

See also: **Eutrophication; Greenhouse Gas Emissions; Isotopes in Soil and Plant Investigations; Nitrogen in Soils: Cycle; Nitrification; Plant Uptake; Symbiotic Fixation; Pollution: Groundwater**

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Nitrification

J I Prosser, University of Aberdeen, Aberdeen, UK

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Introduction

Nitrification is the oxidation of reduced forms of nitrogen, ultimately to nitrate. It is carried out by three microbial groups: (1) autotrophic ammonia oxidizers, (2) autotrophic nitrite oxidizers, and (3) heterotrophic nitrifiers. Autotrophic ammonia and nitrite oxidizers are characterized by their ability to oxidize ammonia sequentially to nitrate under aerobic conditions, but these organisms possess a wider metabolic diversity. This has significant consequences for their ecology, for the occurrence and kinetics of nitrification, and for interactions with other organisms. The major sources of reduced forms of soil nitrogen are excretion and decomposition of organic nitrogen, derived from animals and plants, and application of ammonia-based fertilizers. Many microorganisms and plants require ammonium for growth, while others assimilate nitrate. For both groups, nitrification is important in regulating the supply or loss of nitrogen from the environment. While ammonia has a propensity to bind to soil particles, its conversion to nitrate leads to significant losses of soil nitrogen through leaching and conversion to gaseous forms through denitrification. Nitrification also contributes to soil acidification, with consequent increases in

mobilization of toxic metals, particularly in heavily fertilized and poorly buffered soils. In addition, nitrifiers are involved in production and conversion of a number of greenhouse gases.

Autotrophic Ammonia-Oxidizing Bacteria

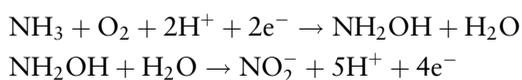
Taxonomy

Autotrophic ammonia oxidizers are Gram-negative bacteria, traditionally placed within the *Nitrobacteriaceae* and characterized by their ability to oxidize ammonia. Further classification is based on a limited number of characteristics, including cell shape, internal membrane structures, flagella, inclusion bodies, salt tolerance, urease activity, and substrate affinity and inhibition. Confirmation of taxonomic groupings and species descriptions is by DNA:DNA hybridization of pure cultures. The type strain, *Nitrosomonas europaea*, has been the subject of the majority of biochemical, physiological, and genomic studies.

More recently, analysis of sequences of 16S ribosomal ribonucleic acid (rRNA) and *amo* A genes (encoding the active subunit of ammonia monooxygenase) has enabled phylogenetic classification of cultured ammonia oxidizers. This places *Nitrosococcus oceanus* in the γ -proteobacteria and other known autotrophic ammonia oxidizers in seven lineages within a monophyletic group within the β -proteobacteria: *Nitrospira*, *Nitrosomonas europaea*/*Nitrosococcus mobilis*, *Nitrosomonas communis*, *Nitrosomonas marina*, *Nitrosomonas oligotropha*, *Nitrosomonas cryotolerans*, and *Nitrosomonas* sp. Nm143. Difficulties in cultivating ammonia oxidizers limit available physiological data, but links exist between these phylogenetic groups, their physiological characteristics, and their environmental origin. Analysis of 16S rRNA gene sequences also indicates evolutionary relationships with photosynthetic bacteria and other groups possessing intracytoplasmic membrane structures, suggesting that ammonia oxidizers evolved from photosynthetic organisms. 16S rRNA sequence analysis suggests only peripheral associations with methane oxidizers, but similarities exist between *amoA* genes of γ -proteobacterial ammonia oxidizers (*N. oceanus*), and *pMMO* (particulate methane monooxygenase), suggesting a common ancestral origin.

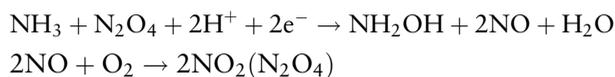
Biochemistry

Ammonia oxidation by β -proteobacteria involves two steps:



In the first step, ammonia is oxidized to hydroxylamine by a membrane-bound ammonia monooxygenase. The reaction is endergonic and requires oxygen and a source of reducing equivalents. Hydroxylamine is then oxidized to nitrite by hydroxylamine oxidoreductase, using oxygen from water in an exergonic reaction. Two electrons are required for ammonia oxidation, while the remainder pass down the electron transport chain. The $\text{NO}_2^-/\text{NH}_3$ redox potential is -340 mV and reduced nicotinamide adenine dinucleotide (NADH) is synthesized by reverse electron flow using adenosine triphosphate (ATP). Hydrogen ions generated by the second step lead to acidification of unbuffered growth medium, limiting sustained growth.

