4.4 Denitrification

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4.4.1 Introduction

Denitrification is the dissimilatory reduction of soil NO$_3^-$ to the nitrogenous gases NO, N$_2$, and N$_2$O. It is carried out by a wide variety of heterotrophic bacteria that use NO$_3^-$ as a terminal electron acceptor when O$_2$ is unavailable. Hence, denitrification occurs in soil environments where C and NO$_3^-$ are available during periods of restricted O$_2$. In wetland soils, these conditions may exist most of the time. In upland soils, these conditions occur mainly following rainfall and within soil aggregates and decomposing litter.

At a global scale, denitrification is a crucial part of the overall N cycle. It is the only point in the cycle in which fixed N reenters the atmosphere as N$_2$. Thus, denitrification closes the N cycle. Without denitrification, atmospheric N$_2$ would eventually be drawn down to nil by N fixers (Section 4.1), and the biosphere would be awash in NO$_3^-$. Globally, denitrification in soil may account for >60% of total N$_2$ + N$_2$O production (Bowden, 1998; Asaka et al., 1992).

Denitrification is important for several reasons as well. It is a major source (perhaps the major source) of atmospheric NO$_x$, an important, radiatively active greenhouse gas that also consumes O$_3$ once it reaches the stratosphere. Nitrogen oxides are an important source of NO in the atmosphere to which cells are continually exposed. Nitrogen oxides are one of the three biogenic trace gases targeted for reduction at the recent International Framework Convention on Climate Change in Kyoto (Bolin, 1998).

At an ecosystem scale, denitrification can rival the amount of N inputs in upland soils and can exceed hydrologic losses of N both in wetlands and in arid and semiarid environments following rainfall or irrigation events. From a management perspective, denitrification can be a positive attribute when it is desirable to remove excess NO$_3^-$ from soil prior to its movement to surface or groundwater (Lowrance et al., 1984). More often, however, managers seek to minimize denitrification in order to further conserve N for plant uptake. Another ecosystem-level consequence of denitrification is the tendency to enhance soil acidification. By removing a NO$_3^-$ anion from the soil solution, denitrification effectively consumes acidity, an effect that can be particularly high in highly

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weathered, variable charge soils, in which CEC is largely governed by soil pH (Sollins et al., 1988; Robertson et al., 1988). Many tropical soils are dominated by variable charge minerals (Uehara and Gillman 1981), and denitrification appears to be an especially active process in the humid tropical soils examined thus far (Robertson and Tiedje, 1988; Großmann, 1995).

Complicating our understanding of denitrification in ecosystems with well-drained soils is denitrification’s extreme spatial variability. Not only does denitrification appear to cluster temporarily around rain events (e.g., Sesselman et al., 1985), it also is highly variable spatially on the order of centimeters to meters (Fotinos and Rolston, 1985; Robertson et al., 1988).

### 4.4.2 Denitrifiers

Denitrification is carried out by a broad array of bacteria, including mostly organotrophs, but also chemo- and photolithotrophs. N₂ fixers, thermophiles, halophiles, and various pathogens. It is remarkable that denitrification occurs in so broad an array of microbial taxa; over 50 genera with over 25 denitrifying species have been identified (Zumft, 1992). In soil, most cultivable denitrifiers are facultative anaerobes from only 3-6 genera, principally *Pseudomonas* and *Alcaligenes* and to a lesser extent *Bacillus*, *Agrobacterium*, and *Flavobacterium* (Tiedje, 1994). Typically, denitrifiers constitute 0.1 to 5% of the total cultivable soil population (Tiedje, 1988).

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

$$2\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$$  \[(4.4.1)\]

Each step is catalyzed by individual enzymes, namely, nitrate reductase (NAR), nitrite reductase (NiR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS). Each is inhibited by O₂, and the organization of these enzymes in the cell membrane has been worked out for Gram-negative bacteria as described in Fig. 4.10. At any step, in process intermediate products can be exchanged with the soil environment, making denitrifiers a significant source of N₂O in soil solution, and important sources of NO and N₂O gas fluxes.

