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13

Nitrogen Transformations

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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. However, several forms of N are also pollutants, so soil microbial transformations of N also affect human and environmental health, sometimes far away 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 forms in soil corresponding to different oxidative states (Table 13.1). Dinitrogen gas (N_2) is by far the most abundant form of N in the biosphere but is unusable by most organisms, including plants. Biological N_2 fixation, whereby N_2 is transformed to organic N (described in detail in Chap. 14), is the dominant process by which N first enters soil biological pools. All subsequent transformations are covered in this chapter: *N mineralization*, which is the conversion of organic N to inorganic forms; *N immobilization*, which is the uptake

TABLE 13.1 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_{NH3}	−3

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

or assimilation of inorganic forms of N by microbes and other soil heterotrophs; *nitrification*, which is the conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and then nitrate (NO_3^-); and *denitrification*, which is the conversion of nitrate to nitrous oxide (N_2O) and then dinitrogen gas (N_2). Other N species (Table 13.1) are involved in these conversions primarily as intermediaries, and during conversion can escape to the environment where they can participate in chemical reactions or be transported elsewhere for further reactions.

Löhnis (1913) first formulated the concept of the N cycle, which formalizes the notion that N species are converted from one form to another in an orderly and predictable fashion, and that at global equilibrium as much dinitrogen gas that is fixed each year by N_2 fixation must be annually converted back to N_2 gas via denitrification (Fig. 13.1).

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 modern pollutant (Galloway *et al.*, 2003). Making managed ecosystems more N conservative and removing N from waste streams are major environmental challenges that require a fundamental knowledge of soil microbial N transformations (Robertson, 1997).

Although the microbiology, physiology, and biochemistry of N cycle processes have been extensively studied for many decades, it is important to note that much of our understanding of these processes has been derived from molecular and organismal scale studies. In some cases, data from the laboratory have impaired our ability to understand and evaluate these processes in nature. Laboratory studies have characterized the nature and regulation of the processes that we discuss in this chapter, but the reductionist nature of these studies has caused us to overlook sometimes surprising possibilities for activity and regulating factors in the natural environment. The occurrence of denitrification (an anaerobic process) in dry and even desert soils is but one example: theory and years of laboratory work suggested that denitrification ought to occur only in wetland and muck soils, but when new field-based methods became available in the 1970s it became abundantly clear that almost all soils denitrify.

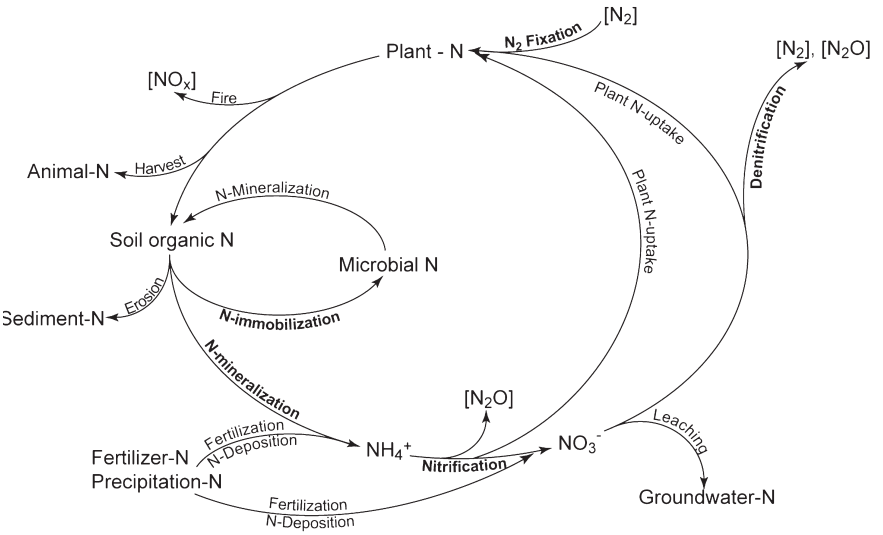


FIGURE 13.1 Schematic representation of the major elements of the terrestrial nitrogen cycle. Those processes mediated by soil microbes appear in bold. Gases appear in brackets.