An alternative mechanism for the first step has recently been proposed, involving nitrogen dioxide and tetroxide, based on studies of *N. eutropha*:



This mechanism does not appear to involve ammonia monooxygenase, and studies have also demonstrated reduction in lag phase and the stimulation of growth, activity, and cell yield in cultures supplied with nitric oxide. Any reduction in lag phase will provide ammonia oxidizers with advantages in soil where ammonia supply is intermittent.

Carbon Metabolism

Organic carbon is obtained by nitrifiers via carbon dioxide fixation, and all strains studied as of 2003 possess type I Rubisco genes, but with sequence variation between strains. Rubisco enzymes in some strains are located in carboxysomes. Energy generated by ammonia oxidation is only just sufficient for growth, particularly as carbon dioxide fixation requires significant reducing equivalents, and generation of reducing equivalents consumes ATP.

Ammonia monooxygenase has broad substrate specificity, and ammonia oxidizers can oxidize and assimilate, but not grow on, a number of organic compounds, including methanol, bromoethane, ethylene, propylene, cyclohexane, benzene, and phenol. Acetate, formate, and pyruvate can increase biomass yield by as much as 130%, but higher concentrations inhibit growth. The ecological implications for this are not clear, but assimilation of soil organic carbon may provide a selective advantage, while ammonia oxidizers may be important in biodegradation of organic pollutants in oligotrophic environments, where alternative carbon sources are scarce.

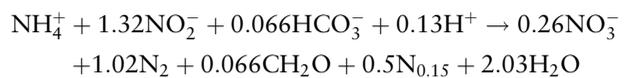
Ammonia monooxygenase is similar to the membrane-bound methane monooxygenase of methanotrophic bacteria. Ammonia oxidizers can oxidize but

not grow on methane and carbon monoxide, and the enzymes have common inhibitors. Incorporation of methane is inhibited by ammonia, and vice versa, and complete methane oxidation may be prevented by inhibition of hydroxylamine oxidation by formaldehyde, produced during methane oxidation. In general, ammonia oxidizers have higher oxidation rates and lower K_m values for ammonia than methanotrophs, while the converse is true for oxidation and affinity for methane. Competition between the two groups for ammonia and methane therefore appears unlikely, but high levels of ammonia-based fertilizer may suppress methanotroph activity. Despite these considerations, ammonia oxidizer 16S rRNA and *amoA* sequences have been found in ^{13}C -labeled nucleic acids extracted from grassland soils treated with ^{13}C -labeled methanol and methane, suggesting their role in soil methane oxidation. Ammonia oxidizers may also have a role in the oxidation of another greenhouse gas, carbon monoxide. They have greater affinity for carbon monoxide than carboxydobacteria, and may provide an important carbon monoxide sink. Carbon monoxide oxidation only occurs during ammonia oxidation, which is required as an energy source, even though carbon monoxide and ammonia compete for the ammonia monooxygenase enzyme.

Ammonia Oxidation under Anaerobic Conditions

Although traditionally considered to be aerobic, autotrophic ammonia oxidizers maintain activity at low oxygen concentrations and under anaerobic conditions. *N. europaea* possesses a nitrite reductase and can denitrify nitrite under anaerobic conditions using hydrogen or organic compounds as electron donors. (They can also denitrify under oxic conditions, using ammonia as electron donor.) This results in the production of nitric and nitrous oxides, and dinitrogen gas. At atmospheric oxygen concentrations, nitrogen oxides constitute 0.1–0.5% of the total N oxidized, but proportions rise to 2.5–10% at low oxygen concentrations.

Anaerobic ammonia oxidation by *N. eutropha* has recently been demonstrated in which nitrogen dioxide or nitrogen tetroxide acts as an electron acceptor, generating nitric oxide and hydroxylamine. The latter is converted to nitrite with release of dinitrogen gas. In addition, anaerobic ammonia oxidation is carried out by autotrophic planctomycetes, which utilize nitrite as an electron acceptor, generating nitrate and nitrogen, with hydrazine and hydroxylamine as intermediates. The process is sensitive to oxygen and nitrite, and the calculated mass balance is:



However, neither of these anaerobic processes has been detected in soil, where their significance is unclear. Both require substrates which are produced under oxic conditions (nitrogen dioxide and nitrite) but the processes may occur at oxic–anoxic interfaces, such as in biofilms formed on soil particulate material.