Each denitrification enzyme is inducible, primarily in response to O₂ partial pressure (PₐO₂), soil pH, and substrate C:N ratio. 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. 4.11); in bulk soil, lags can be substantially longer, and differences in lags among groups comprising the complex soil microbial community, together with substrate abundance, may significantly affect the contribution of denitrifiers to fluxes of the intermediates NO and N₂O to the atmosphere. The fact that induced enzymes degrade at different rates, and more slowly than they are induced, also leads to a hysteresis response around environmental conditions that induce denitrification: whether a soil has denitrified recently, i.e., identifying enzymes are present, may largely determine the soil's response to newly favorable conditions for denitrification. Rainfall onto soil that is moist, for example, will, all else being equal, lead to a faster, and perhaps stronger denitrification response than will rainfall onto the same soil when it is dry (Goldman and Middelburg, 1988).

By the same token, denitrifier N₂O production following rainfall on a previously dry soil may be initially stronger than N₂O production in a previously wet soil, because the dry soil there will be a lag between the onset of rainfall and the induction and synthesis of nitrous oxide reductase (Nar; Fig. 4.30). That means that the moisture regime immediately prior to rainfall may partly determine the nitrous fraction of gas product that is N₂O (or the N₂O/N₂ ratio). Whether or not these lags are significant contributors to differences in N₂O production among different habitats is unknown and under active investigation.

Also contributing to differences in the molar ratio of N₂O/N₂ flux in soil may be population-level differences among the bacterial species comprising the denitrifier community. Denitrifier differ in their ability to utilize N₂O as a nutrient (Abou Seada and Assman, 1985; Munch, 1991), and recent evidence suggests that these differences, coupled with differences in denitrifier community composition among different habitats, may help to explain differences among habitats in rates of N₂O flux that do not appear to be directly related to environmental conditions (Bartlett, 1998).

4.4.3 Controls on Denitrification

For decades after its discovery as an important microbial process, denitrification was assumed to be important in nitrogen-cycling soil environments. Only in sediments and wetland soils was denitrification recognized to be important to ecosystem function. In fact, it was not until the advent of whole ecosystem simulations and the use of ¹⁵N to trace the fate of fertilizer N that denitrification was considered potentially important in soils other than wetlands. It was these studies (Atkinson, 1955) that suggested the role for denitrification to upland soils, though until the development of the accurate
block technique in the 1970s (Yoshinari and Knowles, 1976; Smith et al., 1978), the importance of denitrification in nonagronomic systems, where $^{15}$N could not be used effectively, could not be confirmed. Acetylene selectively inhibits nitrous oxide reductase ($N_{\text{ox}}$), allowing one to assess $N_2$ production by following $N_2O$ accumulation in an acetylene treated soil core (Tiedje, 1994) or soil monolith (Ryden and Dawson, 1982; Rolston et al., 1982).

Today, denitrification is known to be an important $N$ cycle process wherever $O_2$ is limiting and $C$ and $NO_3^-$ are available. In unsaturated soils, this frequently occurs within soil aggregates (Sextone et al., 1985b), in decomposing plant litter (Parkin, 1987), and in rhizospheres (Prade and Troldenier, 1990; Nieder et al., 1989). Soil aggregates vary widely in size but, in general, are comprised of small mineral particles and pieces of organic matter < 2 mm diam. glued to one another with biologically derived polysaccharides (Oades, 1993). Like most particles in soil, aggregates are surrounded by a thin water film that impedes gas exchange. Modeling efforts in the 1970s and 1980s (Arah and Smith, 1989) 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 experimentally confirmed in 1985 (Fig. 4.12) (Sextone et al., 1985b), 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 (Gamble et al., 1977).