Key problems have also arisen from evaluating microbial N cycle processes in isolation from other biogeochemical processes in nature (e.g., carbon (C) metabolism and plant nutrient uptake), from underestimating the physiological flexibility of bacteria in nature (e.g., nitrifying denitrifiers, aerobic denitrifiers, anammox bacteria), and from focusing almost exclusively on those microbes that can be cultivated in the laboratory. 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. It is when we attempt to scale up information from the microbial scale, to address real questions relating to plant growth, water pollution, and atmospheric chemistry at ecosystem, landscape, and regional scales, that this problem becomes obvious and important.

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 other microbes. This conversion is carried out by microorganisms that release, or mineralize, nutrients as a by-product of their consumption of detritus. While microorganisms attack detritus primarily as a source of energy and carbon 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, microorganisms must scavenge additional N from their surroundings, taking up or immobilizing N in their biomass.

The key to understanding mineralization–immobilization is to "think like a microbe" that is attempting 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 N is retained by the microbes and even more N must be immobilized from the soil solution. Indeed, it has been shown that microbes invest energy in the synthesis of enzymes (e.g., amidases to acquire N and phosphatases to acquire P) to acquire nutrients that they need while decomposing substrates of low quality. Microbial N uptake is also affected by organism growth efficiency. For example, fungi have wider C:N ratios in their tissues than bacteria and therefore—because of their lower N needs—can grow more efficiently on low-N substrates.

Mineralization results in an increase, while immobilization results in a decrease, in simple, plant-available forms of N in the soil. Traditionally, ammonium has been viewed as the immediate product of mineralization. In fact 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 Bennet, 2004). Plants from a variety of habitats have been shown to take up amino acids and other organic N forms; mycorrhizas 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.

It is important to recognize that mineralization and immobilization are occurring at the same time within relatively small volumes of soil. While one group of microbes might be consuming a protein-rich and therefore nitrogen-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 also 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 is increasing, i.e., there is net mineralization. When gross immobilization exceeds gross mineralization, inorganic N in the soil is decreasing, i.e., there is net immobilization.

Mineralization and immobilization are carried out by a wide array of microorganisms—aerobes, anaerobes, fungi, and bacteria. 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

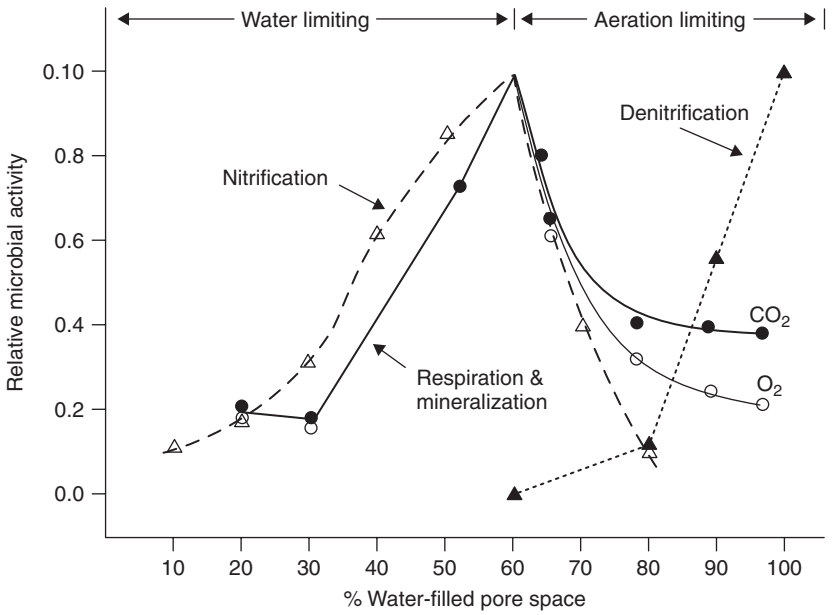


FIGURE 13.2 The relationship between water-filled pore space (a measure of soil moisture availability) and relative amount of microbial activities (redrawn from Linn and Doran, 1984).

populations of bacteria and fungi, and they can create or modify habitats for a wide array of organisms, e.g., earthworms create burrows and termites macerate wood. Mineralization and immobilization are widely distributed because they are so fundamental—all heterotrophic soil organisms consume organic materials for energy and C and immobilize and mineralize N as a by-product.