Autotrophic Nitrite-Oxidizing Bacteria

Nitrite oxidizers are classified into four genera within the proteobacteria: *Nitrobacter* (the most studied; α -proteobacteria), *Nitrococcus* (γ -proteobacteria), *Nitrospira* (δ -proteobacteria), and *Nitrospina* (forming its own subdivision). *Nitrobacter* strains are related to several denitrifying organisms, while *Nitrobacter* and *Nitrococcus* possess intracytoplasmic membranes and are related to photosynthetic bacteria. δ -Proteobacterial nitrite oxidizers do not contain intracytoplasmic membranes and are not related to photosynthetic organisms. Only *Nitrobacter* and *Nitrospira* have been detected in soil. Nitrite oxidation is catalyzed by nitrite oxidoreductase, the $\text{NO}_2^-/\text{NO}_3^-$ redox potential is 430 mV, and reducing equivalents are generated by reverse electron flow, consuming ATP:



Nitrite oxidizers fix CO_2 for growth but, unlike ammonia oxidizers, many are capable of mixotrophic and heterotrophic growth on a range of simple organic substrates. Heterotrophic growth is slow (doubling times of 30–150 h) but leads to greater biomass yields. Nitrite oxidoreductase enables heterotrophic growth under anaerobic conditions, with nitrate as the electron acceptor and production of nitrite and ammonia, and nitrogen oxides. This metabolic versatility may explain greater abundance of nitrite oxidizers than ammonia oxidizers in soil.

Heterotrophic Nitrification

Heterotrophic nitrification is the oxidation of inorganic and organic reduced forms of N, to nitrate, by a wide range of fungi and heterotrophic bacteria. In some organisms, the mechanism is similar to that in autotrophic ammonia oxidizers and is linked, in some strains, to aerobic denitrification. The second mechanism, termed fungal nitrification, is linked to lignin degradation and involves reaction of reduced organic compounds with hydroxyl radicals produced in the

presence of hydrogen peroxide and superoxide. There is little evidence that heterotrophs gain energy or other benefit from nitrification, and cellular rates of heterotrophic nitrification activity are significantly lower than for autotrophs. Nevertheless, it may be important in acid soils or where C:N ratios and heterotroph biomass are high.

Analysis of Nitrifiers and Nitrification

Nitrification Activity and Rates

Nitrification rates in soil are traditionally measured as ammonia consumption or nitrite plus nitrate production during incubation of soil or soil slurry. Zero-order kinetics result from short-term measurements at nonlimiting ammonia concentrations and measure potential nitrification, which can be used to estimate nitrifier abundance. Short-term measurements at limiting substrate concentrations generate Michaelis–Menten kinetics. Long-term incubation of nonenriched soil leads to exponential increases in nitrite plus nitrate owing to unrestricted growth, at nonlimiting substrate concentrations, or growth at submaximal and decreasing rates at limiting substrate concentrations. Nitrate-production kinetics are frequently fitted to the logistic equation. Kinetics can be interpreted differently, however, if nitrifiers are considered to be distributed heterogeneously, as clusters growing on discrete sources of ammonium within the soil. If nitrification is measured in individual soil aggregates, the observed nitrate production curves fit well with the cumulative distribution curves for lag periods. Bulk nitrate concentrations therefore reflect not cumulative product of a uniformly growing population but the continued activation of clusters of nitrifying bacteria. The increase in nitrate concentration then depends on the distribution of lag periods prior to the onset of nitrification. Further evidence for heterogeneous distribution of ammonia oxidizers in soil is provided by molecular analysis of communities

in grassland soils. Regular application of inorganic nitrogen fertilizer reduces heterogeneity in ammonia oxidizer communities, in association with similar reduction in the heterogeneity of soil pH and ammonia concentration. Analysis of nitrification kinetics during incubation is therefore important in providing information on soil population levels and substrate concentrations. Growth parameters are more easily obtained from laboratory cultures and typical values are given in [Table 1](#).

Other nitrogen cycle processes, e.g., denitrification, immobilization, and ammonification, will influence concentrations of ammonium, nitrite, and nitrate. Rigorous measurement of soil nitrification rates, and distinction between autotrophic and heterotrophic nitrification therefore necessitate the use of metabolic blocks and ^{15}N -based techniques. The former involve measurement of nitrate production in the presence of specific nitrification inhibitors e.g., nitrapyrin and acetylene. The second involves applying ^{15}N as NH_4^+ or NO_3^- and following changes in the concentration of label in the respective pools.

