Groundwater	Pollution of drinking water
	Fertilizer		Coastal eutrophication
	Disturbance that stimulates nitrification		
	Combustion (acid rain)		
Ammonia (NH_3, NH_4^+)	Fertilizer	Surface runoff	Pollution of drinking water
	Animal waste	Atmosphere	Eutrophication
Nitrous oxide (N_2O)	By-product of nitrification, denitrification, anammox	Atmosphere	Greenhouse gas
		Groundwater	Ozone destruction in stratosphere
Nitric oxide (NO)	By-product of nitrification, denitrification, anammox	Atmosphere	Ozone precursor in troposphere
Dissolved organic N	By-product of mineralization	Surface runoff	Eutrophication (?)
		Groundwater	

TABLE 14.4 Criteria for Determining if a Site is a Source or Sink of N in the Landscape

Criteria	Determinants
Is the site N rich?	Fertilized
	Fine texture (clay)
	Legumes
	Wet tropics
Is the site highly disturbed?	Disturbance of plant uptake (e.g., harvest)
	Stimulation of mineralization (e.g., tillage)
	Disturbance of links between plant and microbial processes (e.g., tillage)
Does the site have a high potential for denitrification?	Wet soil
	Well-aggregated
	High available organic matter
Does the site have a high potential for NH_3 volatilization?	High pH (>8.0)

From Groffman (2000).

One approach to thinking about microbial N cycle processes at large scales is to ask a series of questions that attempt to determine if a particular ecosystem is a source or sink of particular N species of environmental concern (Table 14.4). Sites that are N-rich either naturally or following disturbance have a high potential to function as sources of most of the reactive N forms identified in Table 14.1 because mineralization and nitrification, the processes that produce most of these reactive forms, occur at high rates.

Nitrogen sinks are defined as habitats that have a high potential to remove reactive N from the environment, preventing its movement into adjacent ecosystems. Ecosystems such as wetlands that are wet and rich in organic materials, for example, have a great potential to function as sinks because of their ability to support denitrification. In many cases these sink areas retain reactive N produced in source areas of the landscape. Riparian buffer zones next to streams, for example, can be managed to retain nitrate moving out of crop fields in groundwater (Lowrance et al., 1984). This nitrate can be stored in plant tissue or in soil organic matter as organic N or can be denitrified to N gas and thereby released to the atmosphere, preferably as N_2, a nonreactive form.

Humans have doubled the circulation of reactive N on Earth, creating a nitrogen cascade in which added N flows through the environment, leading to degradation of air and water quality and coastal ecosystems in many areas (Galloway et al., 2008). Solutions to landscape, regional, and global N enrichment problems often rely heavily on managing microbial N transformations. For example, coastal areas of the Gulf of Mexico suffer from eutrophication and hypoxia that have been linked to excess N from the Mississippi River Basin (Rabalais et al., 2002). Proposed solutions to this problem include better management of microbial N-transformations in crop fields as well as the creation of denitrifying wetland sinks for excess N moving out of agricultural areas (Mitsch et al., 2001).

Source-sink dynamics of N ultimately depend on the juxtaposition of different ecosystems in the landscape and the hydrologic and atmospheric transport vectors that link them—a complex topic that requires knowledge of hydrology and atmospheric chemistry in addition to soil ecology and microbiology. Because soil microbes play a crucial role in forming and consuming reactive N in the environment, their management can be an important and even crucial means for regulating N fluxes at local, regional, and global scales.

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