Chapter 14

Nitrogen transformations

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Chapter outline

14.1 Introduction

Nitrogen (N) is essential for life on Earth. Soil biota are responsible for its accumulation, persistence, and loss from ecosystems. Biotic N transformations in soil include its capture from the atmosphere, mineralization from soil organic matter (SOM), nitrification into forms more likely to be taken up by plants or lost, and denitrification back to atmospheric forms. Our understanding of N cycles in soil has been transformed in recent years with the discoveries of new microbial taxa via the application of modern genomic technologies, new processes via the application of new analytical approaches, and new insights into the functional importance of biotic biodiversity. Understanding N cycle transformations in soil is key to understanding the terrestrial and thus the global N cycle, including the cumulative environmental impact of reactive N — i.e., N that is environmentally active — as it accelerates plant productivity, contributes to climate change, and suppresses biodiversity in ecosystems worldwide.

The importance of N to ecosystem productivity is most evident in agriculture, where the amount of N fertilizer added to the biosphere each year to support crop growth (112 Tg N; FAO 2019) now exceeds the amount of N added naturally from other terrestrial sources (61 Tg; Vitousek et al., 2013). This has enormous implications for both human welfare — we are now feeding more than 7 billion people — and

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the environment. Consequences of more anthropogenic N in the biosphere are legion, ranging from degraded ground and surface water quality and increased greenhouse gas loading of the atmosphere to plant biodiversity loss and poor rural air quality.

What do soil biota have to do with this? Nitrogen exists in more forms than any other element essential for life on Earth (Table 14.1), all of which are affected by microbial activity. In fact, once N enters the biosphere in a form that is biologically available, microbes largely control its transformation from one form to another. Even with the advent of chemical fertilizers, microbes remain largely responsible for their entry into the biosphere. Understanding the cycling and fate of reactive N, whether at global or local scales, thus requires understanding the organisms responsible for driving each part of the cycle.

It is a daunting and exciting task. Of the 14 discrete N transformations known to be mediated by microbes, 4 have been discovered in only the past decade (Kuypers et al., 2018). Whole classes of microbes with newly recognized metabolic capacities have been revealed by recent genomic advances, yet we are only now learning how plants can affect — perhaps even control — their N environment by altering their microbiome. Soil microbiology thus plays another crucial role in life on Earth by regulating the form and availability of N to all terrestrial and many aquatic and marine organisms. Since N often limits plant productivity, it follows that soil microbes often regulate net primary production, the productive capacity of ecosystems, whether agricultural or natural. Understanding N transformations and the soil organisms 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 (BNF), whereby N_2 is transformed by microbes into simple organic compounds, is the dominant natural process by which N enters soil biological pools. All other soil N transformations happen subsequently: (1) N mineralization, the conversion of organic N to inorganic forms; (2) N immobilization, the uptake or

TABLE 14.1 Main forms of nitrogen in the environment and their oxidation states.

Name	Chemical formula	Oxidation state
Nitrate	NO_3^-	+5
Nitrogen dioxide (g)[a,b]	NO_2	+4
Nitrite	NO_2^-	+3
Nitric oxide (g)[b]	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	-3

[a] *Gases (g) occur free in both the soil atmosphere and dissolved in soil water.*
[b] *NO and NO_2 are collectively known as NO_x.*

assimilation of inorganic N into biomass by plants, microbes, and other soil organisms; (3) nitrification, the conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and then nitrate (NO_3^-); and (4) denitrification, the conversion of NO_3^- to nitrous oxide (N_2O) and then N_2, closing the global cycle. Other forms of N (Table 14.1) are primarily involved in these conversions as intermediaries. During conversion, they can escape into the environment where they can participate in chemical reactions or be transported elsewhere for further transformation.

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) with no global loss. That is, to maintain atmospheric equilibrium at the global scale, the same amount of N_2 that is fixed each year must either be permanently stored in geologic reservoirs or converted back to N_2 via denitrification.

Nitrogen fixation — both biological and industrial — now far outpaces historical rates of denitrification and is the principal reason reactive N has accumulated in the biosphere to become a major pollutant (Galloway et al., 2008). Making agricultural and other managed ecosystems more N-conservative, and removing excess N from soils, water bodies, and urban waste streams, are major environmental challenges that require a fundamental knowledge of microbial N transformations (Robertson and Vitousek, 2009).

The microbiology, physiology, and biochemistry of N cycle processes have been studied for over a century, and much of our understanding has been derived from molecular and organismal scale studies in the laboratory. Laboratory observations and experiments have been used to characterize the nature and regulation of N transformations, 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

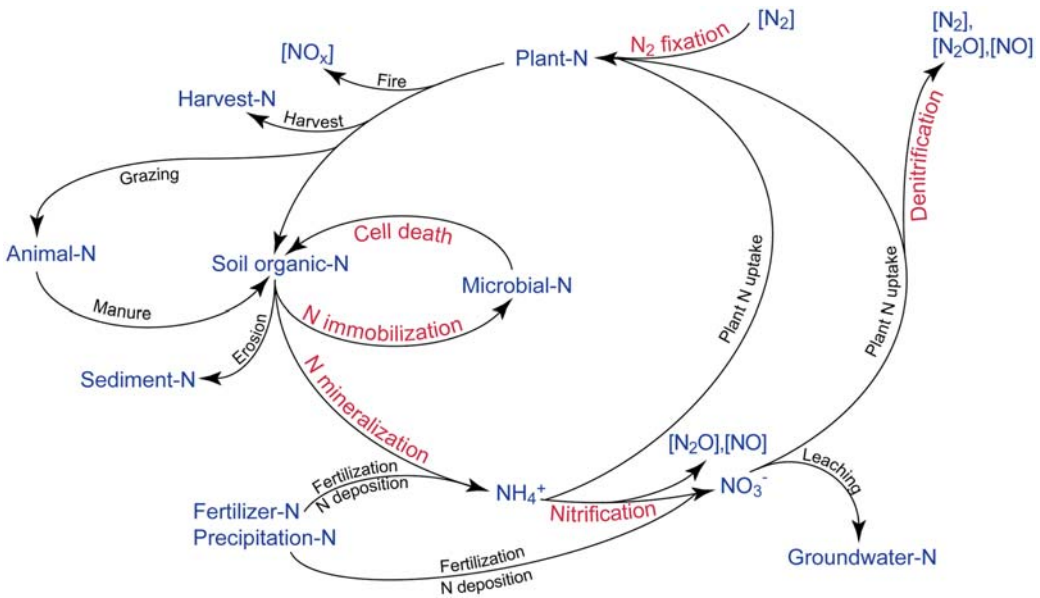

FIGURE 14.1 Schematic representation of the major elements of the terrestrial nitrogen cycle. Those processes mediated by soil microbes appear in red. *(Gases appear in brackets.)*

ecological significance of these processes. Genomic advances have allowed us to overcome some of these limitations by revealing the occurrence and activity of N cycle genes in surprising places; even so, we are still dependent on laboratory observations and experiments to define the functional significance of such genes. For example, theory and years of laboratory work suggested that denitrification ought to occur only in anaerobic wetland and muck soils, but when new field-based methods became available in the 1970s, and genomic methods in the 2000s, it became clear that almost all soils, including dry and even desert soils, support active denitrifiers.

The genomic revolution has also challenged our taxonomic understanding of soil N microbes. Whereas traditional taxonomy often classified microbial taxa on the basis of metabolic capabilities — nitrifiers, denitrifiers, N_2 fixers, and such — we now know of many crossover examples, such as N_2 fixers or nitrifiers that can also denitrify, or the occurrence of N_2 fixation genes in many taxa for which N_2 fixation has not been documented. Archaeal nitrifiers were unknown 20 years ago but are now known to numerically dominate nitrifier populations in almost all soils. Taxonomic classification based on N cycle processes is no longer useful or applicable.

In this chapter we will detail the major soil processes responsible for driving the terrestrial N cycle: N_2 fixation, N mineralization, nitrification, and denitrification. An understanding of these four processes forms the foundation for understanding N in the environment. That said, at the end of the chapter we also consider several other processes that can be important in specialized environments.

14.2 Nitrogen fixation

Nitrogen is rarely present in soil parent materials, creating the basis for widespread limitation of primary production by N. Although atmospheric N_2 is abundant, it is also extraordinarily stable, and large amounts of energy are required to convert it to a form useable by plants and other organisms. Thus the global N cycle depends on energy-intensive mechanisms, either natural or anthropogenic, to "fix" N from the atmosphere.

Biological nitrogen fixation (BNF) dominates natural inputs to the terrestrial biosphere (Table 14.2), but because of the rise of industrial fertilizer production, today it comprises approximately 35% of total global sources. The environmental cost of the shift from biological to synthetic N production is substantial — N fertilizers represent the principal source of greenhouse gas costs in most fertilized cropping systems and contribute to low system-wide N use efficiency (Robertson and Vitousek, 2009). There is thus substantial interest in moving fertilized agriculture toward systems that rely more on BNF to provide the N needed for high yields while increasing N use efficiency and lowering N losses from soil.

