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DEDICATION

We dedicate the second edition of Nitrogen in the Marine Environment to our spouses, Linda Duguay, Bill Norton, Skip Stiles, and Gerdi Weidner, for their constructive input to this volume and to our children (Jennifer and Rebecca Capone, Zach, Zane and Zoë Norton, Maeve and Patrick Mulholland Stiles, Daniel Carpenter and Rachel Poccia) for enduring our endless conversations revolving around nitrogen and the oceans.
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A Timely Book for Interesting Times

Since the first edition of this book in 1983, several revolutions have occurred vis à vis scientific studies of the marine nitrogen cycle. Just consider some of the words and phrases, that appear in this edition but were unknown or seldom-used by marine scientists in 1983. Here are just a few: anammox, archaea, polymerase chain reaction, isotopomer, trace metal limitation. In 1983, we were in the early stages of just being able to properly enumerate the bacteria occurring in sea water. Now we talk about their inherent genetic capabilities of bacteria and archaea, and we are beginning to understand that viruses also play an important role. With respect to the oceanic fixed-N budget several source and sink terms have been increased several-fold, suggesting much more rapid turnover than envisioned in 1983. Thus this book appears at an “interesting time” vis à vis changes in our understanding of nitrogen in the marine environment.

While these massive changes in understanding would be more than sufficient to justify an update of the first edition, the articles in this book have perhaps a more important raison d’etre, because of the crucial role that nitrogen cycling plays in atmospheric chemistry and in aquatic productivity. In 1983, we were well aware that anthropogenic greenhouse gas emissions were likely to cause global warming, but it is probably fair to say that the prevailing view was of a process that involved gradual change. Now, we have ice-core data suggesting the possibility of massive climate change on sub-decadal time scales, and we are witnessing great perturbations in the marine ecosystem. In my view, the time is not far off when every scrap of understanding about marine ecosystems will be needed to help adapt to and mitigate global change. Because nitrogen plays such a key role, the chapters in this book are timely as a vehicle for accelerating understanding.

The editors and authors are to be commended for taking on the difficult task of providing a comprehensive update of the deliciously complex subject of nitrogen in the marine environment, and producing a timely book for interesting times!

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The debut of the first edition of Nitrogen in the Marine Environment in 1983 chronicled the tremendous growth in knowledge concerning the cycling of nitrogen in the sea to that point and heralded the explosive expansion in work on oceanic nitrogen cycling that quickly followed (Fig. 1). Nitrogen in the Marine Environment, second Edition (long overdue!) is the natural witness to that growth. The page count alone has doubled relative to the original edition, and this edition is still insufficient to cover all the threads of ongoing research on nitrogen in the sea.

Unprecedented discoveries over the last decade have changed the way we view the marine nitrogen cycle. These include the recognition that phytoplankton in the sea are not uniformly limited by nitrogen, and that the critical limiting nutrient or nutrients (we now recognize co-limitation) vary within and among systems as a result of differences in external forcings and the biogeochemically important functional groups present and consequently selected. The critical role of picoplankton in marine ecosystems and biogeochemical cycles (including nitrogen dynamics) is now well established.

Figure 1. Number of papers by year with topics including “nitrogen and marine or nitrogen and ocean” from the Web of Science database. We assume the jump in citation rate from 1989 to 1991 represents changes in the data base itself (e.g., new journals included) rather than being a result of publication of the first edition of Nitrogen in the Marine Environment. The steady growth from 1992 is evident, and the number of papers doubled from 1993 to 2003.
We have gained a new appreciation of the diversity and the quantitative importance of marine nitrogen fixers. We now recognize the significance of Archaea and the processes they mediate (e.g., nitrification) and the importance of anammox bacteria in some marine ecosystems. In addition, our traditional view of just who does what in the marine nitrogen cycle is shifting as we discover that taxonomic groups often don’t fit squarely into the functional group they’ve been assigned and furthermore that the biogeochemical roles of bacteria and phytoplankton often overlap. Even our understanding of what constitutes the labile fraction of the dissolved nitrogen pool has changed. We have identified and begun to quantify how many previously unrecognized biotic and abiotic processes affect the marine nitrogen cycle (e.g., extracellular enzymes, use of novel dissolved organic nitrogen compounds, photochemical processes, etc.). This illustrates just how much we have learned and are still discovering about the marine N cycle. We could go on—that’s why the book is so long!

Numerous factors have fueled this revolution. Perhaps first and foremost is the appreciable growth of practitioners in this field bringing new tools and unique perspectives. This growth has occurred in tandem with the arrival of new technologies enabling us to address questions we couldn’t even imagine or articulate two decades ago. Advances in molecular biology, analytical techniques, remote sensing, sensor technology, and computational methods and power have all had an impact. Moreover, with the burgeoning availability of time-series data sets, these diverse factors have synergistically led to the steady expansion of data collection and synthesis over the last twenty plus years. We have developed the capacity and infrastructure to measure more things in greater numbers and at lower cost.

The pace of discovery continues to accelerate. So what does the future hold? We believe we can safely predict that our resolution of the temporal and spatial scales of variability of the populations and processes relevant to understanding the nitrogen cycle will steadily increase with the improvement and deployment of moored arrays and remote observation systems with novel nutrient and biological sensors. Numerous metagenomic surveys are underway and the prospect that novel organisms and pathways will be revealed is practically a given. Such novel observations will inform and stimulate a new generation of experimentalists as well.

Alarmingbly, the sea itself is changing at an unprecedented rate, in large part due to human influences. Increasing CO₂ concentrations, temperatures, coastal eutrophication, atmospheric nitrogen deposition, and decreasing pH will continue or even accelerate over the upcoming decades. What does this portend for the marine nitrogen cycle? What feedbacks within the system may minimize or aggravate the nature and rate of changes in marine ecosystems? Can, and should, the marine nitrogen cycle be manipulated to mitigate these changes? These are all questions on the table to be addressed by current and future cohorts of marine “azotonauts.”

We expect the third edition will necessarily follow soon!

Douglas G. Capone
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Margaret R. Mulholland
Edward J. Carpenter
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Nitrification is the process whereby ammonium (NH$_4^+$) is oxidized to nitrite (NO$_2^-$) and then to nitrate (NO$_3^-$). It thus links the most oxidized and most reduced components of the nitrogen (N) redox cycle and helps determine the overall distributions of these important nutrients. Ammonium rarely occurs at significant concentrations in oxygenated habitats. It is recycled rapidly between heterotrophic and N$_2$ fixing organisms (which excrete NH$_4^+$ directly or release organic N that is microbially degraded to NH$_4^+$) and many heterotrophic and photosynthetic plankton (which utilize NH$_4^+$ as a N source) in the surface ocean.
Similarly, NO$_2^-$ rarely accumulates in oxygenated habitats (see below for exceptions), although NO$_2^-$ is an essential intermediate in several oxidation and reduction processes in the N cycle. Nitrate, the end product of nitrification, however, accumulates in the deep ocean, and seasonally in the deep water of lakes, where there is no demand for inorganic N by phytoplankton.

The oxidation of NH$_3$ to NO$_2^-$ and of NO$_2^-$ to NO$_3^-$, although thermodynamically favorable when linked to reduction of oxygen, has long been considered a biological process, with only minor known abiotic contributions. For example, photochemical oxidation of NH$_4^+$ to NO$_2^-$ was debated in the 1930s (Rakestraw and Hollaender, 1936; ZoBell, 1933). It was eventually demonstrated that NO$_2^-$ that results from high doses of UV radiation actually comes from reduction of NO$_3^-$ (Armstrong and Tibbitts, 1968). Photochemical oxidation likely occurs in the surface ocean, but is a minor flux. Oxidation of NH$_3$ and amino-level N in organic compounds to N$_2$ gas linked to reduction of manganese oxide has been demonstrated in sediments (Hulth et al., 1999; Luther et al., 1997) and this pathway may have significant ramifications for the N cycle of sediments. Whether the Mn$^{+++}$ reduction pathway represents abiotic nitrification or anaerobic biological nitrification (see below) remains to be resolved.