Enumeration

Dilution-plate enumeration of viable nitrifiers is not feasible because of poor growth on solid media and enumeration is traditionally achieved using the most probable number (MPN) method. This involves multiple inoculation from dilutions of a soil suspension into mineral-salts medium containing ammonium and a pH indicator. After incubation for several (typically 4) weeks, growth is assessed by nitrite production, ammonium loss, and/or acidification, and cell concentrations are calculated using statistical tables. The requirement for laboratory cultivation leads to underestimation of cell concentrations. In addition, growth may be biased by ammonium concentrations in the medium, with inhibition of some strains at high concentrations ([Figure 1](#)). MPN counts for typical soils range between 10^3 and 10^5 cells g^{-1}

Table 1 Reported ranges for kinetic constants derived from pure cultures of ammonia and nitrite oxidizers

	Ammonia oxidizers	Nitrite oxidizers
Maximum specific growth rate (h^{-1})	0.02–0.088	0.018–0.058
Doubling time (h)	8–35	12–39
Biomass yield ($\text{g biomass mol}^{-1}$ substrate)	0.42–1.72	1.11–1.51
Cell yield (cells mol^{-1} substrate)	1.85–10.6	
Carbon yield (ratio of CO_2 fixed: NO_2^- or NO_3^- produced)	0.014–0.096	0.013–0.031
Cell activity (fmol NO_2^- or $\text{NO}_3^- \text{ cell}^{-1} \text{ h}^{-1}$)	0.9–31.3	5.1–42
Biomass activity (fmol NO_2^- or $\text{NO}_3^- \text{ g biomass}^{-1} \text{ h}^{-1}$)	4–200	15.7–25.2
Saturation constant for growth ($\text{mmol l}^{-1} \text{ NH}_4^+$ or NO_2^-)	0.12–14	0.045–0.178
Saturation constant for activity ($\text{mmol l}^{-1} \text{ NH}_4^+$ or NO_2^-)	0.05–0.07	1.6–3.6

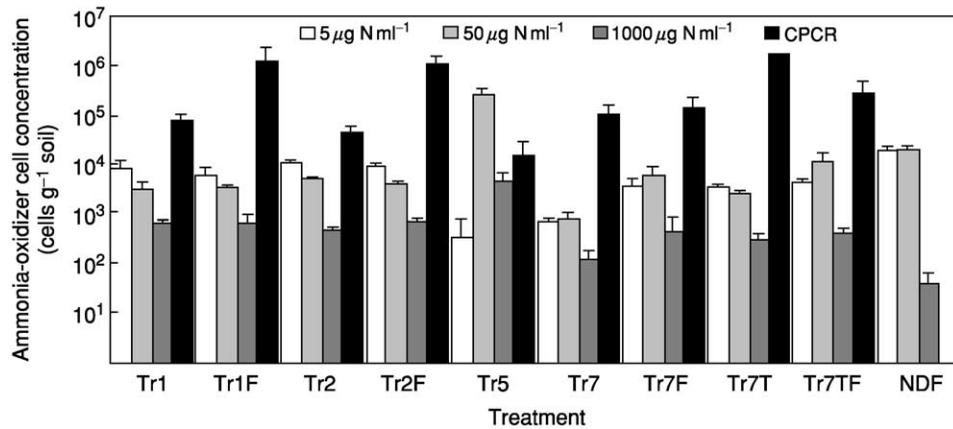


Figure 1 Enumeration of ammonia-oxidizer cells in long-term ecological research (LTER) soils using the most probable number (MPN) method with medium containing 5, 50, or 1000 mg of $\text{NH}_4^+\text{-N ml}^{-1}$ and competitive polymerase chain reaction (C-PCR). Treatments are: Tr1, conventional tilling; Tr2, no tilling; Tr5, *Populus* perennial cover crop; Tr7, historically tilled; NDF, natural deciduous forest. Error bars represent the standard errors. Suffixes 'T' and 'F' indicate tillage and fertilization, respectively. (Reproduced with permission from Phillips CJ, Harris D, Dollhopf SL *et al.* (2000) Effects of agronomic treatments on the structure and function of ammonia oxidising communities. *Applied and Environmental Microbiology* 66: 5410–5418.)

soil for ammonia oxidizers, with nitrite oxidizers slightly more abundant. Quantitative molecular techniques (e.g., competitive PCR (cPCR), real-time PCR) avoid cultivation bias and detect the total nitrifier populations, giving cell concentrations 1–2 orders of magnitude greater than MPN counts (Figure 1).

Enrichment and Isolation

Autotrophic ammonia-oxidizing bacteria are readily enriched by inoculation of an inorganic medium, containing ammonium with soil, and incubation for several weeks. Isolation of pure cultures is more difficult, due to faster growth of heterotrophs on organic carbon contaminants or by-products of nitrifier growth. This typically takes several months and cultures frequently do not survive continued subculturing. Laboratory selection also reduces the likelihood of obtaining dominant or representative environmental strains, but enrichment of a strain is necessary for unequivocal evidence of its presence and viability in a particular soil.