BNF in natural systems represents the primary process of N availability in plants. Atmospheric N deposition via precipitation is minor in most ecosystems (certainly in preindustrial times) and SOM (important in all ecosystems) represents the legacy of past BNF. In unfertilized systems an understanding of BNF and its consequences is crucial for understanding ecosystem function. This may become especially important as climate change solutions that involve sequestering C in ecosystems will necessarily also sequester N — creating a new demand for N that could be difficult to satisfy without additional BNF or fertilizer inputs (Hungate et al., 2003).

Biological nitrogen fixation, mediated exclusively by microbes with the enzymatic capacity to reduce atmospheric N_2 to ammonia (NH_3), is subsequently assimilated into amino acids or leaked into the soil

TABLE 14.2 Sources of nitrogen to the terrestrial biosphere.

Inputs	Tg N
Natural	
Biological N_2 fixation (BNF)	44
Lightning fixation	4
Rock N	10
N in aerosols from ocean	3
Total	61
Anthropogenic	
N fertilizer	112
Crop biological N_2 fixation	48
Fossil fuel combustion	25
Other industrial production	22
Total	207

From Vitousek et al. (2013) and (FAO 2019).

environment as NH_4^+. Nitrogen (N_2) fixers fall into three functional groups described by life history habits that evolved in response to the high energy requirements for converting N_2 to NH_3: (1) symbiotic N_2 fixers that live in close association with a host that provides their symbiont fixed C and a BNF-compatible microenvironment in exchange for assimilable N; (2) associative N_2 fixers that live in close proximity to plants, often in the plant rhizosphere (adjacent to roots), use C that is provided by the plants (purposefully or not) to fix N_2; and (3) free-living N_2 fixers that use C available to all heterotrophs, or in the case of phototrophic cyanobacteria, fix their own through photosynthesis.

14.2.1 The biochemistry of biological nitrogen fixation

BNF occurs via the nitrogenase enzyme complex:

$$N_2 + 8H^+ + 8e^- + 16Mg-ATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16Mg-ADP + 16\,P_i. \quad \text{(Eq. 14.1)}$$

NH_3 is toxic to cells in high concentrations, thus in free-living diazotrophs (archaea and bacteria capable of BNF), NH_3 is quickly assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway (Bottomley and Myrold, 2015):

$$\text{Glutamate} + NH_3 + ATP \xrightarrow{\text{glutamine synthetase}} \text{Glutamine} + ADP + P_i \quad \text{(Eq. 14.2)}$$

$$\text{Ketoglutarate} + \text{Glutamine} + \text{NADH} \xrightarrow{\text{glutamate synthetase}} 2\ \text{Glutamate} + \text{NAD}_{ox}. \quad \text{(Eq. 14.3)}$$

In symbiotic and associative diazotrophs the NH_3 is excreted and quickly assimilated by plant enzymes into amino acids. NH_3 can also escape into the soil solution, where it can be nitrified or otherwise assimilated by heterotrophs.

The nitrogenase complex is comprised of both dinitrogenase plus dinitrogenase reductase and requires metallic cofactors − most commonly molybdenum (Mo) but, in some nitrogenases, vanadium (V) and iron (Fe). Complete assembly of nitrogenase requires multiple *nif* genes: *nif*H encodes dinitrogenase reductase, *nif*D and *nif*K Mo nitrogenase, and *nif*K Fe nitrogenase. A variety of other *nif* genes (including B, F, I, J, L, LA, N, Q, S, U, V, W, X, Y, and Z) serve to regulate oxygen (O_2), sense inorganic N, assemble iron-sulfur clusters, and perform various other functions crucial for effective N_2 fixation (de Bruijn, 2015). At least nine *nif* genes are required for complete BNF.

Equations 14.1 to 14.3 show that BNF is energetically taxing. A total of 16 ATP molecules are necessary to reduce 1 molecule of N_2 to $2NH_3$, although some diazotrophs possess uptake hydrogenase, so the 4 ATP molecules needed to reduce $2H^+$ to H_2 can be recycled back to protons and electrons to power additional N_2 reduction. Energy is also required to maintain a large number of genes and their products necessary to synthesize and support a completely functional N_2-fixing enzyme system. Remarkably, nitrogenase can comprise ~10% of total cell protein in a functioning diazotroph (Bottomley and Myrold, 2015). With all said, the energetic cost of assimilating N_2 via nitrogenase vs. NH_4^+ free in the soil solution ranges from a factor of 1.8 to 5.4 (Hill, 1992).

Additional to the cost of maintaining and activating nitrogenase are the costs of maintaining an anaerobic microenvironment for BNF. Nitrogenase is exquisitely sensitive to O_2, which irreversibly denatures it; thus diazotrophs or their symbionts must invest in ways to exclude O_2. In the legume-rhizobia symbiosis this cost is largely borne by the plant, which creates a novel root nodule designed specifically to house diazotrophs. O_2 is actively excluded by transporting it away from the nodule via leghemoglobin − a red-colored protein with a high affinity for O_2, similar to hemoglobin in human blood. In actinorhizal symbioses (see below), although the plant creates nodules, O_2 exclusion appears mostly achieved by the vesicle wall of the bacterium itself.

In most free-living cyanobacteria (photosynthetic bacteria formerly known as blue-green algae) BNF occurs in vegetative cells called heterocysts, where thick walls and respiration protect nitrogenase from O_2, and light is used to directly power BNF. Cyanobacteria, with or without heterocysts, can also perform BNF at night when respiration consumes unwanted O_2. Free-living and associative diazotrophs in soil may be at the greatest O_2 disadvantage, which may limit BNF activity to episodic bursts following significant rainfall (Roley et al., 2019) or to high-respiration, low-O_2 soil microsites.

14.2.2 The diversity of biological nitrogen fixers

Genomic tools have massively expanded our knowledge of the number of organisms that can perform BNF. The ability to synthesize nitrogenase and fix N_2 remains exclusively with microbes − primarily bacteria, but also a few methanogenic archaea. Legume symbionts, collectively called rhizobia, are predominantly α-proteobacteria lineage proteobacteria. These include the well-studied genera *Rhizobium* and *Bradyrhizobium*, especially important in legumes cultivated for food such as soybean (*Glycine max* L.) and common bean (*Phaseolus vulgaris* L.). Since 1990, we have discovered scores of new genera that

can partner with these and other legumes (Roy et al., 2020), almost all understudied, which has provided a wealth of opportunities to better understand this important association.

Symbiotic BNF also arose in a different bacterial phylum, the actinobacteria (Fig. 14.2). Approximately eight different nonleguminous plant families contain genera capable of hosting actinobacteria, such as *Frankia* and *Parasponia* (Table 14.3). So-called actinorhizal plants are globally distributed and tend to be woody shrubs (e.g., *Myrica* and *Ceanothus*) or trees (e.g., *Alnus* and *Casuarina*) that colonize early successional forests or shrublands following ecological disturbance but can also persist in aggrading forests (Binkley et al., 1992), where rates of N_2 fixation can rival those in leguminous field crops.

Free-living diazotrophs belong to a wide phylogenetic range of bacteria that includes the α-proteobacteria, β-proteobacteria (e.g., *Burkholderia*, *Nitrospira*), δ-proteobacteria, γ-proteobacteria (e.g., *Pseudomonas*, *Xanthomonas*), firmicutes, and cyanobacteria (Gaby and Buckley, 2015). Free-living diazotrophs also include symbiotic and associative N fixers that must persist in soil prior to associating with their plant hosts. Metagenomic analysis based on the presence of *nif*H genes in native switchgrass (*Panicum virgatum* L.) rhizospheres (Bahulikar et al., 2020) revealed the cooccurrence of diazotrophs from at least five phyla. Renewed interest in switchgrass and other native grasses for bioenergy production is fueling new interest in associative nitrogen fixation as a means for providing N to crops without the economic and environmental costs of N fertilizers (Robertson et al., 2017).

A phylogenetic map of *nif*H genes (Fig. 14.2) illustrates the broad diversity of BNF among archaea and bacterial phyla. The presence of *nif*H in archaea and bacteria worldwide is striking, with about as many representatives in marine as in terrestrial habitats. While most legume symbionts (rhizobia) are in the α-proteobacteria and nonlegume symbionts like *Frankia* in the Frankia, Paenibacillus, and

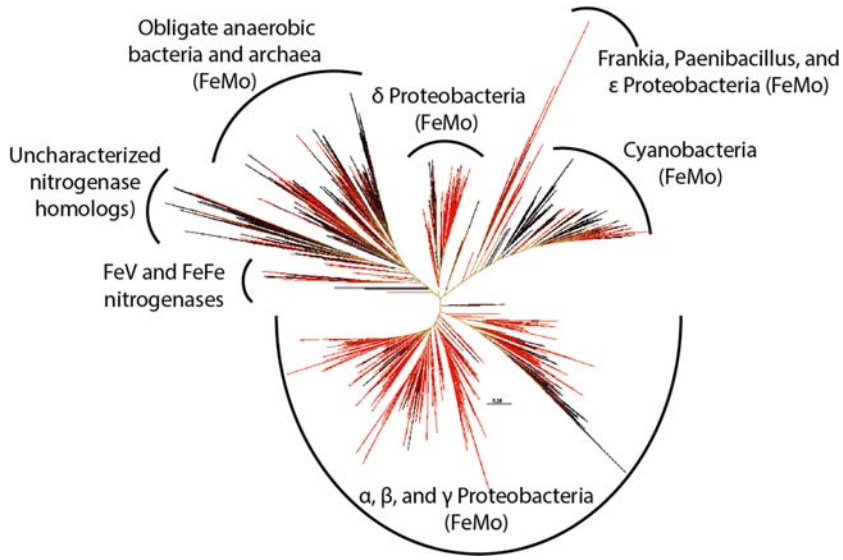

FIGURE 14.2 Phylogenetic distribution of *nif*H sequences. Red branches are those associated with N_2-fixing soil bacteria and archaea; black branches are from marine environments. Most legume symbionts (Rhizobia) are α-proteobacteria; nonlegume symbionts, such as actinobacteria, are in the *Frankia*, *Paenibacillus*, and ε-proteobacteria lineage. Free-living diazotrophs cut across all groups. *(Redrawn with permission from Gaby and Buckley, 2011.)*

TABLE 14.3 Families and genera of N_2-fixing plant-*Frankia* actinorhizal associations.