Ammonium often accumulates to quite high concentrations in anoxic sediments and in stratified waters where oxygen concentrations are very low, which is consistent with the obligate aerobic physiology of conventional nitrifying bacteria. Recently, however, a novel process referred to as anammox (for anaerobic NH$_4^+$ oxidation) has been elucidated and shown to be capable of oxidizing NH$_4^+$ to N$_2$ under anoxic conditions (Mulder et al., 1995; van de Graaf et al., 1995). This process is in fact a form of denitrification, because its function is to remove fixed N from the system. Perhaps we should refer this process as oxidative denitrification or NH$_4^+$ denitrification, to distinguish it from conventional nitrification, which does not result directly in the loss of fixed N from the system. Anammox is elaborately discussed in the Chapter on denitrification by Devol, this volume.

The most important organisms in aerobic nitrification are the so-called nitrifying bacteria and the NH$_3$ oxidizing archaea (AOA). Until very recently, the known nitrifiers were restricted to the Proteobacterial phylum. The Proteobacterial genera are not all closely related to each other, but appear to have arisen from a photosynthetic ancestor, diverging before the ability to nitrify was developed in various groups (Teske et al., 1994). There are two functionally distinct groups of nitrifiers: those that oxidize NH$_4^+$ to NO$_2^-$ (NH$_3$-oxidizing bacteria and archaea, AOB and AOA) and those that oxidize nitrite to NO$_3^-$ (NO$_2^-$-oxidizing bacteria, NOB). No organism is known to carry out both reactions. These unique metabolic traits are not without costs; the chemolithoautotrophic lifestyle enables nitrifiers to exploit a unique niche in natural systems, but also confers constraints such as slow growth and inflexible nutritional requirements.

A new group of aerobic NH$_3$-oxidizing nitrifiers was recently discovered, first by detection of NH$_3$ oxidizing genes of apparent Archaeal origin in environmental metagenomic libraries (Schleper et al., 2005) and subsequently verified with the cultivation of a strain of Archaea (NH$_3$-oxidizing archaea, AOA) that oxidizes NH$_3$ to NO$_2^-$, apparently using a pathway very much like that known in AOB
(Konnecke et al., 2005). It now seems likely that AOA are more abundant than AOB in marine systems (Wuchter et al., 2006), and are also prevalent in soils (Leininger et al., 2006).

Several other useful reviews are available (Hagopian and Riley, 1998; Kaplan, 1983; Ward, 1986, 2000, 2002; Ward and O’Mullan, 2005). The 2002 review contains expanded information on the molecular ecology of nitrifiers, the 2005 chapter contains detailed information on methods for both rate measurement and molecular ecological investigations of AOB, and the present chapter contains new information on the NH₃-oxidizing Archaea, which were unknown at the times of the earlier reviews.

2. Nitrifying Microorganisms

2.1. Aerobic nitrification

2.1.1. Autotrophic nitrification

Most of the aerobic nitrification that occurs in natural habitats is thought to be performed by obligately autotrophic, or in a few cases, mixotrophic, bacteria and archaea. The classical autotrophs are best known and have been assumed to be responsible for the major fluxes in this pathway, so we shall consider them first.

Most of our knowledge of autotrophic nitrifiers derives from studies on cultivated strains, and the best known of those are the NH₃-oxidizing genus *Nitrosomonas* and the NO₂⁻-oxidizing genus, *Nitrobacter*. The cultured autotrophic nitrifiers, both NH₃ oxidizers and NO₃⁻ oxidizers, depend on CO₂ as their major carbon source and fixation is carried out via the Calvin cycle. It is estimated that CO₂ fixation accounts for about 80% of the energy budget of an autotroph (Forrest and Walker, 1971; Kelly, 1978). In addition, the use of NH₄⁺ or NO₃⁻, respectively, as the sole source of reducing power for this autotrophic growth is relatively inefficient: about 35 mol of NH₄⁺ or 100 mol of NO₃⁻ must be oxidized to support fixation of a single mole of CO₂ (Baas Becking and Parks, 1927). This “perverse insistence” on fixing their own CO₂, utilizing a unique but low yield energy source (Wood, 1986), accounts for their well deserved reputation for slow growth, even under optimal conditions in the laboratory. The commonly cultivated strains of *Nitrosomonas* and *Nitrobacter* have minimal generation times of 7–13 h (Bock et al., 1989).

The only cultivated AOA, *Nitrospumilus maritimus*, depends on CO₂ as its only carbon source and the presence of even low levels of organic carbon were inhibitory to growth. The pathway of CO₂ fixation is, however, unknown. Hyperthermophilic Crenarchaeota generally utilize a 3-hydroxypropionate pathway or a reductive TCA cycle for autotrophic carbon fixation. Another cultivated marine Crenarchaeota strain, *Cenarchaeum symbiosum*, a sponge symbiont, appears to use the 3-hydroxypropionate pathway. It cannot be concluded on this basis which pathway is used by the AOA, but it very likely that is not the Calvin cycle. *N. maritimus* had a minimal generation time of 21 h, longer but roughly on the same scale as AOB.
The overall reaction for NH₃ oxidation (Eq. (5.4)) shows that the process consumes molecular oxygen and produces hydrogen ions, in addition to NO₂⁻. A requirement for molecular oxygen occurs in the first step of the oxidation (Eq. (5.1)), which is catalyzed by a monooxygenase (NH₃ monooxygenase, AMO). The uncharged gaseous NH₃ is the actual substrate for AMO, as demonstrated by the pH dependence of the reaction rate (Suzuki et al., 1974; Ward, 1987a). Synthesis and activity of AMO in N. europaea, a terrestrial model organism, respond directly to NH₃ concentration (Stein et al., 1997), albeit at levels much greater than observed in the marine water column. Some AOB accumulate very high levels of NH₄⁺ internally, however (Schmidt et al., 2004a), so the effective concentrations might not be those detected in the environment. Ammonia monooxygenase has never been completely purified and assayed in cell free conditions, although its gene sequence has been derived for both Nitrosomonas and Nitrosococcus type AOBs (Alzerreca et al., 1999; Klotz and Norton, 1995; Sayavedra-Soto et al., 1998). AMO contains copper and probably also iron in its active form (Zahn et al., 1996).

The immediate product of AMO is hydroxylamine, which is further oxidized by hydroxylamine oxidoreductase (HAO) to NO₂⁻ (Eq. (5.2)). AOA apparently do not possess the hydroxylamine reductase gene, so the pathway of ammonia oxidation in these organisms must be quite different from that outlined here for AOB. Oxygen is also consumed by the terminal oxidase (Eq. (5.3)), as a result of electron transport generating ATP for cellular metabolism.

\[
\text{NH}_3 + \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O} \quad \text{(ammonia monooxygenase)}
\]  
\[\text{(5.1)}\]

\[
\text{NH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + 5\text{H}^+ + 4e^- \quad \text{(hydroxylamine oxidoreductase)}
\]  
\[\text{(5.2)}\]

\[
2\text{H}^+ + 0.5\text{O}_2 + 2e^- \rightarrow \text{H}_2\text{O} \quad \text{(cytochrome oxidase)}
\]  
\[\text{(5.3)}\]

\[
\text{NH}_3 + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + \text{H}^+
\]  
\[\text{(5.4)}\]