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 water contents, similar to respiration in Fig. 13.2, although it is important to recognize that significant activity is likely to occur 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.

Water-filled pore space (WFPS) is a useful measure of 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

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

TABLE 13.2 C:N Ratios in Various Organic Materials (from Tisdale *et al.*, 1993 and Hyvönen *et al.*, 1996)

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

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 rock—sand grains and other soil mineral particles.

What controls the balance between N mineralization and N 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 materials with different C:N ratios to soil (Table 13.2). When one adds manure, with a relatively low C:N ratio (ca. 20:1), to soil, for example, 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 are keen to obtain the energy and C in the sawdust but cannot degrade this material without additional N because the sawdust does not have sufficient N to allow the microbes to build proteins. So the microbes must immobilize N from their environment, resulting in a decrease in plant-available N in the soil.

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, while those with a C:N ratio $<$25:1 stimulate mineralization (Table 13.2). The exception to this rule is highly decomposed substances with a low C:N ratio, e.g., soil organic matter (humus or compost) in which labile C and N have been depleted and the remaining C is in complex forms inherently resistant to decomposition (see Chap. 12) and therefore resistant to mineralization.

There are a wide variety of methods for measuring mineralization and immobilization (see Hart *et al.*, 1994; Robertson *et al.*, 1999). Measurement of net mineralization and immobilization rates is much easier and more common than is measurement of gross rates. Measurement of net rates usually involves quantifying

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 label is used as a basis for calculating the gross production and consumption of ammonium.

NITRIFICATION

Nitrification is the microbial oxidation of reduced forms of nitrogen 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 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:

$$NH_4^+(aq) \rightleftharpoons NH_3(aq) + H^+(aq).$$

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, can be easily transported out of the rooting zone by water when precipitation exceeds evapotranspiration.

Nitrate is also subject to denitrification, in which denitrifying bacteria convert nitrate to N gas. Additionally, nitrification in many soils is a major source of soil acidity, which can have multiple effects on ecosystem health, including the hydrologic loss of base cations as hydrogen ions displace other cations from exchange sites. And 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 nil. 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 can also be direct sources of the atmospheric gases NO_x and N_2O.

THE BIOCHEMISTRY OF AUTOTROPHIC NITRIFICATION

Autotrophic nitrification is a two-step process, carried out by separate groups of bacteria: 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. NH_3 oxidation is characterized as

$$NH_3 + 1\frac{1}{2}\,O_2 \rightarrow NO_2^- + H^+ + H_2O.$$

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, non-polar low-molecular-weight compounds, including phenol, methanol, methane, and halogenated aliphatic compounds such as trichloroethylene:

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

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

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

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:

$$2H^+ + \tfrac{1}{2}O_2 + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O.$$

Intermediary compounds formed during the oxidation of hydroxylamine to nitrite can result in the formation of NO (Fig. 13.3), which can escape to the atmosphere and influence the photochemical production of ozone (O_3) and the abundance of hydroxyl (OH) radicals in air, primary oxidants for a number of tropospheric trace gases including methane. Ammonia oxidizers also appear 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! Denitrification is described later in this chapter.

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:

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

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 respired via the Calvin cycle; growth efficiencies of the nitrifiers are correspondingly low. This

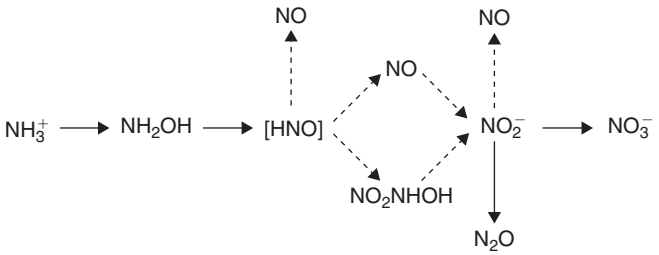


FIGURE 13.3 Autotrophic nitrification pathways including pathways for gas loss. Broken lines indicate unconfirmed pathways (from Firestone and Davidson, 1989).

explains in part their poor ability in many soils to compete with heterotrophs and plants for ammonium.