Family	N_2-fixing genera
Betulaceae	*Alnus*
Casuarinaceae	*Casuarina*
Coriariaceae	*Cariaria*
Datiscaceae	*Datisca*
Elaeagnaceae	*Elaeagnus, Hippophae*
Myricaceae	*Myrica and Comptonia*
Rhamnaceae	*Ceanothus*
Rosaceae	*Cercocarpus and Purshia*

From Bottomley and Myrold (2007)

ε-proteobacteria lineages, free-living diazotrophs cut across all groups: α-, β-, δ-, γ-, and ε-proteobacteria, as well as the firmicutes and cyanobacteria. While the presence of *nif*H in DNA does not necessarily reflect its use, it seems unlikely that coding for a protein this complex would be genetically conserved without consistent selective pressure. Notwithstanding, linking metagenomic knowledge to functional activity — in this case, quantitative N_2 fixation — remains a significant exciting challenge in soil microbial ecology.

14.2.3 Environmental control of biological nitrogen fixation

The conservative nature of the nitrogenase complex means that all diazotrophs are potentially constrained by the same set of ecological factors (Vitousek et al., 2013): low available energy, abundant inorganic N and O_2, and an insufficient supply of key resources, such as phosphorus (P), Fe, potassium, and Mo. Perhaps the most important environmental control on BNF is N availability. Given the high energetic cost of maintaining and using nitrogenase, it makes little evolutionary sense for a microbe to fix N_2 for assimilation into amino acids and proteins when more available forms of N are available. It is better to use energy for growth and reproduction than to fix N_2 when N is available in other useable forms. In fact, NH_4^+ is well known to inhibit nitrogenase synthesis and therefore BNF in pure culture, and in plant symbioses the plant takes the same tack. For example, BNF in soybeans declines to almost nil when soil inorganic N is available (Fig. 14.3), relieving the plant of much of the C cost of N assimilation. Conversely, BNF is especially important in infertile soils, whether naturally low in N or degraded. Exceptions to BNF N inhibition appear in some symbiotic plants and lichens (Binkley et al., 1992; Drake, 2011; Menge and Hedin, 2009), suggesting opportunities for a better understanding of the genetic and physiological basis for this functional trait.

Carbon or available energy can be an equally important constraint to BNF, especially in free-living and associative diazotrophs. Root exudates, known to be significant signaling compounds for legume nodulation, may also stimulate associative N_2 fixers (Coskun et al., 2017), whether intentionally or not. Evidence

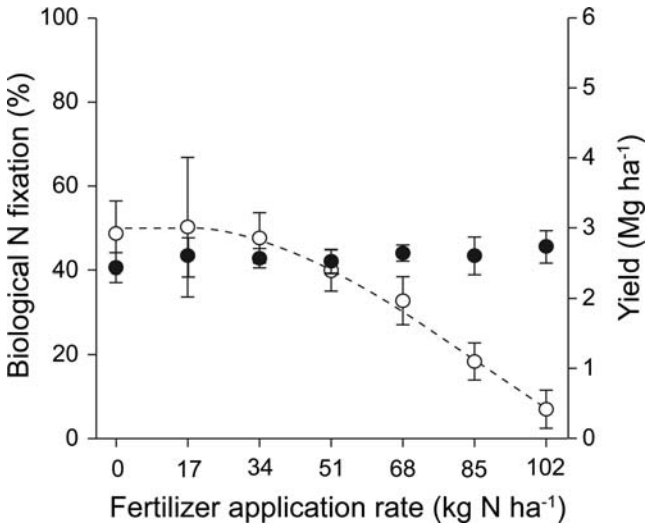

FIGURE 14.3 Biological N_2 fixation (open circles) and grain yield (filled circles) in soybeans grown in the field at different fertilizer rates. *(Redrawn with permission from Gelfand and Robertson, 2015.)*

for resource constraints other than N and energy primarily comes from resource addition experiments. BNF in alfalfa (*Medicago sativa* L.), for example, can be responsive to added Mo. Phosphorus has been observed to increase BNF in herbaceous legumes and forests (both in trees and epiphytic cyanolichens), although for nutrients that also limit plant productivity, it can be difficult to separate nutrient limitations on plant productivity and subsequent delivery of fixed C to diazotrophs from direct diazotrophic limitations on BNF per se. However, from a management standpoint, it may not matter.

Given that N more often than any other nutrient limits plant productivity in most terrestrial ecosystems, it can be interesting to ask why BNF, especially symbiotic BNF, is not more widespread. Shouldn't plants that partner with diazotrophs have a competitive advantage over plants that rely exclusively on atmospheric N deposition and SOM mineralization? Several hypotheses have been advanced to explain this apparent paradox (Vitousek et al., 2013): (1) in forests N_2-fixing plants tend to be shade intolerant due to the high energetic demands of BNF and thus are able to establish only following canopy-clearing disturbances when plant competitors are temporarily suppressed; (2) legumes and other N_2-fixing plants have higher leaf N and protein contents, making them more palatable than nonfixing plants to herbivores, especially in grazed systems; and (3) higher non-N nutrient demands of symbiotic N_2 fixers (Mo, Fe, perhaps P) place them at a competitive disadvantage. It is curious, however, that despite the higher energetic costs of BNF to symbiotic plants, at least in moderately fertile soil there seems to be no agronomic yield penalty for a greater reliance on BNF than on N fertilizer (Fig. 14.3).

14.3 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 the soil organisms (Chapter 12). This conversion is carried out by microbes and other soil organisms that release or mineralize

nutrients as a by-product of their detritus consumption. Although microbes consume detritus primarily as sources of energy and C to support the growth of new microbial biomass, they also have a need for nutrients, especially N, to assemble proteins, nucleic acids such as DNA, and other cellular components. If plant detritus is rich in N, microbial needs are easily met, and net N release or net N mineralization occurs. If plant detritus is low in N, microbes must scavenge additional inorganic N from their surroundings, leading to its net immobilization into microbial biomass.

The key to understanding mineralization-immobilization is to "think like a microbe": that is, think about a microbe's attempt to make a living by obtaining energy and C from detritus. Sometimes the detritus has all the N needed, so as detritus is consumed for its C, any extra N is released (mineralized) to the soil solution. Sometimes the detritus does not have enough N to meet microbial growth needs, so as detritus is consumed, additional N must be immobilized from the soil solution. Likely these two scenarios are happening simultaneously within even relatively small volumes of soil. While one group of microbes might be consuming a protein-rich and therefore N-laden bit of organic matter (think legume leaves), another group, perhaps <100 μm away, might be consuming detritus rich in C but low in N (think plant stalk). The first group is mineralizing N, while the second is immobilizing N, 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 worth making a distinction between gross and net mineralization and immobilization. Gross N mineralization is the total amount of soluble N released by soil biota, 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). This effect is readily apparent in compost management. Compost that has a high C-to-N (C:N) ratio, such as decomposing wood or wheat straw (Table 14.4), will lead to net N immobilization when applied to soil, whereas compost with a low C:N ratio, such as decomposing clover or lawn-grass clippings, will lead to net N mineralization. The difference will strongly affect plant N availability.

There is also an energetic cost to decomposition. Microbes invest more energy in the synthesis of enzymes to obtain nutrients (e.g., amidases to acquire N and phosphatases to acquire P) when decomposing substrates of low quality. Microbial N uptake is also affected by organism growth efficiency or the proportion of metabolized C that becomes microbial biomass (Chapter 9). Fungi have higher C:N ratios in their tissues than bacteria and archaea and so can grow more efficiently on low N substrates.

Traditionally, NH_4^+ 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 from a variety of habitats can take up simple, soluble organic forms of N leads us to broaden our definition of mineralization products to include any simple, soluble form of N that can be taken up by plants (Moreau et al., 2019). 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 (Chapter 4).

Soil fauna also play an important role in mineralization and immobilization (Chapter 5). They are responsible for much of the preliminary decomposition of detritus; feed on and can regulate populations of archaea, bacteria, and fungi; and can create or modify habitats for a wide array of organisms. For example, isopods shred leaf litter, earthworms create castings and burrows, and termites macerate wood. Often their own consumption is aided by gut microbes − wood-feeding termites, for example, rely on protozoan, bacterial, and fungal symbionts to digest cellulose. All heterotrophic soil organisms consume organic materials for energy and C and, at the same time, immobilize and mineralize N.