The overall reaction is energy yielding, and allows sufficient ATP production to support reverse electron transport for CO₂ fixation. However, the first step, oxidation of NH₃ to hydroxylamine, requires the input of reducing power. The second step, hydroxylamine oxidation, yields four electrons. These join the electron transport chain at the level of ubiquinone, from which two are shunted back to AMO for activation of NH₃. The N oxidation and electron transport pathways in Nitrosomonas are linked in the cytoplasmic membrane and periplasmic space; detailed information from the N. europaea genome (Chain et al., 2003) is consistent with the previous biochemical characterizations of the system (Whittaker et al., 2000). Depending on conditions (and enhanced at low oxygen concentrations), nitric oxide (NO), nitrous oxide (N₂O) and even dinitrogen gas (N₂) have been reported as secondary products.
in autotrophic NH$_3$ oxidation by both marine and terrestrial strains (Schmidt et al., 2004b; Zart and Bock, 1998). Although N$_2$O and NO can be produced in vitro by HAO from hydroxylamine (Hooper and Terry, 1979), reduction of NO$_2^-$ appears to be the dominant pathway in whole cells (Hooper et al., 1997; Poth and Focht, 1985; Remde and Conrad, 1990). Nitrite reductase activity has been demonstrated in *Nitrosomonas europaea* (DiSpirito et al., 1985; Miller and Wood, 1983) and in several marine AOB (Casciotti and Ward, 2001). Genes with homology for the copper type nitrite reductase and the nitric oxide reductase of heterotrophic denitrifiers have been detected in a group of marine *Nitrosomonas* isolates (Casciotti and Ward, 2001, 2005). Thus it appears that the marine AOB produce N$_2$O by a pathway that is homologous with that in denitrifiers (“nitrifier denitrification”). The pathway for nitrous oxide production in AOB remains controversial, however, because *N. europaea* mutants with nonfunctional NO$_2^-$ reductase genes can also produce N$_2$O (Beaumont et al., 2002), implying that an alternative pathway is present in some strains. It remains to be verified whether marine nitrifiers exhibit the same phenomenon. There is evidence from the genomes of AOB that *N. europaea, N. marina* and *N. oceani* contain different suites of electron transport molecules and quite different NO$_2^-$ reductase genes (Chain et al., 2003; Klotz et al., 2006), which may imply they also have different mechanisms for N$_2$O production. It remains to be determined whether AOA have similar pathways or capabilities.

The biochemistry of NO$_2^-$ oxidation is simpler than NH$_3$ oxidation because it is only a two electron transfer and involves no intermediates. The additional oxygen atom in NO$_3^-$ is derived from water (Eq. 5.5), and the molecular oxygen that is involved in the net reaction (Eq. 5.7) results from electron transport involving cytochrome oxidase (Eq. 5.6).

\[
\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2\text{H}^+ \text{ (nitrite oxidoreductase) } \quad (5.5)
\]

\[
2\text{H}^+ + 0.5\text{O}_2 \rightarrow \text{H}_2\text{O} \text{ (cytochrome oxidase) } \quad (5.6)
\]

\[
\text{NO}_2 + 0.5\text{O}_2 \rightarrow \text{NO}_3^- \quad (5.7)
\]

The energy yield of NO$_2^-$ oxidation is even less that that of NH$_3$ oxidation, necessitating the oxidation of vast amounts of NO$_2^-$ in order to fix small amounts of CO$_2$. For both processes, therefore, the biogeochemical impact is greater on the N cycle than on the C cycle.

The biomass produced by primary fixation of CO$_2$ by nitrifiers can be significant in some habitats. Under sea ice in Antarctica (Horrigan, 1981), an anchialine cave in the Yucatan Peninsula, Mexico (Pohlman et al., 1997) and the Mississippi River Plume in Gulf of Mexico (Pakulski et al., 2000b) are examples. In these cases, the significance of autotrophic nitrification as an in situ source of organic carbon is enhanced relative to the production of organic matter by photosynthesis. Photoautotrophic carbon production is minimal due to the absence of light caused by ice cover, the geometry of the cave itself, and in the Mississippi River system, high levels of suspended matter, respectively. Chemoautotrophy might also be a significant source of in situ production on sinking
particles (Karl et al., 1984) and in hydrothermal plumes where elevated NH$_4^+$ (and perhaps methane) concentrations could support NH$_3$-oxidizing bacteria (Cowen et al., 1998; Lam et al., 2004). It now appears that AOA comprise a significant fraction of the prokaryotic cells in seawater (Wuchter et al., 2006), but it is not known to what extent these cells are obligate autotrophs. If they possess a wider metabolic repertoire than the typical bacterial nitrifiers, i.e., if they predominantly access carbon and energy sources other than CO$_2$ and NH$_4^+$, they may make a very important contribution to microbial biomass that is not linked to nitrification.

a. Phylogeny of bacteria and archaea involved in nitrification: The history of the study of nitrifying bacteria has been described elsewhere (Ward, 2002; Watson et al., 1989) and only the current status of their diversity and evolutionary relationships will be summarized here. The classic species distinctions were based on cell shape and the arrangements of internal membranes (Koops and Moller, 1992; Watson et al., 1989). These have been largely superceded by the evolutionary relationships deduced from 16S rRNA sequencing.

The phylogeny of bacterial nitrifiers (Teske et al., 1994) shows that most of them are descendants of a common ancestor that was photosynthetic, rather than descending from a common ancestral nitrifier. Extensive phylogenetic trees depicting the relationships among cultivated nitrifiers, and the relationships of sequences obtained without cultivation from environmental samples, are available in the publications cited in this section and are not reproduced here.

The NH$_3$-oxidizing bacteria (AOB), including the marine AOB, fall into two major phyla in the Proteobacteria (Koops et al., 2003; Purkhold et al., 2003). The β subdivision species, containing genera known as Nitrosospira and Nitrosomonas, have been the subject of many recent studies. The main tool for investigating them in both culture and environmental samples is polymerase chain reaction (PCR) followed by cloning and DNA sequencing. On the basis of 16S rRNA sequence analysis, several clusters and a large amount of microdiversity within clusters has been detected in a wide variety of environments, both terrestrial and aquatic. Stephen et al. (1996) surveyed enrichment cultures and gene libraries of soils and marine sediments and found that Nitrosomonas sequences were more often associated with enrichment cultures and Nitrosospira with libraries. Smith et al. (2001) compared the phylogenetic affiliations and diversity of AOB 16S rRNA clone libraries and enrichment cultures obtained from the same marine sediment samples from a seawater loch being used for salmon farming. Only one (a Nitrosomonas type) of 18 enrichment cultures was represented in the 40 AOB clones retrieved without culturing. Nitrosospira-like sequences are often dominant in NH$_3$-oxidizer sequence clone libraries retrieved from the oceanic environments (Bano and Hollibaugh, 2000; O’Mullan and Ward, 2005).

A few generalities linking species or strains to environmental conditions have emerged. For example, McCaig et al. (1999) investigated the community structure of β-subdivision AOB in marine sediments underlying fish farms, and found a novel Nitrosomonas group whose distribution correlated with elevated NH$_4^+$ concentrations. Purkhold et al. (2000) summarized the ecological characteristics of the known cultivated strains and the major clusters defined by environmental
sequences for the betaproteobacterial AOB. None of the major clades are restricted to seawater. There are two major clades, one in the *Nitrosospira* cluster and one in the *Nitrosomonas* cluster, which have been reported primarily from marine environments for which no cultivated representatives are known. These findings suggest that the *Nitrosomonas* lineages are less common in natural samples than *Nitrosospira*, and raise concerns about biases introduced by relying on pure culture techniques.