Three major environmental controls act in concert to regulate denitrification fluxes in soil, namely, $C$, $NO_3^-$, and $O_2$. Carbon is important because in most ecosystems most denitrifiers are heterotrophs (Poth and Focht, 1985) and require $C$ as the electron donor. Nitrate serves as the electron acceptor, and must be provided via nitrification (Section 4.3) or from external sources such as atmospheric deposition or fertilization (Section 4.5). Oxygen is a preferred electron acceptor because of its high energy yield as compared to alternate acceptors such as $NO_3^-$ or $SO_4^{2-}$, and thus $O_2$ must be depleted before denitrification occurs. In most soils, the majority of the denitrifiers present are facultative

![Fig. 4.12 Measured oxygen profile through a 1.2 cm soil aggregate [Redrawn from Sextone et al., 1985b. Soil Sci. Soc. Am. J. 49:645-651, with permission of the Soil Science Society of America]
anerobes (Tiedje, 1988; Knowles, 1991) that will simply avoid synthesizing denitrifier enzymes until O₂ steps below some critical threshold.

In the field, O₂ is by far the dominant control on denitrification (NO₂⁻ → N₂) flux from soils. It is an easy matter to reduce denitrification in an aerobic soil by removing O₂ and to reduce denitrification in saturated soil by drying or otherwise aerating it. The relative importance of C and NO₃⁻, the other major controls, will vary by ecosystem. Under saturated conditions, such as those found in wetlands and lowland rice paddies, NO₃⁻ limits denitrification because nitrifiers, the principal source of NO₂⁻ in unfertilized ecosystems, are inhibited at low O₂ concentrations. Consequently, denitrification occurs only in the slightly oxygenated rhizosphere and at the sediment-water interface, places where nitrifiers have sufficient O₂ to oxidize NO₂⁻ to NO₃⁻, which can then diffuse to the denitrifiers in the increasingly anaerobic zones away from the root surface of sediment-water interface. It is often difficult to find NO₂⁻ in persistently saturated soils not only because of low nitritation, but also because of the tight coupling between nitrifiers and denitrifiers (Patric, 1982; Reddy and Patric, 1980; Monieret et al., 1990). In wetlands with fluctuating water tables or with significant inputs of NO₃⁻ from groundwater, NO₂⁻ may be less limiting.

In unsaturated soils, on the other hand, C availability more often limits denitrification. In these soils, carbon supports denitrification both directly, by providing electron donors to denitrifiers, and indirectly, by stimulating O₂ heterotrophic consumption. 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 excess C stimulates denitrification (Bremmer and Shaw, 1954a,b; Knowles, 1991), although the C added must be in an available form and must not lead to N immobilization sufficient to decrease NO₂⁻ availability (Fiestone, 1982)

Where the effect of C on denitrification is indirect through its effect on O₂ availability, C can be considered a secondary control, similar in effect to other controls on denitrification that serve to affect denitrification rates via their effects on the availability of the three primary controls of substrate
C, NO₂⁻, and O₂. Tiedje (1988) and Groffman et al. (1988) used the terms distal and proximal to describe the hierarchical nature of major controls on denitrification. In this scalar characterization (Fig. 4.13), different ecological systems affect C, NO₂⁻, and O₂ availability differently, with these attributes themselves affected by physical and biological phenomena that occur at larger spatial scales. Water, for example, affects denitrification principally via its influence on O₂ availability and on the diffusivity of NO₂⁻ and C, water, in turn, is affected by (among other factors) soil porosity and transpiration rates, which are influenced, in turn, by (among other factors) the plant community and soil microbiological activity. Eventually, of course, all controls evolve from climate and land use influences. The usefulness of this scheme is in its identification of new ecosystem disturbance, whether delivered by management or by nature, might affect denitrification fluxes.