TABLE 14.4 C:N ratios of various organic materials.

Organic material	C:N ratio
Soil microorganisms	8:1
Sewage sludge	9:1
Soil organic matter	10:1
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).

The widely distributed nature of mineralization and immobilization 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 rates of respiration (see Fig. 14.4), yet it is important to recognize that significant activity often occurs at extremes of both temperature and moisture. Globally, in most soils the quantity and quality of detrital inputs are the main factors that control the rates and patterns of mineralization and immobilization (Li et al., 2019). 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), moisture or temperature can limit microbial activity, and SOM and the organic N it contains will accumulate due to low rates of mineralization.

Water-filled pore space 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. The calculation of the percent of water-filled pore space is:

$$\frac{\text{soil water content} \times \text{bulk density} \times 100}{1 - (\text{bulk density}/2.65)} \qquad \text{(Eq. 14.4)}$$

Soil water content is determined gravimetrically (g H_2O g^{-1} dry soil), bulk density (g cm^{-3}) is the dry mass of a given soil volume, and the value 2.65 is the density (g cm^{-3}) of rock, which by definition has no pores. In most soils microbial activity (respiration) tends to be highest at a water-filled pore space of ~60% as first documented by Linn and Doran (1984) (Fig. 14.4).

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

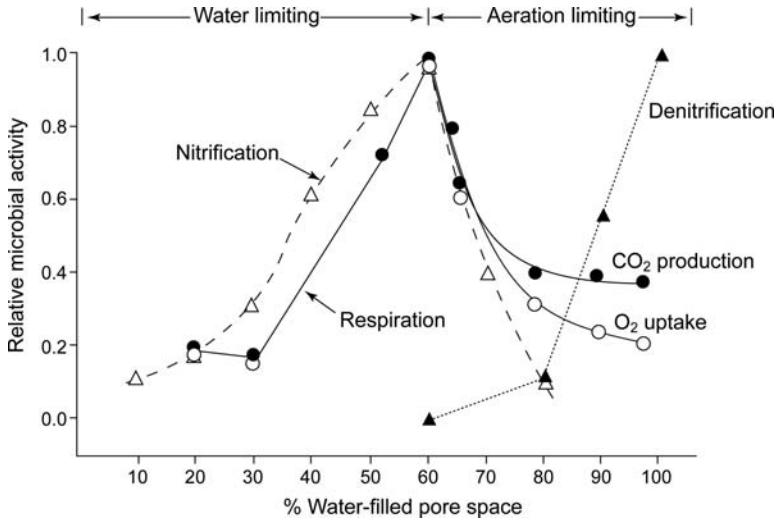

FIGURE 14.4 The relationship between water-filled pore space (a measure of soil moisture availability) and the relative amount of microbial activity in soil. *(Redrawn with permission from Linn and Doran, 1984.)*

adding various organic materials with different C:N ratios to soil (Table 14.4). When one adds manure to soil, with its relatively low C:N ratio of about 20:1, microbes have little 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 with its high C:N ratio (625:1) to soil, the microbes could not degrade this material without additional N because the sawdust has only 1 g of N for every 625 g of C, well below the amount of N needed to build proteins or other biomass constituents. Thus the microbes must acquire N from soil, resulting in a decrease in plant-available soil N. If there is no N to immobilize, microbial growth is slowed.

The balance between mineralization and immobilization is also affected by organism N use efficiencies or C:N ratios. As noted earlier, fungi have wider C:N ratios in their tissues than bacteria and will therefore have a lower need for N and, subsequently, will 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 14.4). Highly decomposed substances, such as SOM, 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 and inherently resistant to decomposition; thus mineralization also proceeds slowly.

There is 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 the measurement of gross rates. Gross rates are measured using isotope dilution methods, whereby small amounts of ^{15}N-labeled NH_4^+ are added to 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 NH_4^+.

Measurement of net rates usually involves measuring changes in inorganic N levels in some type of whole soil incubation. The accumulation of N during incubation is considered net mineralization, whereas

the loss of N is net immobilization. In most cases these incubations are conducted in well-aerated containers with no plant uptake or leaching losses. Changes in inorganic N levels are measured by periodic extractions of incubated 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. Net N mineralization assays are a powerful means for understanding a soil's capacity for meeting plant N needs and are a common way to compare soil N availability through time and across ecosystems, landscapes, and even continents.

14.4 Nitrification

Nitrification is the microbial oxidation of NH_3 to less reduced forms, principally NO_2^- and NO_3^-. Nitrifying bacteria, first isolated in the late 1800s (Frankland and Frankland, 1890; Winogradsky, 1890), gain as much as 440 kJ of energy per mole of NH_3 oxidized when NO_3^- is the end product. The discovery of archaeal nitrifiers in 2005 (Könneke et al., 2005) and in 2015 a bacteria capable of oxidizing both NH_3 and NO_2^- in a single cell (Daims et al., 2015; van Kessel et al., 2015) has had a paradigm-shifting impact. We now know that nitrifiers are much more ubiquitous and diverse than earlier imagined, including bacteria, fungi, archaea, in addition to heterotrophs as well as autotrophs.

The importance of nitrifiers to ecosystem function is considerable. Even though some NO_3^- enters ecosystems as atmospheric N deposition or fertilizer, in most ecosystems NO_3^- is formed in situ via nitrification. This includes fertilized agricultural systems, insofar as the vast majority of chemical fertilizers are NH_3 based and organic fertilizers are first mineralized to soil NH_4^+. Because NO_3^- is an anion, in most soils it is substantially more mobile than NH_4^+, the ionized source of NH_3 in soil water:

$$NH_4^+\ (aq) \rightleftarrows NH_3\ (aq)\ +\ H^+\ (aq). \qquad \text{(Eq. 14.5)}$$

As a positively charged ion, NH_4^+ can be held on cation-exchange sites associated with SOM, 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 or irrigation exceeds evapotranspiration. An exception occurs in highly weathered soils, such as in much of the tropics, where variable charge minerals at low pH have anion exchange sites that can hold NO_3^-.

Nitrification is also a major source of soil acidity (Coleman and Thomas, 1967), which can have multiple effects on ecosystem health, including the mobilization of toxic metals and the hydrologic loss of base cations due to hydrogen ions' displacing other cations on exchange sites. In soils dominated by variable-charge minerals, which include most highly weathered tropical soils, soil acidity largely controls cation-exchange capacity (CEC) (Sollins et al., 1988), which can be driven to very low levels by nitrifier-generated acidity. Additionally, many plants (Moreau et al., 2019) and heterotrophic microbes (Jones and Richards, 1977) prefer one form of inorganic N over the other, implying a potential effect of nitrifiers on plant and microbial community composition. Finally, nitrifiers themselves can 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.

14.4.1 The biochemistry of autotrophic nitrification

Autotrophic nitrifiers obtain their C from CO_2 or bicarbonate (HCO_3^-) rather than from organic matter and are obligate aerobes. Until recently, it was thought that autotrophic nitrification is necessarily a two-step

process, carried out by separate groups of bacteria and archaea called NH_3 and NO_2^- oxidizers. We are now aware that so-called comammox bacteria (combined or complete ammonia oxidizers) in the genus *Nitrospira* can perform complete nitrification – both NH_3 and NO_2^- oxidation – within the same cell, although canonical nitrification, carried out sequentially by two separate taxa, appears to be far more common.

Ammonia oxidation is characterized as:

$$NH_3 + 1\frac{1}{2}\,O_2 \rightarrow NO2^- + H^+ + H_2O. \quad \text{(Eq. 14.6)}$$

The first step in this oxidation is mediated by the membrane-bound enzyme ammonia monooxygenase, 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:

$$NH_3 + 2H^+ + O_2 + 2e^- \xrightarrow{\text{ammonia monooxygenase}} NH_2OH + H_2O. \quad \text{(Eq. 14.7)}$$

This reaction is irreversibly inhibited by small quantities of acetylene, which inhibits ammonia monooxygenase and provides a straightforward means for experimentally differentiating autotrophic from heterotrophic nitrification in soil.

Until recently, hydroxylamine was thought to be further oxidized directly to NO_2^- by hydroxylamine oxidoreductase. It has now been shown for ammonia-oxidizing bacteria (AOB) (Caranto and Lancaster, 2017) that hydroxylamine dehydrogenase (HAO) oxidizes hydroxylamine to NO, which is then oxidized to NO_2^- by an unidentified nitric oxide oxidoreductase (NOO) (Fig. 14.5):

$$NH_2OH + H_2O \xrightarrow{NH_2OH\ \text{dehydrogenase}} NO + 3e^- + 3H^+ \quad \text{(Eq. 14.8)}$$

$$NO + H_2O \xrightarrow{\text{nitric oxide oxidoreductase}} NO_2^- + 1e^- + 2H^+. \quad \text{(Eq. 14.9)}$$

NO is likewise an essential metabolite in ammonia-oxidizing archaea (AOA), but whether it is similarly produced or is a cosubstrate with hydroxylamine for the production of NO_2^- is as yet unclear (Stein, 2019).