Soil Communities and Diversity

Information on 16S rRNA and *amoA* gene sequences from cultured ammonia oxidizers has led to the design of oligonucleotide polymerase chain reaction (PCR) primers specific for β -proteobacterial autotrophic ammonia oxidizers. These have been used to amplify ammonia-oxidizer gene fragments from nucleic acids extracted directly from soil. Gene fragment sequences may then be analyzed by cloning, sequencing of clone library representatives, and phylogenetic analysis. Alternatively, fingerprinting techniques such as

denaturing or temperature gradient gel electrophoresis (DGGE, TGGE) and terminal restriction fragment length polymorphism (T-RFLP) analysis may be used. These approaches do not require laboratory cultivation and thereby eliminate many of the disadvantages of cultivation-based techniques, and difficulties in purifying and identifying ammonia-oxidizing bacteria.

The application of molecular techniques has greatly expanded our knowledge of nitrifier communities in soils. Phylogenetic analysis of amplified sequences reveals considerable diversity within soil ammonia oxidizers. Duplicate sequences and sequences identical to pure cultures are rarely obtained, although this partly reflects the few pure culture sequences in databases and, potentially, sequencing and cloning errors. Sequences are sometimes homologous to those of laboratory cultures enriched from the same environment and the majority fall within the lineages obtained with sequences from cultivated organisms. One exception is the possible subdivision of *Nitrosospira* into four clusters, and there is evidence of differences in physiological characteristics of pure and enriched cultures representative of these different *Nitrosospira* clusters.

Soils are dominated by β -proteobacterial ammonia oxidizers, with γ -proteobacterial sequences apparently restricted to marine environments. The links between 16S rRNA-defined lineages and physiological characteristics aid in the interpretation of the distribution of these organisms under different soil conditions. This approach also indicates that *Nitrosospira* may be more abundant than *Nitrosomonas* in

soil. If so, more studies are required of *Nitrosospira* physiology, rather than basing interpretation on information on *Nitrosomonas europaea*.

Fingerprinting techniques, particularly DGGE, have been used to study the effects of environmental factors and soil treatments on ammonia-oxidizer communities. This provides more rapid assessment and greater replication than cloning and/or sequencing approaches. In addition, bands of interest on DGGE gels may be excised, cloned, and sequenced and information on identity obtained by comparison with database sequences. These approaches can also be used to assess quantitative or semiquantitative changes in ammonia-oxidizer community structure.

Comparison of 16S rRNA gene sequences in enrichment cultures with those amplified from nucleic acids extracted from the same soil provides an indication of selection during laboratory cultivation. The relative abundances obtained using these two approaches differ but, in one study, 16% of sequences were common to both enrichment cultures and clones analyzed. Molecular analysis of cultures obtained during MPN enumeration also indicates selection, with frequent domination by *Nitrosomonas* strains in samples from environments which appear, from molecular analysis, to be dominated by *Nitrosospira*. Molecular- and cultivation-based techniques therefore provide different views of diversity and species composition within soil ammonia-oxidizer communities. However, molecular techniques are also subject to bias and additional techniques are required. In this respect, direct detection of cells using fluorescent *in situ* hybridization (FISH) with specific ammonia-oxidizer probes would be useful. This approach, however, is limited by autofluorescence and the relatively low cell concentrations of ammonia oxidizers in soil.

Influence of Soil Characteristics on Ammonia-Oxidizer Communities

Soil pH

Ionization of ammonia at low pH values reduces substrate availability for ammonia oxidizers, and laboratory batch growth of ammonia oxidizers does not occur at pH values of less than 6.5. However, autotrophic ammonia oxidizers with neutral pH optima are frequently isolated from acid soils, and autotrophic nitrification has been demonstrated in soils with pH values as low as 3.5. In some situations, nitrification in acid soils is due to heterotrophic nitrification. Coupled denitrification–nitrification by heterotrophic nitrifiers is unlikely, due to the requirement for high substrate concentrations, and heterotrophic nitrification involving ammonia mono-oxygenase-based reactions suffers from reduced ammonia availability at low pH. However, acidophilic fungal heterotrophic nitrifiers have been characterized and fungal nitrification is significant in some acid soils. Nevertheless, use of specific inhibitors of autotrophic ammonia oxidation and ^{15}N -based studies have demonstrated active, autotrophic ammonia oxidation in acid soils.