The γ subdivision NH₃ oxidizers are represented by a single genus containing two species, *Nitrosococcus oceani* and *N. halophilus*, which have only been reported from marine or saline environments. Several different strains of *N. oceani* exist in culture, derived from various locations in the Atlantic and Pacific Oceans, but they all appear to be closely related (Ward and O'Mullan, 2002). *N. oceani* has been detected by immunofluorescence and by PCR in saline lakes in Antarctica (Voytek et al., 1999) and in various marine systems (O’Mullan and Ward, 2005; Ward and Carlucci, 1985; Ward and O’Mullan, 2002; Ward et al., 1989a) and by immunofluorescence in the Mediterranean Sea (Zaccone et al., 1996). Two strains of *N. halophilus* were isolated from a salt lake and a salt lagoon, and both have NaCl optima of about 700 mM, compared to 500 mM for *N. oceani* (Koops et al., 1990). Compared with the large amount of data available for the betaproteobacterial AOB, there are few reports of *Nitrosococcus*-like sequences obtained from the marine environment and none from freshwater environments, and no reports of finding *N. halophilus*-like sequences. This lack of information on *Nitrosococcus* may be a matter of PCR primer specificity and limited research effort at present, but it just as likely implies that *Nitrosococcus* is a minor component of the AOB assemblage.

The 16S rRNA sequence on only one AOA is currently available, and that sequence places *Nitrosopulilus maritmus* within the low-temperature marine Crenarchaeota, a group abundant in seawater and distinct from low temperature Crenarchaeota in soils (Konnecke et al., 2005). Phylogenetic analysis of several hundred AOA partial amoA sequences identified major groups that clustered by environment; water column sequences clustered together whether from the Black Sea, Monterey Bay or the eastern tropical North Pacific, while sediment sequences clustered together with other sediment and soil sequences (Francis et al., 2005). It is assumed that these are all derived from Crenarchaeota, but this is a large and more diverse group that previously appreciated, so the phylogeny of AOA remains largely unexplored.

The new phylogeny of NO₂⁻ oxidizing bacteria (Bock and Wagner, 2003), based on 16S rRNA sequences, shows that the best known autotrophic NO₂⁻ oxidizer, *Nitrobacter*, comprises a coherent genus in the α subdivision of the Proteobacteria. This genus is most closely related to non nitrifying genera that contain autotrophic members and strains capable of denitrification (Teske et al., 1994). Like *Nitrosococcus oceani*, *Nitrococcus mobilis* (Watson and Waterbury, 1971) belongs to the γ subdivision of the Proteobacteria, the only example of both NH₃- and NO₂⁻-oxidizing phenotypes occurring in the same subdivision. *Nitrospina gracilis*, the only species in this genus, represented by two isolates (Watson and Waterbury, 1971), is assigned to the δ subdivision of the Proteobacteria. Possibly the most unusual nitrifier is the genus *Nitrospira*, which is represented by only two isolates, and does not share a common lineage with the other nitrifiers. A novel *Nitrospira* strain,
N. moscoviensis, isolated from a heating system in Moscow, Russia, was assigned to a new genus outside of the Proteobacteria (Ehrich et al., 1995). These authors reanalyzed the *Nitrospira marina* sequence, which Teske et al. (1994) had placed in the δ Proteobacteria, and concluded that *Nitrospira* belonged outside the Proteobacteria, in a deeply branching cluster related to *Leptospirillum*. Sequences closely related to this group have now been reported from biofilms and freshwater sediments (Daims et al., 2001), but the only report so far of *Nitrospira*-like sequences from marine clone libraries is from a sediment sample at 2339 m (Li et al., 1999). In stream sediments (Altmann et al., 2004), *Nitrospira* cells (enumerated by fluorescence in situ hybridization) were 5–9 times more abundant than *Nitrobacter* cells. The marine isolate (Watson et al., 1986) was obtained from the Gulf of Maine and the authors reported that similar cells were present in many enrichments, suggesting it is a common member of the marine nitrifier assemblage.

Except for *Nitrospira* and *Nitrospina*, all of the bacterial nitrifiers cluster in phyla that are characterized by photoautotrophic ancestry and are themselves characterized by the possession complex intracytoplasmic membranes. These membranes are thought to be the site of the redox proteins involved in N oxidation, and therefore homologous with the photosynthetic membranes of photosynthetic bacteria and cyanobacteria. Nevertheless, the bacterial nitrifiers are polyphyletic, and the phenotype has apparently arisen independently numerous times. The homology of the functional genes (*amo, hao*) involved in their physiology implies gene transfer events, however, rather than independent evolution of these enzymes. The fact that the *amoA* genes from AOB and AOA are homologous raises the question of the ultimate origin of the NH$_3$ oxidizing phenotype. If the ancestral Crenarchaeota were thermophiles, it is possible that NH$_3$ oxidation originally arose in thermophiles and spread from the archaea to the bacteria. Internal compartmentalization was not observed in *N. maritimus*, but whether some of the AOA possess internal cytoplasmic membranes has not been established.

An understanding of the phylogeny of nitrifying bacteria is relevant to the study of nitrification in aquatic habitats because it has implications for detection and quantification methods. Although the bacterial nitrifiers are polyphyletic, they are not so diverse as to be unmanageable; their affiliation within a small group of lineages makes them amenable to identification and detection using a relatively small suite of molecular probes. This approach forms the basis of much current knowledge on the diversity and distribution of autotrophic nitrifying bacteria, and has already made important contributions to the study of nitrifying archaea as well.

The autotrophic bacterial NH$_3$ oxidizers show significant metabolic and morphological similarities with another group of autotrophic bacteria, the methane oxidizers. They are also closely related phylogenetically to the methane oxidizers, in both the γ and β subdivisions (Teske et al., 1994). Prior to the availability of ribosomal RNA sequence data for determination of phylogenetic relationships, it had been reported that NH$_3$ -oxidizing nitrifiers were capable of methane oxidation and vice versa (Dalton, 1977; Jones and Morita, 1983; Ward, 1987a). Thus it was an interesting verification of the metabolic studies when it was reported that the central genes in the two pathways, genes encoding NH$_3$ monooxygenase in the nitrifiers and methane monooxygenase in the methanotrophs, were evolutionarily related
(Holmes et al., 1995). It has not been possible to determine unequivocally which process is the natural or predominant one for some nitrifiers and methanotrophs in nature (see later text).

2.1.2. Heterotrophic nitrification

The autotrophic NH$_3$ oxidizers are considered to be obligate chemolithoautotrophs with no source of energy other than NH$_3$ and no net source of cellular carbon other than CO$_2$. Obligate autotrophy had been attributed to the absence of one or more enzymes in the tricarboxylic acid cycle, but inspection of the complete genome for *Nitrosomonas europaea* shows that a complete TCA cycle is present (Chain et al., 2003). *N. europaea* has limited genetic capability for transport and metabolism of organic molecules, but the basis for its apparent obligate dependence upon NH$_4$ and CO$_2$ is still not entirely clear.

The autotrophic NO$_2^-$ oxidizers have been reported to exhibit various degrees of heterotrophy, although generation times and time required for adaptation to new substrates is on the order of weeks (Bock, 1976). Recently completed genomes of terrestrial nitrite oxidizers indicate that they too possess a complete TCA cycle, but do not have the complete pathways for utilization of carbon substrates larger than 3 carbons (Starkenburg et al., 2006). The NOB also have very limited organic transport capabilities (Starkenburg et al., 2008). Marine strains for AOB and NOB have not been thoroughly investigated for their heterotrophic capabilities, and the potential for them to exhibit this metabolism in the ocean is unknown. The only cultivated AOA is apparently an obligate chemoautotroph, but uncultivated marine Crenarchaeota are apparently capable of amino acid assimilation (Ouverney and Fuhrman, 2000), so the extent of the AOA metabolic repertoire remains an open question.