Two of the four electrons released in these reactions replace the two used in the first oxidation reaction, leaving a net of two electrons to generate energy for cell growth and metabolism via electron transport:

$$2H^+ + 1\,½O_2 + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O. \quad \text{(Eq. 14.10)}$$

NO produced by AOB can escape into the atmosphere and influence the photochemical production of ozone (O_3) into the troposphere and the atmospheric abundance of hydroxyl (OH) radicals, primary oxidants for a number of important tropospheric trace gases, including methane. This is a good example of nitrification's indirect effect on global atmospheric chemistry.

The NO_2^- produced by AOB can also be used to produce N_2O, an important greenhouse gas that can then escape into the atmosphere by a process known as nitrifier denitrification. In O_2-stressed environments AOB can use NO_2^- as an electron acceptor rather than O_2. There is no current evidence that archaea or comammox nitrifiers can denitrify as they lack NO reductase genes (Prosser et al., 2020), but the NO_2^- produced by both AOA and AOB can, if not consumed by nitrite oxidizers, be abiotically converted to

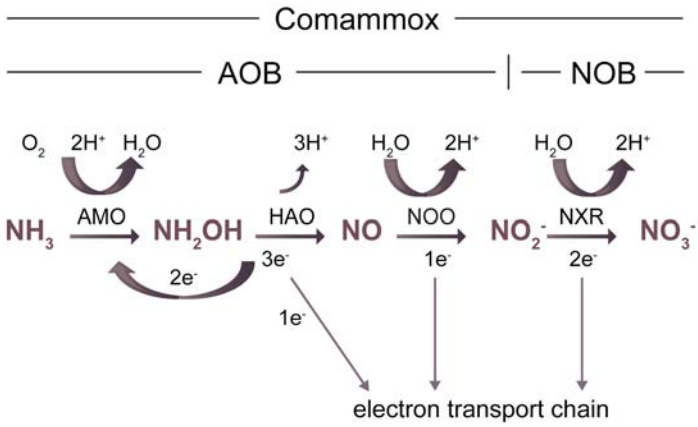

FIGURE 14.5 Autotrophic nitrification pathway for ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and comammox bacteria. AMO = ammonia monooxygenase, HAO = hydroxylamine dehydrogenase, NOO = nitric oxide oxidoreductase, NXR = nitrite oxidoreductase. Some NO can also escape into the atmosphere, as can N_2O, which is produced biotically from NH_2OH oxidation and the reduction of NO_2^- to NO then N_2O, termed nitrifier denitrification. N_2O can also be produced through abiotic reactions of NO, NH_2OH, and NO_2^-. The pathway for AOA is similar to that for AOB, but with different enzymes than HAO and NOO and an unclear role for NO. AOA also produce N_2O but only abiotically. *(Redrawn with permission from Stein, 2019.)*

reactive N gases by a variety of underappreciated chemical reactions resulting in NO_x and N_2O, and difficult to distinguish from biological sources (Heil et al., 2016).

In most soils the NO_2^- produced during canonical NH_3 oxidation is quickly oxidized to NO_3^- by NO_2^--oxidizing bacteria:

$$NO_2^- + H_2O \xrightarrow{\text{nitrite oxidoreductase}} NO_3^- + 2H^+ + 2e^-. \qquad \text{(Eq. 14.11)}$$

These reactions are membrane associated, and because nitrite oxidoreductase is a reversible enzyme, the reaction can be reversed to result in NO_3^- reduction to NO_2^-.

Comammox bacteria differ from the canonical nitrifiers in that they can carry out complete nitrification within a single cell:

$$NH_3 + 2\,O_2 \rightarrow NO_3^- + 2H^+ + H_2O.$$

Up to 80% of the energy produced during nitrification is used to fix C. Growth efficiencies of all nitrifiers are correspondingly very low, which is especially the case for comammox bacteria (Koch et al., 2019).

14.4.2 The diversity of autotrophic nitrifiers

Our taxonomic understanding of nitrifiers has been fundamentally transformed over the past few years by new molecular techniques that have revealed considerable taxonomic diversity, whereas before, there was thought to be 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 domain, the

Archaea. As first noted by Leininger et al. (2006), soil *amoA* gene abundance suggests that archaeal nitrifiers are far more abundant than bacterial nitrifiers in most soils. Comammox bacteria have now been documented in a wide variety of habitats (Koch et al., 2019), including agricultural soils (Orellana et al., 2018; Wang et al., 2020). Keep in mind, however, that greater abundance does not necessarily imply greater activity.

The ecological significance of these discoveries is slowly coming into focus. Inference enzyme kinetics for the few available isolates suggest that archaeal and comammox nitrifiers are favored in soil microenvironments with very low NH_4^+ concentrations (Koch et al., 2019; Prosser and Nicol, 2012), which by inference will have very little NH_3 available to nitrifiers. However, the discovery of AOA isolates with much higher NH_3 substrate affinities (e.g., Lehtovirta-Morley et al., 2016) complicates easy generalizations. Nevertheless, enzyme kinetic studies of whole soils with AOB inhibitors, which allow us to judge the relative importance of each group in different soils without the need to study hard-to-isolate individual populations (e.g., Liang et al., 2020; Taylor et al., 2012), corroborate the general trend of AOA's importance in soils with low NH_4^+ availability. AOA tend to be more active than AOB in soils with low NH_4^+ concentrations, such as low-pH forest soils (e.g., Liang et al., 2020), whereas AOB are more active than AOA in soils with an abundant NH_4^+ supply, such as fertilized agricultural soils. Evidence to date confirms the coexistence of all three groups (AOA, AOB, and comammox) in most soils, underscoring the importance of microsite heterogeneity for promoting microbial diversity.

Prior to 2000, the bacterial nitrifiers were viewed as the single family Nitrobacteraceae, defined by their characteristic ability to oxidize NH_3 or NO_2^-. Early work beginning with Winogradsky (1892) classified the NH_3-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 NH_3-oxidizing bacteria in the beta subclass of the Proteobacteria (Fig. 14.6; Norton, 2011). *Nitrosolobus* and *Nitrosovibrio* are no longer considered distinct from *Nitrosospira*, and *Nitrosococcus* is being reclassified to *Nitrosomonas*. Today, we have nearly complete 16S rRNA gene sequences with >1000 nucleotides for the 14 described species of Betaproteobacteria NH_3 oxidizers, which have a gene sequence similarity of 89% (Fig. 14.7; 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*. Culturable strains tell a very limited story, however.

Culture-free molecular techniques, such as 16S rRNA sequencing and the retrieval of *amoA* clones, have now been widely used to examine the diversity of NH_3 oxidizers in vivo. These techniques avoid the need for pure-culture cultivation and its bias toward those species that can be successfully separated from their native habitat. Although molecular techniques can themselves be biased because of their dependence on effective extraction of nucleic acid from soil and the bias associated with PCR amplification, primers, and cloning methods, they nevertheless suggest that most soils are dominated by archaeal species and *Nitrosospira*, not by *Nitrosomonas* (Prosser, 2011). Archaeal species are diverse and formally defined as class Nitrososphaeria in the phylum Thaumarchaeota, with four basal lineages: Ca. Nitrosocaldales, Nitrososphaerales, Ca. Nitrosotaleales, and Nitrosopumilales (Alves et al., 2018). Members of the Nitrososphaerales lineage appear to dominate soil environments. More than 80% of AOA sequenced from soils and sediments belong to this lineage, with a majority of taxa belonging to just two clades that lack