One explanation for this phenomenon is the possession by some ammonia oxidizers of urease activity, enabling growth on urea as a sole nitrogen source. Urease activity and growth on urea are independent of pH in the range 4–8, possibly through uptake by passive diffusion. Laboratory studies show that, under these conditions, ammonia oxidation can proceed at pH values as low as 4, until urea is exhausted, after which it ceases, even if ammonium is present in solution. This has been demonstrated in a strain of *Nitrosospira* that cannot oxidize ammonium at pH values less than 7 (Figure 2). Urease activity therefore provides

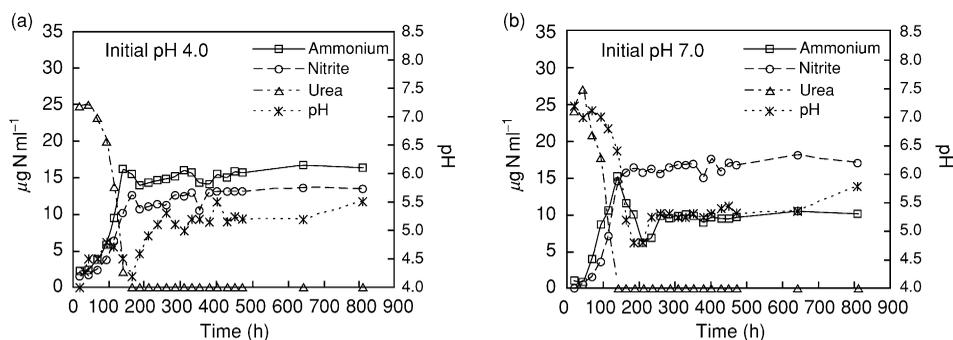


Figure 2 Changes in pH and in concentrations of urea, ammonium, and nitrite during growth of *Nitrosospira* strain NPAV in liquid batch culture on poorly buffered medium containing urea, at initial pH values of (a) 4 and (b) 7. (Reproduced with permission from Burton SAQ and Prosser JI (2001) Autotrophic ammonia oxidation at low pH through urea hydrolysis. *Applied and Environmental Microbiology* 67: 2952–2957.)

the potential for acidophilic ammonia oxidation, but is not a prerequisite for ammonia oxidizers isolated from acid soils.

Autotrophic ammonia oxidizers appear to be protected from low pH in soil when occurring at high cell concentrations and associated with particulate matter. High cell concentrations can be achieved in aggregates, which when inoculated into liquid medium can nitrify at low pH, and nitrification at pH 4 has been demonstrated for cells immobilized in alginate. Biofilm formation on surfaces such as glass slides, glass beads, ion-exchange resin beads, and clay minerals can reduce the pH minimum for growth and activity of *N. europaea* by up to 2 pH units. Surface growth protection increases with the cation exchange capacity of the particulate material and lag phases prior to growth of stationary-phase or starved cells are reduced for attached cells. Protection from low pH may result from preferential growth and nitrification at the clay surface, due to adsorption of ammonium. It is greatest when the only source of ammonium is that bound to clay minerals. This has been demonstrated by growth on ammonia-treated vermiculite (ATV), in which ammonia is fixed to the vermiculite by high-temperature exposure to ammonia. In the presence of ATV, *N. europaea* growth occurs without a lag phase and with greater nitrite yield than for suspended cells, but with no decrease in the pH of the medium. This, and the other clay effects, suggests localized buffering; ammonium released from the clay surface is utilized by adsorbed cells and H⁺ ions are effectively inactivated by exchange for ammonium ions. It is not clear why biofilm growth should reduce the lag phase, but this presents a significant ecologic advantage in competing for transient supplies of ammonium. A possible mechanism involves cell-cell signaling molecules, acyl homoserine lactones, which will accumulate at high cell concentrations. When *N. europaea* biofilms established on glass beads are starved and then resupplied with ammonia, oxidation occurs immediately. However, supply of ammonia to suspended, nonbiofilm cells in liquid medium results in a lag period, prior to ammonia oxidation. The lag period increases with the length of the preceding starvation period but is significantly reduced by addition of acyl homoserine lactones. Signaling compounds are produced by a wide range of soil microorganisms and affect a number of ecologically important processes. These compounds may therefore mediate interactions between nitrifiers and other soil microorganisms, particularly in biofilms and in the rhizosphere, where biomass concentrations are sufficient to lead to high concentrations of signaling molecules.