Heterotrophic nitrification has been reported for bacterial genera including *Bacillus*, *Paracoccus*, *Pseudomonas*, *Thermus*, *Azoarcus*, and the fungus, *Aspergillus*. Heterotrophic nitrification usually refers to the production of NO$_2^-$ or NO$_3^-$ either from NH$_4^+$ or from organic substrates by heterotrophic bacteria, and is best studied in a few denitrifiers where it is linked to aerobic denitrification (Stouthamer et al., 1997). The enzymology of the process is unknown (Nemergut and Schmidt, 2002) and its physiological role is not understood, as it usually cannot support growth and in fact, reduces growth yield compared to denitrification alone.

Liberation of NO$_3^-$ from organic intermediates has also been reported for green algae (Spiller et al., 1976) and N-fixing legumes (Hipkin et al., 2004) but the relevance of these processes in the marine environment is unknown. Although the organisms performing them are autotrophic, the processes might be considered heterotrophic because the released N is derived from an organic intermediate.

It has been argued that heterotrophic nitrification involves enzyme systems that are quite different from those of the autotrophs (Wehrfritz et al., 1993) and that heterotrophic nitrification cannot serve as an energy generating mechanism (Castignetti, 1990), as the autotrophic process does. In the aerobic denitrifiers, which are also capable of nitrification, the initial enzyme, AMO, appears to be quite similar to the enzyme in autotrophic nitrifiers. However, HAO differs significantly and in the heterotrophs, is a smaller, simpler enzyme that performs a two electron transfer (instead of the four electron transfer of the autotrophic HAO) and
releases nitroxy1 as the product, rather than NO\textsubscript{2}\textsuperscript{-} (Richardson et al., 1998). Thus, no electrons are available for reverse electron transport in the heterotrophic system and the purpose of the process appears to be a means of disposing of excess reductant in times of redox stress, rather than harvesting that reductant for cellular energy.

In aquatic systems, the most important heterotrophic nitrifiers are thought to be a class of denitrifiers which are capable of aerobic denitrification, and which can also oxidize NH\textsubscript{3}. The organism in which these physiologies were first described is now known as Paracoccus pantotrophus (formerly known as Thiosphaera pantotropha). P. pantotrophus oxidizes NH\textsubscript{3} to NO\textsubscript{2}\textsuperscript{-} using an enzyme that exhibits important similarities and differences compared to AMO from autotrophic nitrifiers (Moir et al., 1996). The NO\textsubscript{2}\textsuperscript{-} so generated can be released into the medium or denitrified to N\textsubscript{2}. Denitrification of NO\textsubscript{2}\textsuperscript{-} or NO\textsubscript{3}\textsuperscript{-} can occur under atmospheric levels of oxygen (Robertson et al., 1995). Aerobic denitrifiers have mostly been isolated from wastewater treatment systems, and their prevalence and ecological significance is unknown in natural aquatic systems.

Because autotrophic nitrification is such a hard way to make a living, it might seem curious that heterotrophic nitrification is not more common. The amounts of NO\textsubscript{2}\textsuperscript{-} or NO\textsubscript{3}\textsuperscript{-} formed are usually quite small compared to autotrophic nitrification, however, and the energetics are apparently even less favorable than for the autotrophic nitrifiers (Stouthamer et al., 1997). There are no substantive reports of heterotrophic nitrification in the marine environment, despite its mention in anecdotal reports and suggestion of it as a possible explanation for curious observations (Hovanec and DeLong, 1996).

Thermophilic heterotrophic nitrifiers, capable of oxidizing NH\textsubscript{3} to NO\textsubscript{2}\textsuperscript{-}, were isolated from several hydrothermal vent habitats (Mevel and Prieur, 1998). Most of the isolates were NO\textsubscript{3}\textsuperscript{-} reducers or denitrifiers and exhibited a wide diversity related to the genera Thermus and Bacillus. Because they are able to ammonify, nitrify and reduce NO\textsubscript{3}\textsuperscript{-}, they would seem to possess most of the physiologies of importance in the N cycle. Such organisms may be common in mesophilic aquatic habitats, but have not been quantitatively investigated.

Heterotrophic strains capable of oxidizing NO\textsubscript{2}\textsuperscript{-} have also been reported. Sakai et al. (1996) described several classes of heterotrophic strains that oxidized NO\textsubscript{2}\textsuperscript{-} to NO\textsubscript{3}\textsuperscript{-} with variable amounts of NO\textsubscript{3}\textsuperscript{-} accumulation, depending partly on the denitrification capabilities of the strains. Strains which were capable of both oxidation and reduction of N oxides were shown to switch between the two directions of conversion depending on the oxygen tension of the culture (Sakai et al., 1997).

Heterotrophic nitrification has been studied in terrestrial systems, especially acid forest soils, where it has been difficult to document autotrophic nitrification. Experiments using isotopes to differentiate production of NO\textsubscript{3}\textsuperscript{-} from inorganic and organic substrates in a forest system found that heterotrophic nitrification accounted for less than 10% of the total nitrification rate (Barraclough and Puri, 1995). No information of this sort is available on the occurrence or significance of heterotrophic nitrification in aquatic systems. The potential for NH\textsubscript{3} and NO\textsubscript{2}\textsuperscript{-} oxidation by heterotrophic bacteria in aquatic systems warrants further exploration, and the capability may be present in many strains already in culture. If heterotrophic nitrification is common in nature, then a focus on autotrophic nitrification as the
model system and the basis for estimation of rates is too narrow and unrealistic. On a per cell basis, heterotrophic nitrification is much slower than is autotrophic nitrification, and the relative impact on N turnover must be greater by autotrophs due to their total dependence on N oxidation for energy. Nevertheless, widespread abundance of more numerous heterotrophic nitrifiers could compete in importance with the smaller number of slow growing autotrophs. Facultative nitrifiers that grow heterotrophically most of the time would seem to be an advantageous compromise, but mixotrophy is surprisingly uncommon in the microbial world (Whittenbury and Kelly, 1977). It is intriguing to wonder whether AOA will be an exception to this generalization. If heterotrophic nitrification were significant, it might not be detectable in the net stoichiometry of organic matter remineralization.

2.2. Anaerobic nitrification

The interest in anaerobic nitrification and aerobic denitrification arises mainly from the necessity to treat large volumes of wastewater to reduce N loading before release into natural waters. Classical nitrification and denitrification are environmentally incompatible processes, the first being obligately aerobic and the second induced only under conditions of anoxia. It is therefore usual to involve two steps in wastewater treatment, an aerobic step to convert NH$_3$ to NO$_3^-$ and a subsequent anaerobic step, to convert the NO$_3^-$ to N$_2$. If the two steps could be combined in one organism under either aerobic or anaerobic conditions, much time and money would be saved in wastewater treatment. And clearly, if a single organism is capable of combined nitrification and denitrification in a bioreactor or water treatment plant, such an organism could be of considerable importance in the natural environment as well.

The heterotrophic nitrifier mentioned earlier, *P. pantatrophus*, carries out at least part of both processes under aerobic conditions. *P. pantatrophus* was originally isolated from wastewater and its ability to denitrify aerobically, as well as this ability in several other conventional heterotrophic denitrifiers, has been confirmed (Robertson et al., 1995).

Autotrophic nitrifying bacteria exhibit some abilities for anaerobic metabolism as well. Enrichment cultures under chemolithotrophic conditions and very low oxygen concentrations catalyzed the net removal of NH$_4^+$ as N$_2$ (Muller et al., 1995). Bock and coworkers have shown that *Nitrosomonas eutropha* produces gaseous products, mainly NO and N$_2$, during growth on nitrogen dioxide gas (NO$_2$) and NH$_3$ (Schmidt and Bock, 1997). The process proceeds at a slower rate than NH$_3$ oxidation in the presence of a normal air atmosphere and supports cell growth. Additions of NO$_2$ and NO enhanced the complete removal of N in the form of NH$_3$ and organic N without the addition of organic carbon substrates (Zart and Bock, 1998).