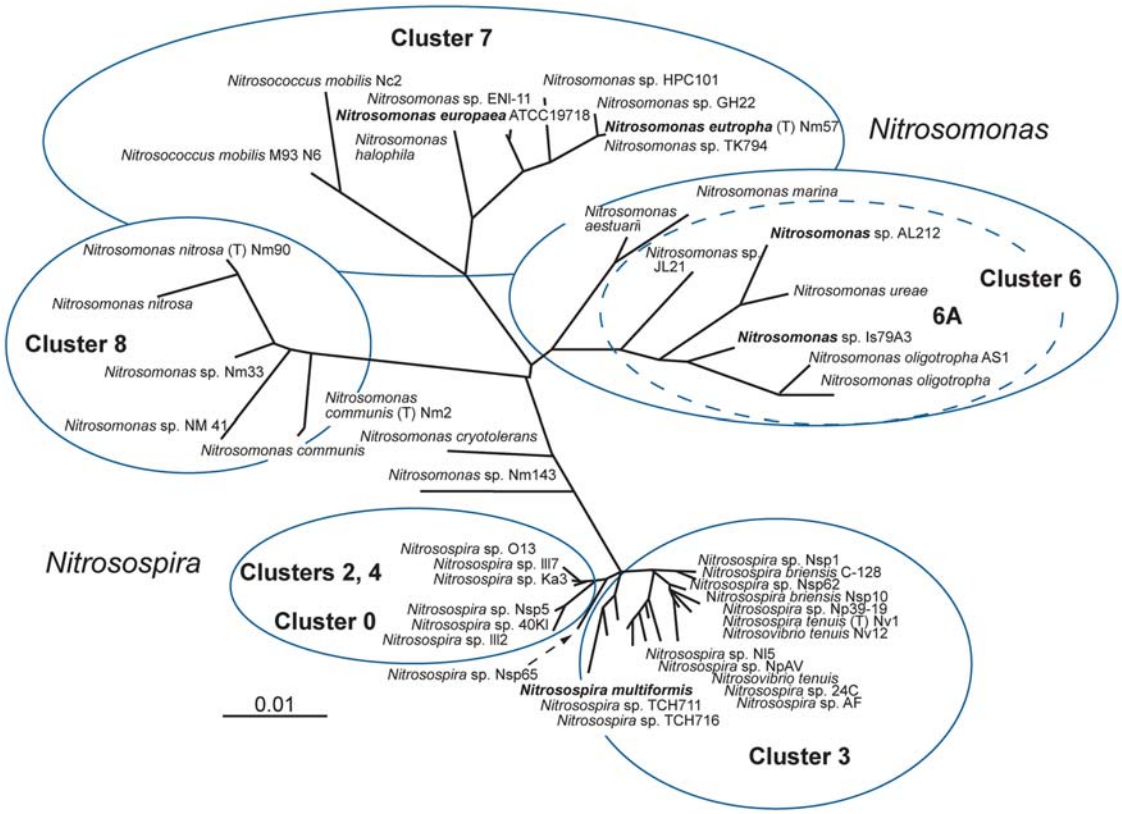

FIGURE 14.6 A 16S ribosomal RNA guide tree for bacterial nitrifiers in the Betaproteobacteria based on isolates. The scale is substitutions per site. *(Redrawn with permission from Norton, 2011.)*

cultivated species. Their recent discovery, ubiquity, numerical dominance in most soils, and unique physiology suggest additional surprises are in store.

Nitrite-oxidizing bacteria also appear in a broad array of phylogenetic groupings, but only the genus *Nitrobacter* and the candidate genus *Nitrotoga* have been cultured from soil (Daims et al., 2011). 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 NO_2^--oxidizing genera are in the delta (*Nitrospina* and *Nitrospira*), gamma (*Nitrosococcus*), and beta (*Candidatus* Nitrotoga) subclasses of the Proteobacteria.

All known comammox bacteria belong to *Nitrospira* lineage II, which is the most environmentally dispersed clade of this diverse genus. Based on 16S analysis, comammox appears comprised of two monophyletic sister clades A and B (Daims et al., 2015). All isolates thus far cultured are in clade A from

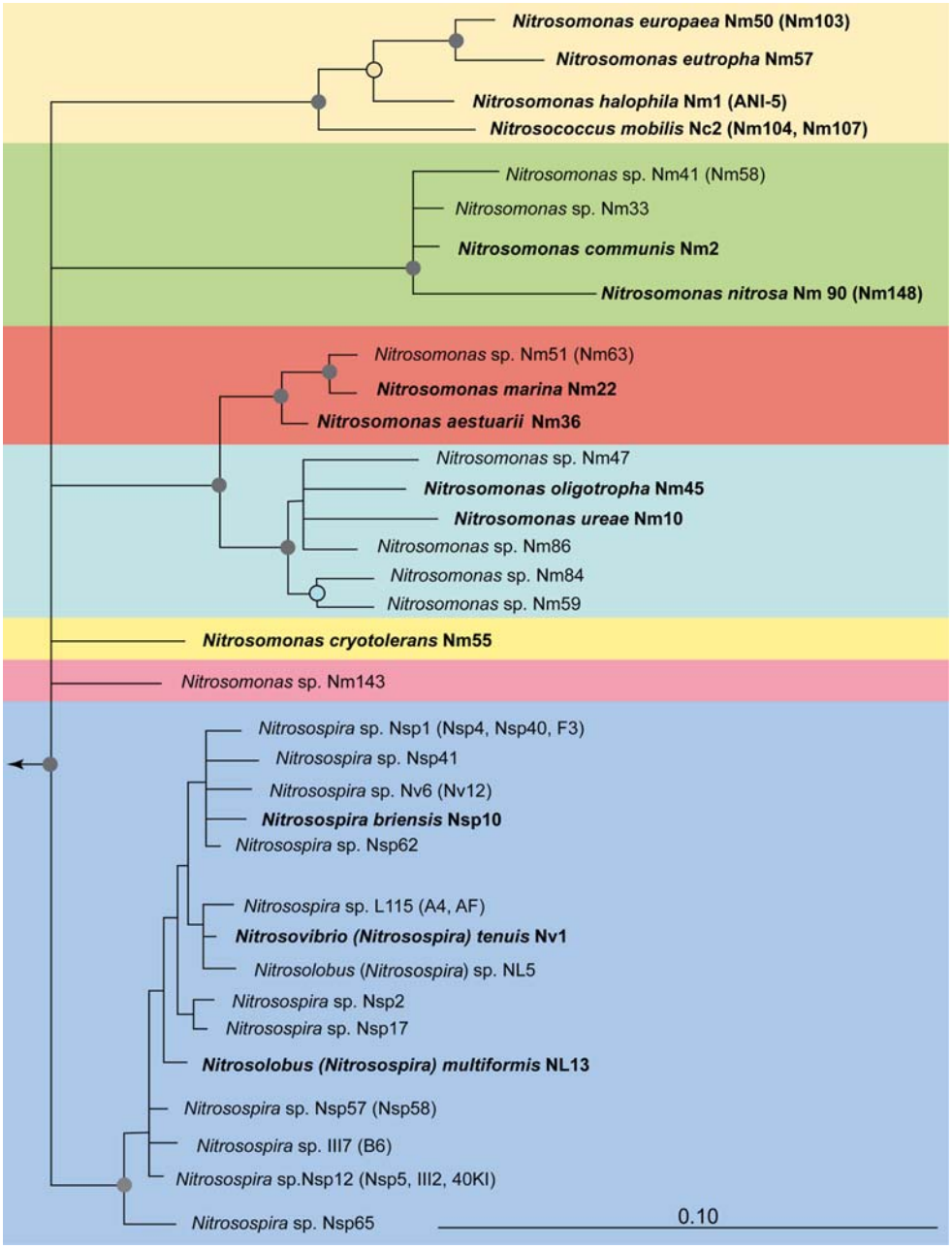

FIGURE 14.7 16S rRNA-based phylogenetic tree of the beta-proteobacterial ammonia oxidizers. The tree includes oxidizers of different genospecies (DNA-DNA similarity <60%) with available 16S rRNA gene sequences longer than 1000 nucleotides. Strains with DNA-DNA similarity >60% are in parentheses after the respective species name. Described species are depicted in bold. Scale bar represents 10% estimated sequence divergence. *(Redrawn with permission from Koops et al., 2006.)*

engineered systems (wastewater treatment plants and deep oil well biofilms), but comammox *Nitrospira* genes have been identified in a wide array of soil environments.

14.4.3 Heterotrophic nitrification

Wide varieties 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 NH_3 oxidation. The first pathway is similar to that of autotrophic oxidation, in that the nitrifying bacteria have similar NH_3- and hydroxylamine-oxidizing enzymes. These enzymes can oxidize a number of different substrates, and it may be that NH_3 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:

$$\begin{array}{ccccccccc} RNH_2 & \rightarrow & RNHOH & \rightarrow & RNO & \rightarrow & RNO_3 & \rightarrow & NO_3^- \\ -3 & & -1 & & +1 & & +3 & & +5. \end{array} \qquad \text{(Eq. 14.12)}$$

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 nitrifying bacteria include *Arthrobacter globiformis*, *Aerobacter aerogenes*, *Thiosphaera pantotropha*, *Streptomyces griseus*, and various other species. The fungus *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 NH_4^+ to acid forest soils were being nitrified to NO_3^- 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), perhaps chiefly performed by acidophilic (Lehtovirta-Morley et al., 2011) and archaeal (He et al., 2012) nitrifiers able to scavenge NH_3 under low-pH conditions. Heterotrophic nitrifiers thus appear to be important in some soils and microenvironments, perhaps where autotrophic nitrifiers are chemically inhibited (see following section), but they are thought now to rarely dominate the soil nitrifier community.

14.4.4 Environmental controls on nitrification

The single most important factor regulating nitrification in the majority of soils is NH_4^+ supply (Fig. 14.8). Where decomposition and thus N mineralization are low, or where NH_4^+ uptake and thus N immobilization by heterotrophs or plants are high, nitrification rates will be low. Conversely, any ecosystem disturbance that increases soil NH_4^+ availability will typically accelerate nitrification unless some other factor is limiting. Examples are tillage, fire, forest clear cutting, waste disposal, fertilization, and atmospheric N deposition — all of which have well-documented effects on NO_3^- production in soils, mostly due to their effects on soil NH_4^+ pools.