THE DIVERSITY OF AUTOTROPHIC NITRIFIERS

For a group of microbes with such functional importance at a variety of scales, the nitrifiers are a remarkably depauperate group from a taxonomic standpoint. Although slow growth rates hinder cultivation-based analyses of their diversity, as do culture techniques that fail to reproduce the diversity of microhabitats in soil (see Chap. 3), even molecular methods fail to find much nitrifier diversity. There is, nevertheless, even in the single phylogenetic group to which most ammonia-oxidizing bacteria belong, significant sequence and physiological diversity. Moreover, the recent discovery of widespread ammonia-oxidizing Archaea bacteria in marine ecosystems (Francis *et al.*, 2005) suggests the potential for similar discoveries of new diversity in soil. Leininger *et al.* (2006) suggest that Archaea may be more numerous than bacterial ammonia oxidizers in soil.

From a taxonomic standpoint, bacterial nitrifiers are viewed as the single Family Nitrobacteraceae, defined by their characteristic ability to oxidize ammonia or nitrite, although biochemical and molecular evidence provides no justification for this view. Early work beginning with Winogradsky (1892) classified the ammonium-oxidizing genera of Nitrobacteraceae on the basis of cell shape and the arrangement of intracytoplasmic membranes. This yielded five genera: *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio.* More recent work based principally on 16S rRNA oligonucleotide and gene sequence analysis places all of these groups in the beta subclass of the Proteobacteria but for a single species of *Nitrosococcus*, which is placed in the Gammaproteobacteria (Purkhold *et al.*, 2000; Fig. 13.4). Today we have almost complete 16S rRNA gene sequences for the 14 species of Betaproteobacteria ammonia oxidizers, which have a gene sequence similarity of 89% (Fig. 13.5; Koops *et al.*, 2003).

Of the ammonia oxidizers thus far isolated from soil all are in the Betaproteobacteria. In arable soils the *Nitrosomonas communis* lineage with strains of *Nitrosolobus multiformis* are numerically dominant. Unfertilized soils usually also contain strains of the *Nitrosomonas oligotropha* lineage and strains of *Nitrosospira*

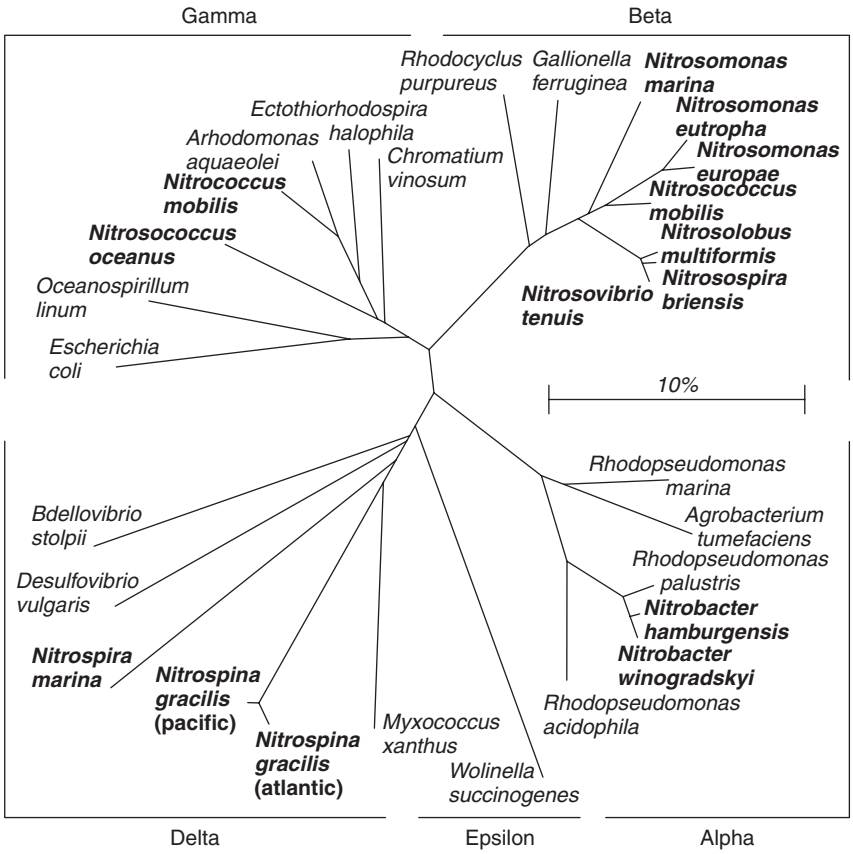


FIGURE 13.4 Distance tree for the Proteobacteria including nitrifiers (in bold). The scale bar corresponds to 0.1 estimated fixed mutation per sequence position (from Teske *et al.*, 1994). More recent work places *Nitrococcus mobilis* in the Betaproteobacteria (see Fig. 13.5).