A completely novel process in which NH$_3$ and NO$_2^-$ are converted anaerobically to N$_2$ was reported several years ago (Mulder et al., 1995; van de Graaf et al., 1995) and the process has been quantitatively described (Jetten et al., 1998; Strous et al., 1999). The organisms responsible for this novel metabolism have been identified as *Planctomyces* (Strous et al., 1999), and the genome of one strain has
been almost completely sequenced (Strous et al., 2006). Referred to as “anammox” (anaerobic NH\(_4^+\) oxidation), the process in wastewater involves a consortium of the planctomycete organism and an autotrophic NH\(_3\) oxidizer such as Nitrosomonas europaea or N. eutropha. Ammonia is oxidized to NO\(_2^-\) by the autotroph under microaerophilic conditions. The NO\(_2^-\) so produced is reduced to N\(_2\) by the planctomycete. Both oxygen and NO\(_2^-\) concentrations are maintained at nearly undetectable levels by the metabolism of the members of the consortium, and while both organisms grow quite slowly (generation times for the planctomycete of ≥11 days are reported), the net removal of NH\(_4^+\) occurs at a rate 25 times faster than that reported for N removal by anaerobic autotrophic nitrification alone (Jøtten et al., 1998).

The anammox process was discovered and characterized in anaerobic wastewater treatment systems, and 16S rRNA sequences identified as belonging to the planctomycete member have been detected in several such systems. Research into the occurrence of the process and presence of the organisms in natural aquatic systems has proceeded rapidly in the last few years. Anammox has now been documented in estuarine (Risgaard-Petersen et al., 2004a; Tal et al., 2005; Trimmer et al., 2003; Trimmer et al., 2005) and marine (Dalsgaard and Thamdrup, 2002; Thamdrup and Dalsgaard, 2002) sediments and in the water column of the Black Sea (Kuypers et al., 2003) and Golfo Duce, an enclosed Bay on the west coast of South America (Dalsgaard et al., 2003) and most recently in the shelf waters of the Benguela upwelling zone (Kuypers et al., 2005), and the OMZs of the eastern tropical South Pacific (Thamdrup et al., 2006; Hamersly et al., submitted for publication). Anammox is an oxidative process and there appear to be some enzymatic similarities between anammox and aerobic NH\(_3\) oxidation (Strous et al., 2006), the ecological significance of anammox is the same as denitrification, i.e., the loss of fixed N in anoxic environments. Therefore, for a full treatment of anammox, please see Chapter 6, Denitrification by Devol, this volume.

Nitrite oxidizers are also reported to possess an anaerobic metabolism and even to grow under denitrifying conditions (Freitag et al., 1987). Subsequent work showed that ATP was not produced during NO\(_2^-\) reduction (Freitag and Bock, 1990). Very little work has been done in this area with marine NO\(_2^-\) oxidizers, but NO\(_2^-\) oxidation has been reported in environments where very little oxygen is present (see later text).

In addition to the unconventional activities of conventional nitrifiers and denitrifiers and the discovery of novel N metabolisms in new organisms, it has also been recently proposed that a short circuit of the nitrification/denitrification couple can also be accomplished abiotically. In marine sediments, which typically contain relatively high manganese levels, N\(_2\) can be produced by the oxidation of NH\(_4^+\) and organic N by manganese dioxide in air. The reduced Mn\(^{++}\) thus formed can be reoxidized by oxygen to continue the oxidation of NH\(_4^+\), or can reduce NO\(_3^-\) to N\(_2\) (Luther et al., 1997). While the free energy of these coupled reactions is shown to be favorable, it remains to be seen whether the abiotic process can be unequivocally identified in natural systems and the degree to which it may compete with the biologically catalyzed processes. Hulth et al. (1999) detected anoxic production of both NO\(_2^-\) and NO\(_3^-\) in marine sediments, concurrent with the production of
reduced manganese. The NO$_3^-$ production was directly proportional to the initial manganese oxide content, and the NO$_3^-$ was subsequently depleted, apparently by denitrification. A series of linked redox cycles in which anoxic nitrification, coupled to manganese reduction, was linked in series to anoxic organic matter oxidation through several biogeochemical reductants, including iron and hydrogen sulfide, was proposed as the mechanism (Hulth et al., 1999). These authors suggested that the lithotrophic nitrification they observed is biologically mediated and potentially of significance in the N cycle at sites where oxidized metals are reworked into anoxic sediments. Other authors have failed to detect significant coupling between Mn$^{2+}$ and NH$_4^+$ in anoxic sediments (Thamdrup and Dalsgaard, 2000). $^{15}$N tracer experiments, including the kind used to demonstrate anammox (e.g., Dalsgaard and Thamdrup, 2002) have also shown that the anaerobic oxidation of NH$_4^+$ via manganese is not a significant flux, at least in some sediments (Risgaard-Petersen et al., 2004a; Thamdrup and Dalsgaard, 2000).

Anoxic NH$_3$ oxidation, whether it results directly in N$_2$ formation (as in anammox) or in NO$_3^-$ production (when linked to manganese reduction), would introduce new links into the aquatic and sediment N cycle. Failure to account for anoxic NH$_3$ oxidation might lead to an underestimate of NH$_4^+$ removal, because the products do not accumulate; they are either lost to the atmosphere immediately, or rapidly reduced by the next step in the anaerobic cycling of organic matter.

3. ROLE OF NITRIFICATION IN THE MARINE NITROGEN CYCLE

Many forms of organic and inorganic N can be utilized by phytoplankton, and the transformation of NH$_4^+$ into NO$_3^-$ by nitrification does not change the absolute inventory of N available for algal nutrition. In soils, the different ionic properties of NH$_4^+$ and NO$_3^-$ are important in determining inorganic N availability in the soil solution, but in aquatic systems, the properties of these ions are less important to their distributions. Because of the different chemical properties and varying preferences, abilities, and metabolic costs of utilizing NH$_4^+$ versus NO$_3^-$, and the role of NO$_3^-$ as a substrate for denitrification, however, this transformation is very important in marine systems.

Because NH$_4^+$ contains N at the oxidation level of proteins, it is readily assimilated by both phytoplankton and bacteria, and is a preferred N source. Ammonia oxidizers may be in competition for NH$_4^+$ with other planktonic organisms. The different physiological requirements of phytoplankton and nitrifiers probably play a role in determining exactly where in the water column NH$_4^+$ assimilation and NH$_4^+$ oxidation occur. As explained below, most nitrification occurs within or near the base of the euphotic zone in the upper 100 or so meters of the ocean. However, there is usually very little NO$_3^-$ in the surface ocean, due to utilization by phytoplankton, except in “high nutrient low chlorophyll” regions and when supplied by episodic events such as regional upwelling. The NO$_3^-$ in the deep water of the oceans has accumulated from nitrification because phytoplankton assimilation is essentially zero below the euphotic zone. It is because of nitrifiers
that N accumulation in the deep waters is in the form of NO$_3^-$, rather than NH$_4^+$. The deep NO$_3^-$ reservoir can be made available to phytoplankton by mixing, upwelling, and seasonal overturn. These physical processes bring cold deep nitrate-rich water up to the surface where, in the presence of light, phytoplankton can assimilate the NO$_3^-$. Thus, although NO$_3^-$ is not usually abundant in surface waters, it is a very important N source for phytoplankton.