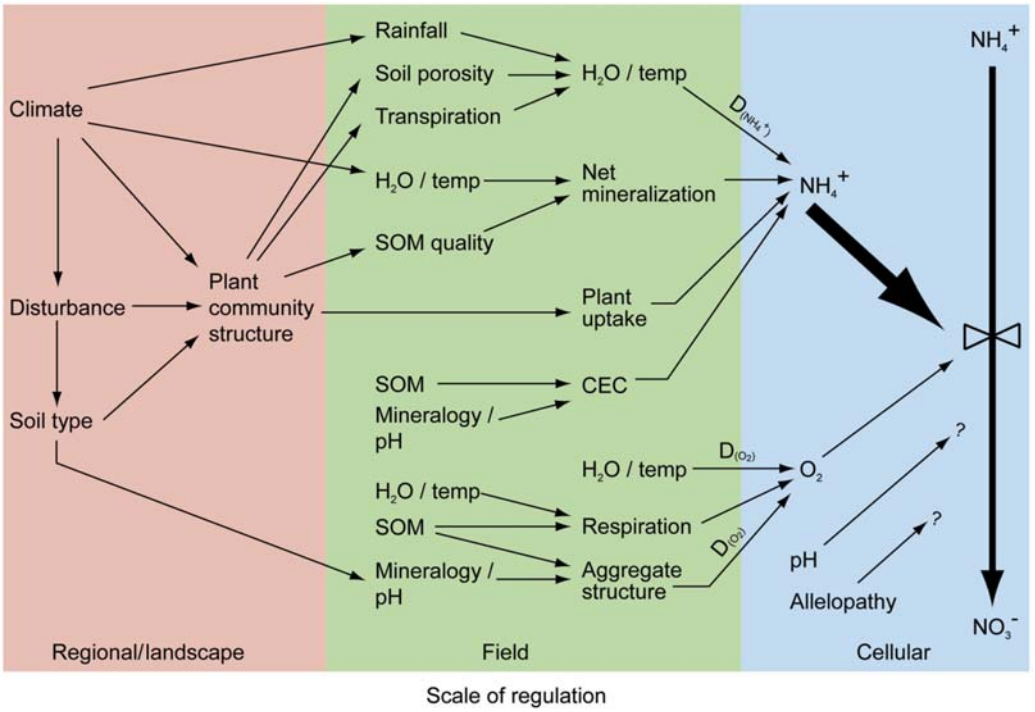

FIGURE 14.8 Environmental controls on nitrification at different scales. *(Redrawn with permission from Robertson, 1989.)*

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 soil solution. This is, in fact, 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 microzone around plant roots and at the water-sediment interface, which is usually only a few millimeters thick. Even though some canonical nitrifiers have the capacity to use NO_2^- rather than O_2 as an electron acceptor during respiration, O_2 is still required for NH_3 oxidation.

Nitrifiers are similar to other aerobic microbes with respect to their response to temperature, moisture, and other environmental variables (Fig. 14.4). Nitrification occurs slowly but readily under snow and in refrigerated soils. 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 to 8 (Prosser, 2011). Rather, low pH often creates substrate limitation. The dissociation of NH_4^+ to NH_3 in soil solution (Eq. 14.5) is orders of magnitude lower in acid than in neutral or alkaline soils, creating an NH_3 limitation for nitrifiers. High rates of nitrification in very acidic forest soils (pH <4.5; Robertson, 1989) are likely due to either acidophilic nitrifiers or to archaea and comammox with very low substrate affinities.

14.4.5 Nitrifier inhibition

Nitrification is unaccountably slow in some soils and, in some circumstances, may be inhibited by natural or manufactured compounds. A wide variety of plant extracts can inhibit culturable nitrifiers in vitro (Rice, 1979), even though their importance in situ has long been challenged (Robertson, 1982). Likewise, commercial products, such as nitrapyrin and dicyandiamide, can be used to inhibit nitrification in soil with varying degrees of success (Beeckman et al., 2018). Neem oil, extracted from the Indian neem tree (*Azadirachta indica* A. Juss), has been used commercially to coat urea fertilizer pellets to slow its nitrification to NO_3^-.

Biological nitrification inhibition has been unequivocally documented in *Brachiaria* spp., a tropical perennial pasture grass where the root exudates appear to inhibit NH_3 oxidizers (Subbarao et al., 2009). It is also suspected in other species, such as sorghum (*Sorghum bicolor* [L.] Moench). However, documenting inhibition in situ is challenging, and to be truly effective, inhibitors must diffuse away from the rhizosphere, where nitrification may already be inhibited by low substrate supply, and must persist in the presence of heterotrophs into periods with little or no plant growth — most of the year in the case of annual crops. Both of these criteria are daunting.

That said, 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, primarily after its conversion to NO_3^- and prior to plant uptake. Thus keeping N in the NH_4^+ form prevents loss via NO_3^- leaching and denitrification, which in most ecosystems are the two principal pathways of unintentional N escape. 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 goal.

14.5 Denitrification

Denitrification is the reduction of soil NO_3^- to the gases NO, N_2O, and N_2. A wide variety of soil microorganisms can denitrify, whereby NO_3^- rather than O_2 is used as a terminal electron acceptor during respiration. Because NO_3^- is a less efficient electron acceptor than O_2, facultative denitrifiers will use NO_3^- only when O_2 is insufficient to meet respiratory demand, and obligate denitrifiers will be at a competitive disadvantage relative to O_2-utilizing heterotrophs. Significant denitrification in soil occurs only when O_2 availability is restricted. This primarily occurs following rainfall as soil pores fill with water and the diffusion of O_2 to microsites is drastically slowed. Denitrification is triggered at water-filled pore space concentrations of 60% and higher (Fig. 14.2) and O_2 concentrations below 2%. In wetlands and lowland

rice paddies this may be the case most of the time, but even in well-aerated soils O_2 demand can exceed supply inside soil aggregates and around rapidly decomposing litter particles, microscale hotspots for denitrification (Kravchenko et al., 2017).

Denitrification was considered the only point in the N cycle where fixed N reenters the atmosphere as N_2 until the 1995 discovery of anaerobic ammonium oxidation (anammox), described later in this chapter. Notwithstanding anammox, denitrification remains the main process that serves to close the global N cycle. In the absence of denitrification N_2 fixers would eventually draw atmospheric N_2 to nil, and the biosphere would be awash in NO_3^- and other reactive forms of N. Denitrification is also the major source of atmospheric N_2O, an important greenhouse gas with ~300 times the global warming potential of CO_2. N_2O also consumes stratospheric ozone, important for shielding the Earth's surface from UV radiation.

Denitrification can also be employed to remove excess NO_3^- from soil prior to its movement as a pollutant to ground and surface waters. This can be accomplished by maintaining natural vegetation in riparian areas and wetlands adjacent to streams. As NO_3^--rich groundwater moves toward streams, it will encounter adjacent anaerobic zones where it can be denitrified to N_2O and N_2, keeping it from polluting downstream surface waters. Conceptually, this process is similar to urban wastewater treatment, which aims to move NO_3^-, produced by the oxidation of organic N in wastewater and its subsequent nitrification to NH_4^+, to anaerobic tanks containing denitrifiers. Anammox has recently been proposed for wastewater N removal (Hu et al., 2013).

Denitrification is less desirable in agricultural systems as it is better to conserve N for plant uptake. In regions with ample rainfall, N losses via denitrification can rival or exceed losses via NO_3^- leaching. Despite its importance, however, there are no technologies to directly inhibit denitrification. Denitrifiers are best managed indirectly by avoiding excess water (e.g., with drainage tiles in crop fields or levees in rice paddies) and excess NO_3^- (e.g., with N-efficient fertilizer technologies or nitrification inhibitors).

14.5.1 Denitrifier diversity and biochemistry

Denitrification is carried out by a broad array of microbes spanning all three domains of life. Best known are prokaryotic denitrifiers, because they have been isolated and described for over a century. Eukaryotes are a more recent addition to our knowledge of denitrifiers, as we've learned over the past 30 years that fungal denitrification is more common than originally thought (Mothapo et al., 2015). Although few archaea are known to denitrify, this is likely an artifact of the difficulty with which archaea are isolated for study. Emerging molecular evidence based on *nirK* gene abundance has revealed a number of archaea with the potential to denitrify.

Denitrifiers are in general quite cosmopolitan. Over 60 genera of bacterial denitrifiers have been identified (Coyne, 2018), including organotrophs, chemotrophs including nitrifiers, photolithotrophs, N_2 fixers, thermophiles, halophiles, and various pathogens. Some 14 different genera of aerobic denitrifiers — prokaryotes able to use O_2 and NO_3^- simultaneously as electron acceptors — have been identified from wastewater treatment systems (Ji et al., 2015). Within the eukaryotes, at least 70 species of fungi spanning 23 taxonomic orders possess denitrifying enzyme genes (Maeda et al., 2015). In soil most culturable denitrifiers are facultative anaerobes from only three to six genera, principally *Pseudomonas* and *Alcaligenes*, and to a lesser extent, *Bacillus*, *Agrobacterium*, and *Flavobacterium*. Typically, denitrifiers can constitute up to 20% of total microbial biomass (Tiedje, 1988).

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

$$2NO_3^- \xrightarrow[nar]{} 2NO_2^- \xrightarrow[nir]{} 2\uparrow NO \xrightarrow[nor]{} \uparrow N_2O \xrightarrow[nos]{} \uparrow N_2 \,. \qquad \text{(Eq. 14.13)}$$

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. The organization of these enzymes in the cell membrane for gram-negative bacteria is described in Fig. 14.9. 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 very 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. 14.10). 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 into the atmosphere. For example, a dried soil recently wet may consume NO_3^- almost immediately, but until *nir* is induced, the NO produced by *nar* will accumulate and escape into the soil atmosphere (see Eq. 14.13). Until *nos* is induced, the N_2O produced by *nor* will escape. This can lead to the nonintuitive effect of drought's stimulating N_2O production.