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 sequences and the retrieval of *amoA* clones have also been used to examine the diversity of ammonia oxidizers *in vivo*, which avoids the need for pure-culture cultivation and its bias toward those species that are cultivatable outside of their native habitat. Although molecular techniques can themselves be biased because of their dependency on the extraction of nucleic acid from soil, PCR amplification, primer bias, and cloning methods, they mostly corroborate pure-culture findings: most *amoA* clones and 16S rRNA based surveys are similar to sequence clusters defined by cultured ammonia oxidizers. Nevertheless, few sequences are completely identical to those of cultured organisms, and since it is not possible to obtain DNA–DNA hybridization data for noncultured organisms, there is no way currently to know if these differences are sufficient to define different species.

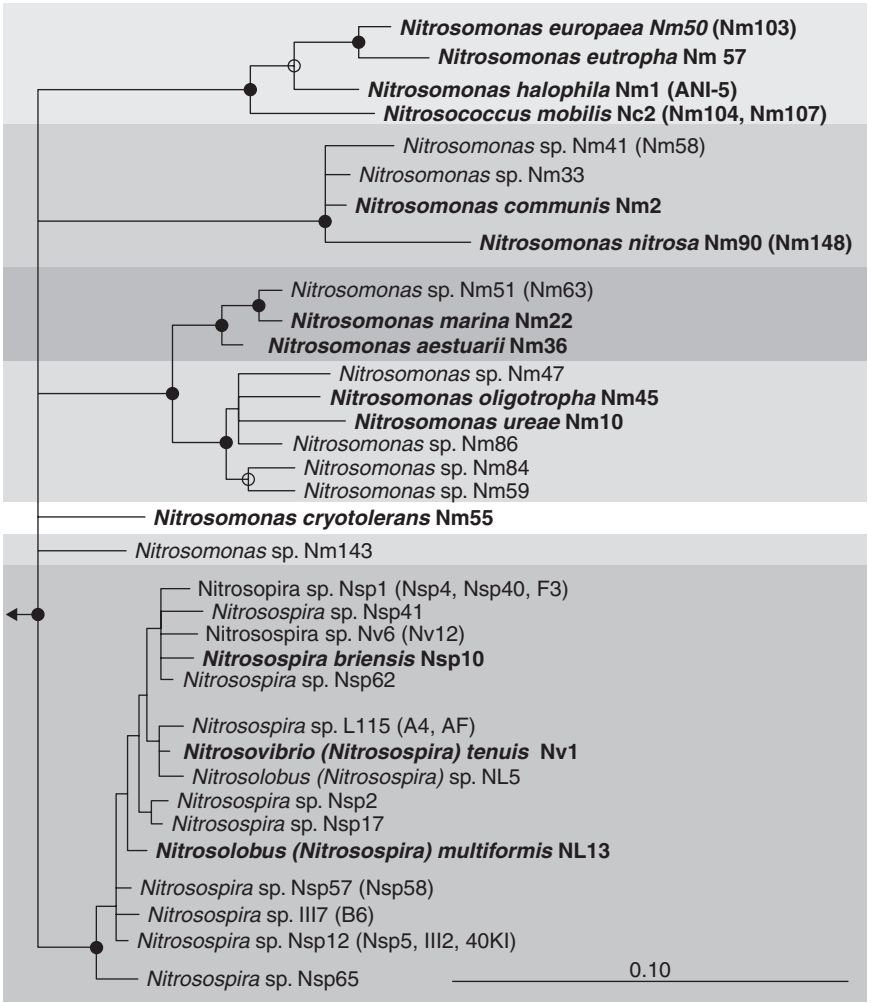


FIGURE 13.5 16S rRNA-based phylogenetic tree of the betaproteobacterial ammonia oxidizers. The tree includes only those oxidizers that have been demonstrated to represent different genospecies (DNA–DNA similarity <60%) and for which 16S rRNA gene sequences longer than 1000 nucleotides are available. 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.*, 2003).