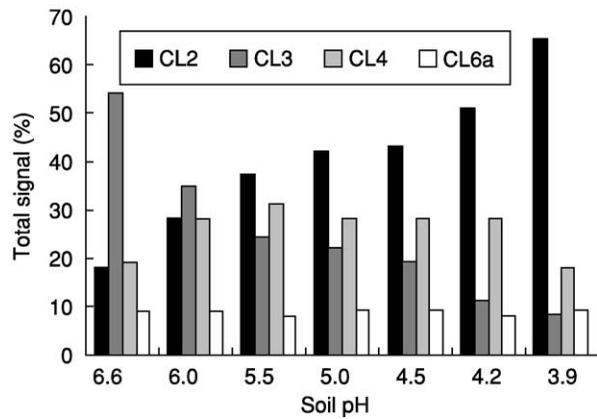


Figure 3 The influence of soil pH on the relative abundances of *Nitrosospira* clusters 2 (CL2), 3 (CL3), and 4 (CL4), and *Nitrosomonas* cluster 6a. Abundances are calculated as relative intensities of denaturing gradient gel electrophoresis (DGGE) analysis of ribosomal ribonucleic acid (rRNA) genes amplified from DNA extracted from soil maintained at a range of pH values, using ammonia oxidizer-specific primers. (Reproduced with permission from Stephen JR, Kowalchuk GA, Bruns MA *et al.* (1998) Analysis of β -subgroup ammonia oxidiser populations in soil by DGGE analysis and hierarchical phylogenetic probing. *Applied and Environmental Microbiology* 64: 2958–2965.)

The development of molecular techniques has enabled assessment of the influence of soil pH on ammonia-oxidizer communities. In one study, DGGE analysis was carried out on β -proteobacterial 16S rRNA gene fragments amplified from DNA extracted from soils maintained for 36 years at pH values in the range 3.9–6.6. Relative abundances of different clusters were assessed as relative intensities of DGGE bands and showed a greater proportion of *Nitrosospira* cluster-2 sequences in acid soils and a greater proportion of *Nitrosospira* cluster 3 at higher pH (Figure 3). Other clusters detected were *Nitrosospira* cluster 4, which may have increased in relative abundance at pH 5.5, and *Nitrosomonas* cluster 6a, which was independent of pH. Molecular studies of agricultural, grassland, and forest soils provide further evidence for the association of *Nitrosospira* cluster-2 sequences with acid soils. However, lack of information on distinctive physiological characteristics specific to representatives of this group, and of physiological diversity within ammonia oxidizers in general, prevents establishment of links between species diversity, functional diversity, and mechanisms for nitrification at low pH.

Nitrogen Fertilization

Sustained nitrogen fertilization and liming increase nitrifier population size, as indicated by cell counts (MPN and cPCR) and nitrification potential

measurements. In addition, evidence from molecular studies of nitrifiers in long-term ecological research (LTER) sites subjected to a number of different fertilization and cultivation conditions indicates an influence on the ammonia-oxidizer communities. Tilled soils were dominated by *Nitrosospira* cluster-3 sequences, while never-tilled soils contained *Nitrosospira* clusters 3 and 4, and *Nitrosomonas* cluster 6. Similar effects of fertilization on the relative proportions of *Nitrosospira* cluster-3 and -4 sequences have been found in chalk grassland soil. Little is known about the persistence of changes after perturbations.

Nitrogen supplied as inorganic fertilizer or, in grazing systems, as animal urine leads to localized, high ammonia concentrations. These concentrations are sufficient to inhibit ammonia-sensitive groups of ammonia oxidizers, while permitting growth of more tolerant strains. Soil microcosm experiments have demonstrated that the kinetics of nitrification of ammonia derived from synthetic sheep urine applied to grassland soil depends on the relative abundances of ammonia-sensitive and ammonia-tolerant strains. Low numbers of the latter result in significant lag periods before nitrification, impact significantly on maintenance of high levels of ammonia for plant growth, and reduce nitrogen losses associated with leaching and denitrification of nitrate.

Inhibition of Soil Nitrification

Specific inhibitors of autotrophic nitrification (e.g., nitrapyrin, dicyandiamide, etridiazole) have been used in the field to reduce losses of fertilizer nitrogen and to reduce nitrate pollution. Inhibitors, particularly acetylene, are also used as metabolic blocks when measuring nitrification rates. Higher concentrations of these compounds are required for inhibition in soil than in laboratory culture because of inactivation and degradation and because attachment of nitrifiers to soil surfaces protects cells from inhibition. Protection is greater with expanding clays with high cation exchange capacity. In many mature coniferous soils, nitrogen is present as ammonium rather than nitrate and net nitrification rates are low. This has led to suggestions of allelopathic inhibition of nitrification by polyphenolic compounds released by roots, which can inhibit laboratory growth of nitrifiers. However, use of ¹⁵N-based techniques has demonstrated high gross nitrification rates in these soils and low nitrate levels are more likely due to greater immobilization of nitrate. There is little direct evidence for specific effects of plant diversity and community structure on ammonia-oxidizer communities, although plants can exert indirect effects through their influence on soil ammonium concentrations.