The fact that induced enzymes also degrade at different rates, and more slowly than they are induced, also leads to a complex response to the environmental conditions that drive denitrification. Whether a soil has denitrified recently (whether denitrifying enzymes are present) may largely determine its response to newly favorable conditions for denitrification. Rainfall onto soil that is already 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 lead to a greater proportion of N product, that is N_2O vs. N_2, because of the presence of *nos* in soil that is already wet (Bergsma et al., 2002).

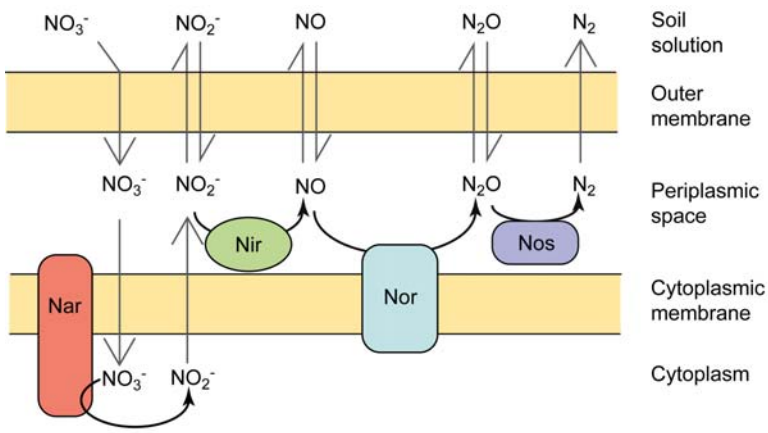

FIGURE 14.9 The organization of denitrification enzymes in gram-negative bacteria. *(Redrawn with permission from Ye et al., 1994.)*

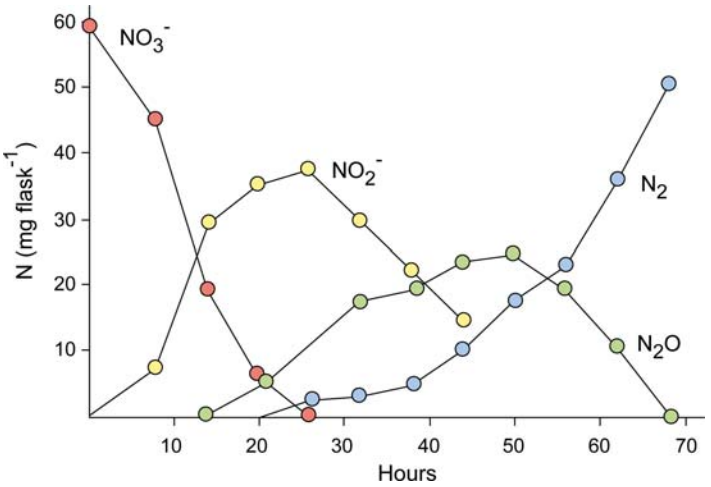

FIGURE 14.10 The sequence of products formed during denitrification in vitro as different enzymes are induced sequentially. *(Redrawn with permission from Cooper and Smith, 1963.)*

14.5.2 Environmental controls on denitrification

For over a century 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 that denitrification was found to be important in unsaturated soils. These studies suggested the importance of denitrification in agricultural soils, and with the development of the acetylene block technique in the 1970s, the importance of denitrification in even forest and grassland soils was confirmed. Acetylene selectively inhibits *nos* (see Eq. 14.13; Fig. 14.9), allowing the assessment of N_2 production by measuring N_2O accumulation in a soil incubated 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 and C and NO_3^- are not. In unsaturated soils this frequently occurs within soil aggregates, and in decomposing plant litter and 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 or other binding agents. Like most particles in soil, aggregates are coated by a thin water film that impedes gas exchange. Oxygen diffuses through water $\sim$10,000 times slower than through air. Quantitative models (Smith, 1980) suggested that the centers of these aggregates ought to be anaerobic owing to a higher respiratory demand in the aggregate's interior than could be satisfied by O_2 diffusion from soil air. This was confirmed experimentally in 1985 (Sexstone et al., 1985), and together with evidence for the importance of organic matter particles for providing anaerobic microhabitats (Kravchenko et al., 2017; Parkin, 1987), a logical explanation is provided for both active denitrification in soils that appear otherwise to be aerobic, as well as for the almost universal presence of denitrifiers and denitrification enzymes in soils worldwide.

The fact that denitrification — or at least N_2O production — occurs in well-aerated soils may also be due to fungal denitrifiers that can denitrify at higher O_2 concentrations than can archaeal or bacterial

denitrifiers (Mothapo et al., 2015). In pure culture their NO_3^- consumption rates are much lower than those of prokaryotes, but their ability to denitrify in drier soils means they may be able to denitrify for longer periods. The fact that fungal denitrifiers also lack a functional *nos* enzyme means that N_2O is their dominant end product, providing added biogeochemical importance.

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 chemolithotrophs like nitrifiers, and photolithotrophs like cyanobacteria. For all, NO_3^- serves as the electron acceptor and must be provided via nitrification, rainfall, or fertilizer. Oxygen, however, is a 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 that can differ substantially by taxa (Cavigelli and Robertson, 2001).

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. In saturated soils, such as those 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 zone 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. Nitrate may be more available in wetlands with fluctuating water tables or with significant inputs of NO_3^- from groundwater.

Under unsaturated conditions such as those in most upland soils, C availability may be a dominant control. For example, in these soils it is easy to stimulate denitrification simply by adding a readily oxidized C source. Carbon supports denitrification, both directly by providing donor electrons to denitrifiers, and indirectly by stimulating O_2 consumption by heterotrophs. It can be difficult to distinguish between these two effects experimentally, yet from a management perspective, there probably is no need to.

The multifactor control of denitrification often creates extraordinary spatial and temporal variation in its activity, which inhibits our ability to produce well-constrained estimates of just how much denitrification is occurring at field, landscape, or regional scales. One approach to addressing this variability is to focus on small areas of intensive activity where controlling factors converge to create episodic periods of high rates of activity that can account for a substantial percentage of overall denitrification at different scales. These spatial and temporal "control points" (Bernhardt et al., 2017) have been effectively targeted in denitrification studies at multiple scales and ecosystem types.

14.6 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 NO_3^- to NO_2^- and then to NH_4^+. 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 NO_3^- is high because DNRA 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 identified in some tropical forest soils (Silver et al., 2001) and in a variety of freshwater sediments (Burgin and Hamilton, 2007). In these environments the flow of inorganic N through DNRA can be as large or larger than the flow through denitrification and nitrification and may help to retain N by shunting NO_3^- into NH_4^+ rather than into 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 NO_3^--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 NH_4^+ and NO_2^- are converted to N_2 (Mulder et al., 1995), is known to occur in sewage treatment plants and marine systems (Kuypers et al., 2005) where anammox 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

$$NH_4^+ + NO_2^- \rightarrow N_2H_2 \rightarrow N_2, \qquad \text{(Eq. 14.14)}$$

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 through several aerobic pathways (Heil et al., 2016). In the Van Slyke reaction amino groups in the α position to carboxyls yield N_2:

$$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2. \qquad \text{(Eq. 14.15)}$$

In a similar reaction NO_2^- reacts with NH_4^+, urea, methylamine, purines, and pyrimidines to yield N_2:

$$HNO_2 + NH_4^+ \rightarrow N_2 + 2H_2O. \qquad \text{(Eq. 14.16)}$$

Chemical decomposition of HNO_2 may also occur spontaneously:

$$3HNO_2 \rightarrow 3HNO_3 + H_2O + 2NO. \qquad \text{(Eq. 14.17)}$$

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 it requires a sterilization procedure that does not itself disrupt soil N chemistry.

14.7 Nitrogen movement in the landscape

Microbial transformations of reactive N (Table 14.5) have great importance for soil fertility, water quality, and atmospheric chemistry at ecosystem, landscape, and regional scales. It is at these scales that

TABLE 14.5 Forms of nitrogen of environmental concern.

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	

differences between what we have learned in the laboratory and what we observe in the environment (see Section 14.1) become most obvious.

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 a particular N species (Table 14.6). 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, nitrification, and denitrification 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 have high potential to function as sinks due to their ability to support denitrification. In many cases these sink areas retain N produced in source areas of the landscape. Riparian buffer zones next to streams can be managed to retain N moving as NO_3^- out of crop fields in groundwater (Lowrance et al., 1984). This NO_3^- can be stored in plant tissue or in SOM as organic N or can be denitrified to N_2O, or better, to N_2.

Humans have doubled the circulation of reactive N on Earth, creating N that then flows through the environment, leading to degraded air and water quality (Robertson and Vitousek, 2009). Solutions to landscape, regional, and global N enrichment problems often rely heavily on managing microbial N

TABLE 14.6 Criteria for determining if a site is a source or sink of nitrogen 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).

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 include better management of microbial N transformations in crop fields as well as the creation of wetland sinks to trap and remove 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 paths that link them − a complex topic that requires knowledge of hydrology and atmospheric chemistry in addition to soil ecology and microbiology. Because soil biota 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. Better management at the ecosystem level is fundamental.

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