The NO$_3^-$ that is produced by nitrification serves as a substrate for denitrification in low oxygen environments in both the water column and sediments (Devol, this volume). Although denitrification involves several semi-independent steps that need not function together, it is common for denitrifiers to begin the sequence with NO$_3^-$ and to produce varying amounts of the other products depending upon the environmental conditions. Thus, although denitrifiers appear to have little in common with nitrifiers, the former are in fact dependent upon the latter – other than lightning and fertilizers, nitrifiers are the only significant source of NO$_3^-$. Similarly, NO$_2^-$ and NO$_3^-$ are the oxidants in the anaerobic oxidation of NH$_4^+$. While NO$_2^-$ can be produced by either nitrification or denitrification, the production of the oxidized forms ultimately depends on aerobic autotrophic nitrification. Thus the role of nitrifiers in the N cycle of marine systems is to link the oxidizing and reducing processes of the N cycle by converting NH$_4^+$ to NO$_3^-$. Nitrification can also be an important sink for oxygen in aquatic environments. NO$_3^-$ is released through the aerobic oxidation of organic matter and nitrification according to the classic Redfield stoichiometry (Redfield et al., 1963):

\[(CH_2O)x(NH_3)y(H_3PO_4) + (x + 2y)O_2 \rightarrow xCO_2 + yHNO_3^- + (x + y)H_2O^-\]

where $x = 106$ and $y = 16$. In this formulation, oxygen consumption should be inversely related to NO$_3^-$ production with a slope of $138/16 = 8.6$. In fully oxygenated sediments (i.e., carbon loading is not sufficient to exhaust the available oxygen), such relationships are often found. Grundmanis and Murray (1982) and Jahnke et al. (1982) reported ratios of 10.1 and 7 respectively, and both state that these are in reasonable agreement with the predicted stoichiometry once differential diffusivities are taken into account. Redfield stoichiometry implies that nitrification is responsible for $32/138 = 23\%$ of the total oxygen consumption associated with organic matter diagenesis. In environments where denitrification and anammox occur, the net O$_2$/NO$_3$ stoichiometries may vary (Devol, this volume).

### 3.1. Distribution and abundance of nitrifiers in the marine environment

Although most strains of NH$_3$-oxidizing and NO$_2^-$-oxidizing bacteria have characteristic intracytoplasmic membrane structures, which can be visualized by electron microscopy, it is not possible to distinguish the otherwise nondescript cells from other bacteria and archaea in water samples using standard microscopic techniques for cell enumeration, e.g., epifluorescence microscopy with DNA fluorochromes.
Most probable number (MPN) methods relying on the appearance or disappearance of NO$_3^-$ in dilution media have been used to estimate the abundance of NH$_3$ oxidizers and NO$_2^-$ oxidizers, respectively, in aquatic environments. MPN approaches are widely used to estimate abundances of nitrifying bacterial populations in soils, but there are very few modern reports using this technique in marine systems. Examples of data on nitrifying bacterial abundances are provided in Table 5.1. Bianchi et al. (1999) estimated the abundance of both NH$_3$ and NO$_2^-$ oxidizers in the water column of the Mediterranean Sea in the Rhone River plume using MPN. Maximum abundances of both types were found in surface water of the stations closest to the river and reached levels of $3.5 \times 10^4$ cells ml$^{-1}$ for NH$_3$ oxidizers and $1.2 \times 10^4$ for NO$_2^-$ oxidizers. Ammonia plus NO$_2^-$ oxidizing cells averaged less than 2% of the total microbial abundance. McCaig et al. (1999) used MPN to estimate the abundance of NH$_3$ and NO$_2^-$ oxidizers in polluted sediments underlying marine fish farms. In the sample from directly underneath the fish cage, NO$_2^-$ oxidizers were abundant and NH$_3$-oxidizers were not detected. Farther away from the cage, NH$_3$ oxidizers were detected, but NO$_2^-$ oxidizers were not present above detection level at these stations. Hall (1986) estimated from data in a number of lake studies that the efficiency of recovery for MPN was 0.05–0.001% for NH$_3$ oxidizers. He also concluded that MPN abundances showed little correlation with observed nitrifying activities. This technique should, however, yield isolates of the most abundant cell type present, assuming it can grow under the enrichment culture conditions. Phillips et al. (2000) compared MPN and a competitive PCR method for enumeration of NH$_3$-oxidizing bacteria in estuarine sediments and detected 1–3 orders of magnitude higher abundances using cPCR (on the order of $10^5 = 10^6$ cells g$^{-1}$).

Immunofluorescence (IF) was first applied to the study of nitrifying bacteria in soil and lake systems by Schmidt and coworkers (e.g., Fliermans et al., 1974; Schmidt, 1978; Smorczewski and Schmidt, 1991; Stanley et al., 1979) and the method was subsequently used by Ward and coworkers to enumerate nitrifiers in seawater (e.g., Ward and Carlucci, 1985; Ward and Perry, 1980). In a small survey of marine and estuarine sites, Ward (1982) reported that *Nitrosomonas* serotypes were more abundant than *Nitrosococcus* and that total abundance of NH$_3$ oxidizers (*Nitrosomonas* plus *Nitrosococcus* serotypes) ranged from $10^5$ cells ml$^{-1}$ in Chesapeake Bay to $10^2$ cells ml$^{-1}$ in inshore ocean waters and $10$–$10^2$ cells ml$^{-1}$ offshore. Maximum abundances ($10^6$ cells l$^{-1}$ for both NH$_3$ and NO$_2^-$ oxidizers) occurred near the bottom of the photic zone in the vicinity of the primary NO$_2^-$ maximum in the Peru upwelling system (Ward et al., 1989a) but such characteristic patterns are not always detected. *Nitrosococcus oceanus* abundance, determined by IF, was positively correlated with temperature, particulate organic carbon and N and total bacterial abundance, and negatively correlated with dissolved oxygen, in brackish Mediterranean lagoons (Zaccone et al., 1996). There was no relationship between IF and MPN counts over the 20 month sampling period of this study.

Immunofluorescence requires that the target organisms be cultivated so that antibodies can be produced against the cells to be enumerated. The antibodies responsible for the cell-staining reaction are those that interact with components of the outer cell membrane, and the reaction can be very specific (Ward and Carlucci, 1985). This is both a strength and a disadvantage of the method. It allows
<table>
<thead>
<tr>
<th>Location</th>
<th>Depth (m)</th>
<th>AOB cell abundance</th>
<th>NOB cell abundance</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediments Freshwater lake sediments</td>
<td>Surficial sediments</td>
<td>$8 \times 10^5$ cells g$^{-1}$</td>
<td>$21 \times 10^5$ cells g$^{-1}$</td>
<td>MPN</td>
<td>Smorczewski and Schmidt, 1991</td>
</tr>
<tr>
<td>Sediments under marine fish farm cages</td>
<td>0.5 cm core top</td>
<td>Up to $0.2 \times 10^6$ cells m$^{-2}$</td>
<td>Up to $25 \times 10^6$ cells m$^{-2}$</td>
<td>MPN</td>
<td>McCaig et al., 1999</td>
</tr>
<tr>
<td>Marine (saline) Water column environments</td>
<td>Oxic water column</td>
<td>Nitrosomonas $2 \times 10^3$</td>
<td>IF</td>
<td>Voytek et al., 1998</td>
<td></td>
</tr>
<tr>
<td>Lake Bonney (Antarctica)</td>
<td>Oxic water column</td>
<td>Nitrosococcus $0.8 \times 10^3$</td>
<td>IF</td>
<td>Voytek et al., 1998</td>
<td></td>
</tr>
<tr>
<td>Mediterranean lagoon</td>
<td>Oxic water column</td>
<td>Nitrosococcus $1 \times 10^3$</td>
<td>IF</td>
<td>Zaccone et al., 1996</td>
<td></td>
</tr>
<tr>
<td>Peru upwelling Ave over water column</td>
<td>Nitrosomonas + Nitrosococcus $0.3 \times 10^3$</td>
<td>Nitro-bacter + Nitrococcus $0.2 \times 10^3$</td>
<td>IF</td>
<td>Ward et al., 1989a</td>
<td></td>
</tr>
<tr>
<td>Southern California Coastal waters</td>
<td>Ave over water column</td>
<td>Nitrosomonas + Nitrosococcus $0.3 \times 10^3$</td>
<td>Nitro-bacter + Nitrococcus $0.3 \times 10^3$</td>
<td>IF</td>
<td>Ward and Carlucci, 1985</td>
</tr>
<tr>
<td>Chesapeake Bay Ave over water column</td>
<td>Nitro-somonas + Nitrosococcus $10^7$</td>
<td>IF</td>
<td>Ward, 1982</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AOB – Ammonia-oxidizing bacteria; NOB – Nitrite-oxidizing bacteria; IF – Immunofluorescence; MPN – Most Probable Number
strain or species specific detection, so that distribution patterns can be discerned for individual types, but therefore does not allow an enumeration of total abundance for all similar physiological types. The necessity for cultivation of the antigenic strain also guarantees that most of the strains that are important in the ocean cannot be tested for cross reactivity and it seems likely that they are not detected by these assays.