Worth noting too is the fact that these techniques do not 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 the spatial distribution of

targeted organisms. Recent work (e.g., Prosser and Embley, 2002) has shown how these techniques can be used to discover nitrifier community change in response to changes in ecosystem management and land use.

Nitrite-oxidizing bacteria appear in a broader array of phylogenetic groupings than do the ammonia oxidizers, but only the genera *Nitrobacter* and *Nitrospira* (Freitag *et al.*, 2005) have been detected in soil; the distribution of the other nitrite-oxidizing genera (*Nitrosococcus* and *Nitrospina*) is not fully known. Members of *Nitrobacter* form an exclusive and highly related cluster in the Alphaproteobacteria (Fig. 13.4). Pairwise evolutionary distance estimates are less than 1%, indicating little genetic diversity within the group (Fig. 13.5), a finding supported by 16S rRNA sequence comparisons (Teske *et al.*, 1994). The other nitrite-oxidizing genera are in the delta (*Nitrospina and Nitrospira*) and gamma (*Nitrosococcus*) subclasses of the Proteobacteria.

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 is similar to that of autotrophic oxidation, in that the nitrifying bacteria have similar ammonia- and hydroxylamine-oxidizing enzymes. In fact 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:

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

These reactions are not coupled to ATP and thus produce no energy. Alternatively, 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. It was assumed until recently

that most of this nitrification was heterotrophic; we know now that most nitrification in acid soils is autotrophic (De Boer and Kowalchuk, 2001), although the exact mechanisms by which nitrification occurs at low pH are not well understood. Heterotrophic nitrification thus appears important in some soils and microenvironments, perhaps particularly where autotrophic nitrifiers are chemically inhibited (see below), but are thought now to rarely dominate the soil nitrifier community.

ENVIRONMENTAL CONTROLS OF NITRIFICATION

The single most important factor regulating nitrification in the majority of soils is ammonium supply (Fig. 13.6). 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. Tillage, fire, clear-cutting, waste disposal, fertilization, atmospheric N deposition—all have well-documented effects on nitrate production in soils, mostly due to their effects on soil NH_4^+ pools.

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, and also following the addition of high C:N residues to agricultural soils because of high microbial (heterotroph) demand for N. In old-growth forests and mature grasslands, plant N demand has diminished and consequently nitrification is usually higher than in midsuccessional communities in which plant biomass is still accumulating, but not usually as high as in early successional communities, in which 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, usually only a few millimeters thick. And although 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. 13.2). Nitrification occurs slowly but readily under snow and in refrigerated soils, and soil transplant experiments (e.g., 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 and 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, 1989). We now recognize that nitrification can be high even in very acid forest soils ($pH < 4.5$;