4.4.4 Managing Denitrification

Efforts to manage denitrification in soil arise from three goals: (1) to maximize the conservation of N in ecosystems to which N is added or otherwise managed to enhance productivity, (2) to remove NO₂⁻ from hydrologic flow paths to surface and groundwater, usually at landscape control points such as sewage treatment plants and riparian zones adjacent to streams, and (3) to reduce fluxes of NO₂ to the atmosphere. This latter goal is relatively new and may well dominate efforts to control denitrification in the coming decades as legislation and policy are developed to meet the provisions of international agreements to reduce greenhouse gas emissions (Bolin, 1998). All three goals are interrelated, but their concomitant objectives are quite distinct from one another: the first goal can be met only by inhibiting or otherwise attenuating denitrification in situ, the second goal can be met principally by stimulating denitrification, and the third by either inhibiting denitrification or by skewing the mole-rat product ratio N₂O:N₂ toward N₂. Goals (1) and (2) are not mutually exclusive only because they are addressed in different parts of the landscape, which emphasizes the need to embrace a management perspective that is greater than the scaled of individual fields.

Cropping systems receive most of the N fertilizer N now produced globally each year (Bumb, 1995). In general, about half of the fertilizer N applied to any given cropping system is lost as NO₃⁻, or gas in the year it is applied (Legg and Moreing, 1982; Robertson, 1997). The portion lost as NO₂⁻ is that portion that is targeted for NO₂⁻ removal downstream (Coal 2 above); thus, conserving N loss is general, rather than simply inhibiting denitrification in situ, obviates the need to control denitrification both upstream and downstream.

How does one conserve N, and thereby, reduce denitrification at the field scale? Fertilizer composition and the rate and timing in which it is applied have a substantial impact on N loss from cropping systems. Myriad technical strategies are available for improving crop N use efficiency (Keeney, 1982; Myers, 1988; Peoples et al., 1995; Robertson, 1997). The most important of these is to synchronize N supply with plant N demand. Typically fertilizer is added to crops prior to the period of maximum crop growth, and prior to uptake this N is available to denitrifiers for transformation to NO₂⁻ and N₂, and is available to wetting fronts for hydrologic transport from the field. Moreover, much of the mineralizable N in crop residues from the previous year is mineralized (transferred from organic to inorganic form [Section 4.2]) prior to crop growth, and this N also increases the soil solution pool available for loss. It is not unusual for the soil NO₂⁻ pool under annual field crops to increase by an order of magnitude prior to significant crop uptake.

The release of N from crop residues can be manipulated directly by tillage, by managing residue quality and quantity, and by the use of cover crops during periods when the field would otherwise be fallow. Cover crops remove organic N from the soil solution, and thereby, act as a temporary buffer against N loss during that portion of the cropping cycle when mineralized N accumulates in the soil solution and is available for uptake or loss. Properly managed, the N retained by the cover crop can
be slowly released by mineralization during the growing season after it is killed when the principal crop is plowed. Unlike fertilizer, which can be added to irrigation water, it is also difficult to manage the timing of fertilizer application. Usually, agronomic considerations dictate fertilizer application in a single pulse at a time when farm machinery can access the field without harming the growing crop or soil structure. Alternatively, slow-release fertilizers such as sulfur-coated urea (Allen, 1984) can improve crop nutrient synchrony, although they generally are not economically practical under most cropping regimes. In situ inhibitors have also been developed for microbially transformed, and can effectively block denitrification in the field. Various nitrification inhibitors, for example, block NO\textsubscript{3} formation, and thereby reduce both denitrification and leaching losses of N. They are not widely used, however, owing to their expense relative to the lower expense of excess N use, and to their inconsistent behavior (Mensing et al., 1983). They do, however, have the added advantage of blocking nitifier-derived N\textsubscript{2}O (Peoples et al., 1996; Klemmedson and Mosier, 1994).