Summary

Nitrifying bacteria convert the most reduced form of soil nitrogen, ammonia, into its most oxidized form, nitrate. In itself, this is important for soil ecosystem function, in controlling losses of soil nitrogen through leaching and denitrification of nitrate. Nitrifiers also contribute to other important processes, including nitrous oxide production, methane oxidation, degradation of organic compounds, and carbon monoxide oxidation. The development of ¹⁵N-based techniques has increased significantly our ability to dissect soil nitrogen transformations and their rates, while molecular techniques now enable characterization of soil nitrifier community structure and changes in species composition. These approaches are increasing our understanding of the ecology of soil-nitrifying bacteria and provide the potential for determining relationships between diversity, community structure, and ecosystem function in this important group of organisms.

See also: Nitrogen in Soils: Cycle; Nitrates; Plant Uptake

Further Reading

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Plant Uptake

A Hodge, University of York, York, UK

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Introduction

Nitrogen (N) is essential for life because it is a component of nucleic acids and amino acids and thus peptides and proteins. In addition, plants also require N for chlorophyll. In most terrestrial ecosystems, N is the primary limiting nutrient. In soils, N is present predominantly in organic form, and inorganic N is made available by the mineralization of organic N to ammonium (NH_4^+) and subsequent oxidation to nitrate (NO_3^-). Both these inorganic N sources can be taken up by plant roots, but roots have to compete with soil microorganisms as well as other plant roots for this N. Recent evidence suggests that plants may be able to access simple organic N sources directly without the need for the mineralization process to release inorganic N, but these N sources also have to be captured in a competitive environment. The distribution of N sources in soil, particularly organic N forms, is both spatially and temporally heterogeneous. Plant roots respond to this heterogeneity in a number of ways in order to capture N from the soil system.

Plant–Microbial Competition for N

Mineralization of organic material to NH_4^+ is carried out by a wide range of soil animals and microorganisms. However, mineralization and the subsequent conversion of NH_4^+ to NO_3^- are generally considered to be the rate-limiting steps in the terrestrial N-cycle. Due to their rapid turnover times and rapid adaptation rate, microorganisms are generally thought to utilize inorganic N sources first, with the plant only being able to access what is left over after microbial uptake. Exceptions are plants such as legumes and alder which form symbiotic associations

with rhizobium and *Frankia* bacteria, respectively. These symbionts form nodules on the roots which are capable of fixing dinitrogen and thus supply the plant with N directly. The majority of plants, however, do not form N_2 -fixing symbiotic associations and must rely on N uptake via their roots or mycorrhizas.

Most transformations of N also depend on the supply of carbon (C), and thus these two nutrient cycles are closely linked. The C:N ratio of the substrate determines the rates at which N (and C) are released and mineral N made available for plant uptake. Also influential are the composition of the decomposer microbial community and its requirements for N. Fungi tend to have a higher substrate assimilation efficiency and a lower N requirement per unit of C assimilated than bacteria. Thus, if the decomposer population is predominantly fungal, more N may be released than if it was predominantly bacterial. However, fungi also tend to turn over less rapidly than bacterial populations, so the N contained in their structural components will be locked up for a longer time than bacterial N. Generally net mineralization, resulting in the release of N, occurs if the C:N ratio of the substrate being decomposed is lower than approx. 25:1. At higher C:N ratios, net immobilization occurs and the microbial decomposer community requires additional N from the soil system to decompose the substrate.

Net immobilization of N reduces the N available for the plant and can reduce overall plant growth. Although other factors influence the release of N, for example lignin content of the substrate, there is a strong inverse relationship between the amount of plant N captured from organic substrates and the C:N ratio of the substrate, as shown in **Table 1**.

In addition to microorganisms soil animals also play an important role in the mineralization process through physical, chemical, and biological processes. Soil animals can physically break up and redistribute organic materials in the soil. They also ingest organic materials which are subsequently broken down by a range of enzymes and/or by microflora present in their guts. Much of the C they consume is used for metabolic processes and the excreted waste products, in the form of NH_4^+ , urea, or amino acids, are a much more readily available form of N for plant uptake than the organic material originally ingested. Soil animals feed on microorganisms as well as other soil animals. The plant benefits from the regulation of the size and activity of the microbial population, and from the release of more N into the soil–plant system. An increase in plant N capture of up to 75% has been reported as a result of protozoa grazing on soil microorganisms. Thus, although microorganisms may