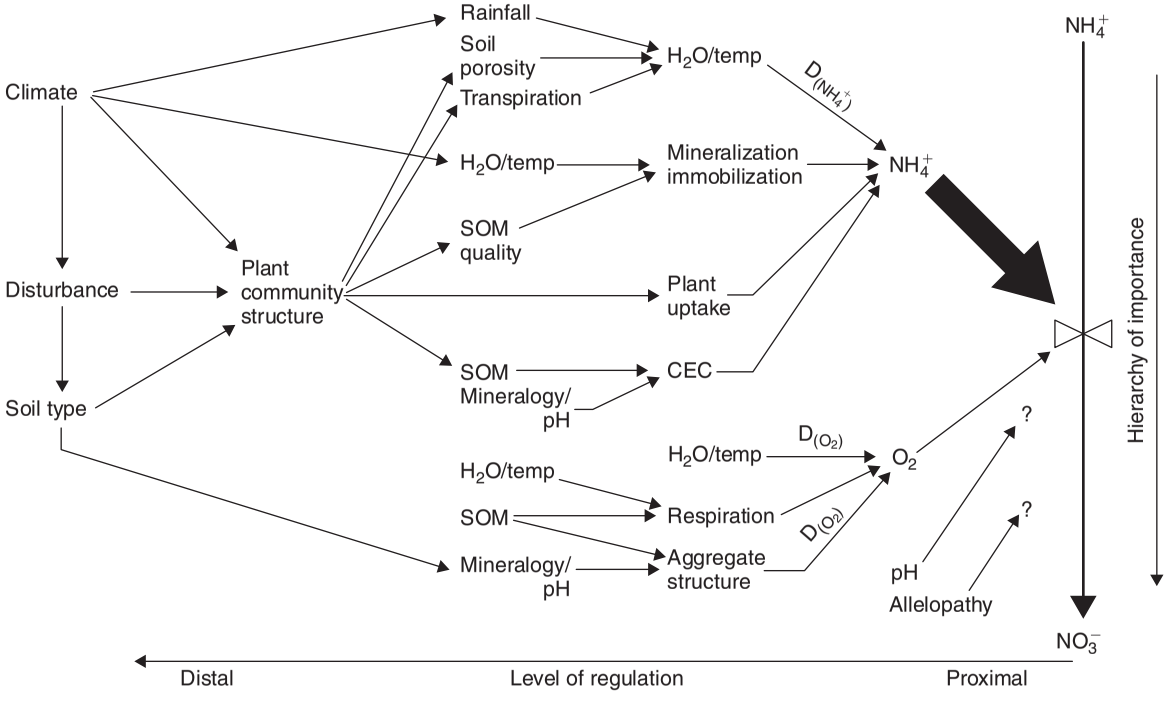


FIGURE 13.6 Environmental controls on nitrification (from Robertson, 1989, after Groffman *et al.*, 1988). The most proximal scale (right side) is at the cellular level.

Robertson, 1989), although the physiological basis for this is still not well understood (DeBoer and Kowalchuck, 2001).

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*, for example, although their importance *in situ* is questionable. Likewise, commercial products such as nitrapyrin and dicyandimide 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. One recent 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. 13.1). 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 by nitrate leaching and denitrification, the two principal pathways of unintentional N loss in most ecosystems. Because many plants prefer to take up N as NO_3^-, it is not desirable to inhibit nitrification completely 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.

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 otherwise unavailable. In most soils this occurs mainly following rainfall as soil pores become water-saturated and the diffusion of O_2 to microsites is slowed drastically. Typically denitrification starts to occur at water-filled pore space concentrations of 60% and higher (Fig. 13.2). In wetland and lowland rice soils 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 at which 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 Chap. 14) would eventually draw atmospheric N_2 to nil,

and the biosphere would be awash in nitrate. Denitrification is also important 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 waste 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.

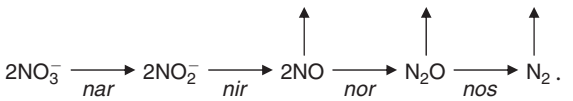
Denitrification can also remove nitrate from groundwater prior to its movement to streams and rivers. In most wetlands and riparian areas nitrate-rich groundwater must move across a groundwater–sediment interface that is typically anaerobic and carbon-rich. As nitrate moves across this interface it can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

In managed ecosystems it is usually desirable to minimize denitrification in order to conserve N further for plant uptake; in regions with ample rainfall ecosystem 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).

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 to 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



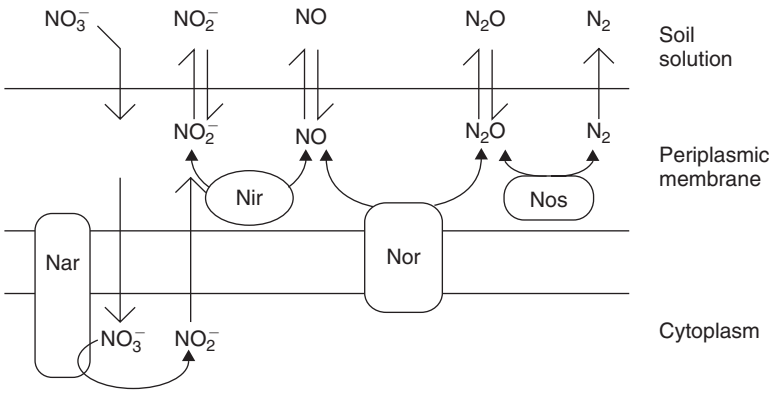


FIGURE 13.7 The organization of denitrification enzymes in the cell membrane for gram-negative bacteria (adapted from Ye *et al.,* 1994).