Denitrification downstream of the agricultural field, in particular at the soil-stream interface can effectively remove NO\textsubscript{3} from the hydrologic flow path (Lowrance et al., 1984; Petersohn and Corell, 1986; Hedlin et al., 1998). Together with NO\textsubscript{2} uptake by vegetation, denitrification in riparian zones and wetlands provides an effective filter to protect surface and groundwater from NO\textsubscript{3} contamination. Thus the design and management of riparian buffer strips have become a particularly important consideration for managers seeking to reduce watershed level NO\textsubscript{3} inputs to coastal waters (Holbark and Lawrence, 1997). Likewise, wetland restoration and water level management may also become an important management tool for reducing downstream NO\textsubscript{3} inputs.

Moving into an era in which fluxes of greenhouse gases become increasingly regulated, one may see a call for reducing N\textsubscript{2}O fluxes as part of efforts to reduce N\textsubscript{2}O emissions (in total N\textsubscript{2}O + N\textsubscript{2}). Management to favor the further reduction of N\textsubscript{2}O to N\textsubscript{2} still, in cropping systems, likely focus on available soil N\textsubscript{2}O concentrations and on water management. For example, in order to reduce N\textsubscript{2}O-N, N\textsubscript{2}O is the preferred electron acceptor when both N\textsubscript{2}O and N\textsubscript{2} are present (Barris, 1984), which leaves N\textsubscript{2}O as a more likely denitrifier in some environments. However, N\textsubscript{2}O is also more likely to occur where soil conditions favor denitrification activity, periods during which denitrifiers lose N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase (N\textsubscript{2}O reductase) 4.4.5 Conclusions Denitrification is a crucial part of the global N cycle and can be a major pathway of N loss from terrestrial ecosystems. As a major source of the atmospheric trace gas N\textsubscript{2}O, denitrification play an important role in atmospheric chemistry. Recent advances in our understanding of denitrification have clarified its biochemical pathway, have provided knowledge of its importance in even well-aerated, upland soils, have demonstrated its ability for helping to mitigate watershed level NO\textsubscript{3} fluxes, and have clarified ecosystem and watershed level controls on rates of denitrification in both natural and managed habitats. Efforts to model denitrification quantitatively have been stymied by an incomplete understanding of how the factors that affect denitrification rates interact in situ, including the influence of denitrifier community composition. Calls for better understanding denitrification and especially the factors regulating and producing composit-
tion (the N₂O₅ ratio) may grow more intense as policymakers respond to international pressure to reduce greenhouse gas emissions globally.

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4.4.6 References


4.5 Nitrogen in the Environment

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4.5.1 Introduction

While N is an extremely valuable and important nutrient within the soils of nearly all ecosystems on the globe (Vitousek and Howarth, 1991), it becomes a highly problematic pollutant once it leaves one ecosystem and moves into another. Given the human alteration of the global N cycle in extreme (Tamm, 1991), there are multiple forms of N that can be considered as pollutants, and that there

<table>
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<tr>
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<th>Source</th>
<th>Dominant reaction vectors</th>
<th>Environmental effects</th>
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<tr>
<td>Nitrate (NO₃⁻)</td>
<td>Fertilizer, Distance, Combustion and rain</td>
<td>Groundwater, Toxic</td>
<td>Europhication</td>
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<tr>
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<td>Fertilizer</td>
<td>Surface runoff, Toxic</td>
<td>Europhication</td>
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<tr>
<td>Ammonium (NH₄⁺)</td>
<td>Animal waste</td>
<td>Atmosphere, Toxic</td>
<td>Europhication</td>
</tr>
<tr>
<td>Nitrous oxide (N₂O)</td>
<td>Byproduct of multiple transformations</td>
<td>Groundwater, Ozone destruction</td>
<td>Ozone precursor, Greenhouse gas</td>
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<tr>
<td>Nitric oxide (NO)</td>
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<td>Atmosphere, Ozone destruction</td>
<td>Ozone precursor</td>
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<td>Byproduct of natural decomposition</td>
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<td>Europhosphorus (?</td>
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