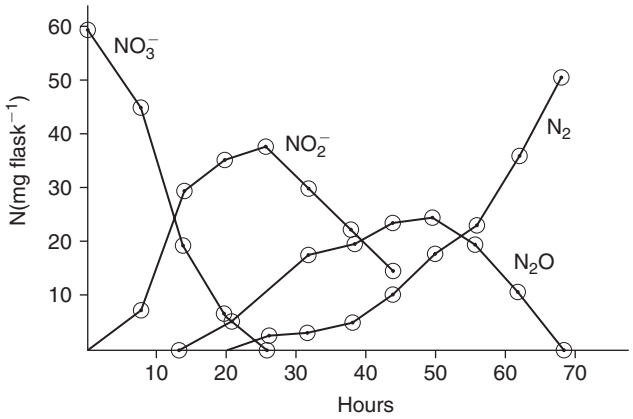


FIGURE 13.8 The sequence of products formed during denitrification (adapted from Cooper and Smith, 1963).

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 gram-negative bacteria is described in Fig. 13.7. 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. 13.8); in the field lags 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 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; Bergsma *et al*. 2002).

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 even forest and grassland soils was confirmed. Acetylene selectively inhibits nitrous oxide reductase (*nos*; see Fig. 13.7), 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 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 and 1980s 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^-. C 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 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.

In unsaturated soils, on the other hand, the availability of soil C 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 distinguish between these two effects experimentally; from a management perspective, there probably is no need to. It is well recognized that exogenous C stimulates denitrification, although the C added must be in an available form and must not lead to N immobilization sufficient to deplete NO_3^- availability.

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, but the ecology of DNRA is much less well understood than that of denitrification. A capacity for DNRA has been found in facultative and obligately fermentative bacteria and has long been thought to be restricted to highly anaerobic environments such as anaerobic sewage sludge bioreactors, anoxic sediments, and the bovine rumen. More recently, however, DNRA has been found to be common and important in some tropical forest soils (Silver *et al.*, 2001). 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. The importance of DNRA in other soils is not clear because few measurements have been made due to the difficulty of measuring DNRA in the presence of other active N-cycle transformations.

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).

Anaerobic ammonium oxidation (anammox), in which ammonium and nitrite are converted to N_2, has been only recently discovered (Mulder *et al.*, 1995; Jetten, 2001) and thus its environmental significance is not fully known except in oceanic systems (Kuypers *et al.*, 2005). Anammox bacteria grow very slowly in enrichment culture and only under strict anaerobic conditions; anammox thus is likely to be a significant soil process only in periodically or permanently submerged soils. One pathway, involving the combination of ammonia with nitrite, has been shown to occur in three or four obligate anaerobes, including *Brocadia anammoxidans* and *Scalindua* spp. A second pathway involves the nitrifier *Nitrosomonas* spp., in a nitrogen tetraoxide (N_2O_4)-dependent reaction that also produces significant amounts of NO and N_2O (Schmidt *et al.*, 2002).

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:

$$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2.$$

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

$$HNO_2 + NH_4^+ \rightarrow N_2 + 2H_2O.$$

Chemical decomposition of HNO_2 may also occur spontaneously:

$$3HNO_2 \rightarrow HNO_3 + H_2O + 2NO.$$

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 nitrogen chemistry.

NITROGEN MOVEMENT IN THE LANDSCAPE

Microbial transformations of reactive N (Table 13.3) have great importance for soil fertility, water quality, and atmospheric chemistry at ecosystem, landscape, and regional scales. It is at these scales that the disconnect between what we have learned in the laboratory and what we observe in the environment (see Introduction) becomes 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 a sink of particular N species of environmental concern (Table 13.4).

TABLE 13.3 Forms of N of Concern in the Environment

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

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 13.1 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 absorb reactive N produced in source areas of the landscape. Riparian buffer zones next to streams, for example, can be managed to absorb 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 (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). 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

TABLE 13.4 Criteria for Determining if a Site Is a Source or a Sink of N in the Landscape (from Groffman, 2000)

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)

and hypoxia that have been linked to excess N from the Mississippi river basin (Turner and Rabalais, 1994). 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, however, their management can be an important and even crucial means for regulating N fluxes at local, regional, and global scales.

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