The molecular methods that have been used to detect and investigate the diversity of NH₃ oxidizers in the ocean have not been widely adapted for their quantitative analysis and enumeration. This is in great contrast to the study of AOB and NOB in soils and freshwater systems, particularly wastewater bioreactor and biofilm environments (reviewed by Schramm, 2003), where competitive and quantitative PCR assays based on both 16S rRNA and amoA genes that are specific for nitrifiers have been used to enumerate cells of various subgroups of nitrifiers. In a study designed to test the adaptation of fluorescence in situ hybridization (FISH) identification of specific cells to automated enumeration by flow cytometry, Sekar et al. (2004) detected both *Nitrosospira* and *Nitrosomonas* sequences in their 16S rRNA clone libraries. They subsequently used an enhanced sensitivity method (catalyzed reported deposition [CARD-FISH]) to enumerate cells of both types in water samples from the coastal North Sea. Probes for *Nitrosospira* and *Nitrosomonas* detected 3.4% and 4.0% of the total DAPI-stained cells in a sample from March 2003, but the same probes did not detect any cells from samples taken in August, September, and November 2002 at the same site (Sekar et al., 2004). Lam et al. (2004) enumerated betaproteobacterial AOB using FISH in and near neutrally buoyant plumes emanating from hydrothermal vents in the Endeavor segment of the Juan de Fuca Ridge. Along with many other reduced substrates, the plumes often contain high NH₄⁺ concentrations and thus might be expected to provide an enriched environment for NH₃ oxidizers. AOB reached maximal abundances of 1–1.4 × 10⁴ cells ml⁻¹ in the plume itself and were present at much lower levels (minimum of 0.04 × 10⁴ cells ml⁻¹) in background (non-plume) samples. The cells were preferentially associated with particles, constituting up to 51% of the total cells enumerated by DAPI in the >3 μm particle fraction, whereas AOB constituted up to 4.9% of the free living cells enumerated by DAPI (Lam et al., 2004). These numbers are 10-fold higher than previous AOB counts obtained by immunofluorescence from shallower depths in the open and coastal ocean (Ward et al., 1982, 1989a) and comparable to those from nutrient rich environments such as Chesapeake Bay (Ward, 1982), suggesting an important role for nitrifiers in this environment. Depending on the specificity of oligonucleotide probes, genetic methods should detect higher numbers than IF, because of the specificity and culturability issues mentioned earlier.

Clone libraries may give some indication of the relative abundance of target organisms. In this case, it would be instructive to know the percentage of a total bacterial 16S rRNA clones that were identified as AOB or NOB– like sequences. AOB sequences are easily retrieved when targeted directly with AOB–specific primers, but are very rare in total 16S rRNA libraries.

The results from FISH and IF measurements show that nitrifying bacteria generally contribute a minor fraction of the total microbial assemblage in natural waters, with estimates ranging from 0.1% to 7.5% of the total cell number in the
water column. This generalization is consistent with the autotrophic nature of nitrifiers, which requires that individual cells process relatively large amounts of N for minimal return in terms of carbon assimilation. The abundances estimated for the hydrothermal vent plumes are quite high (Lam et al., 2004), but consistent with environmental parameters that favor the autotrophic NH$_3$ oxidizing metabolism. Abundance data for NO$_2^-$ oxidizers are almost non-existent and for NH$_3$ oxidizers very sparse. This is an area in which quantitative PCR and enhanced sensitivity single cell detection methods (e.g., CARD-FISH) could be very usefully applied. FISH and quantitative PCR assays for 16S rRNA and $amoA$ genes detected up to 1000 fold greater abundances of Crenarchaeota than AOB in coastal North Sea water (Wuchter et al., 2006), indicating that unlike AOB, the AOA can be a major contribution to the total microbial assemblage.

3.2. Methods for measuring nitrification rates in water and sediments

As with most biogeochemically important processes, there is no perfect method for direct measurement of nitrification rates. Potential artifacts arise from the use of incubations, but incubation free methods can also be problematic.

3.2.1. Changes in din concentration and specific inhibitors

The easiest experimental design might be simply to incubate samples and measure the concentrations of NO$_2^-$ or NO$_3^-$ over time. This approach provided some of the earliest evidence for the occurrence of biologically mediated nitrification (Rakestraw, 1936; von Brand et al., 1937). Vaccaro (1962) estimated nitrification rates by measuring changes in NO$_2^-$ and NO$_3^-$ in incubated samples, from depths up to 800 m in the Sargasso Sea, in the presence of added NH$_4^+$ or mixed whole plankton. He reported that both additions stimulated the net production rate of NO$_2^-$ or NO$_3^-$. In such an experiment, accumulation of NO$_2^-$ or NO$_3^-$ indicates net nitrification. A decrease in the concentration over time could be observed, however, even when nitrification is occurring, if consumption of NO$_3^-$ or NO$_2^-$ exceeds production in the incubation bottle. This is likely to be the case in surface waters where the low concentration of fixed N relative to high biological demand means that large fluxes can be obscured by tight coupling between production and consumption terms.

The simple nutrient measurement approach can be modified by the use of specific inhibitors. Chemicals that specifically inhibit either NH$_4^+$ oxidation (e.g., acetylene, allylthiourea, methyl fluoride, N-serve) or NO$_2^-$ oxidation (chlorate) are added to replicate incubation bottles (Bianchi et al., 1997; Billen, 1976). The bottles must be incubated in the dark to prevent assimilation of by phytoplankton. A decrease in the concentration of NO$_2^-$ over time in the bottles in which NH$_4^+$ oxidation was inhibited provides an estimate of the NO$_2^-$ oxidation rate. The rate of NO$_2^-$ increase in the bottles to which NO$_2^-$ oxidation inhibitor was added approximates the rate of NH$_4^+$ oxidation. Aside from the overall limitations of
bottle incubations, the potential artifacts associated with the inhibitor approach relate to the conditions of the incubation:

1. Preventing production by phytoplankton probably has cascading effects on the activities of other microbes in the bottle, such that the rate of NH$_4^+$ mineralization is reduced, therefore changing the source term for the nitrification substrate. (1)

2. Incubating in the dark may release the nitrifiers from light inhibition such that the measured rate exceeds the *in situ* rate. (2)

3. Incubations typically last 48 h, which is sufficient to overcome the lag induced by light inhibition, but is also long enough to create quite unnatural conditions. (3)

The search for precisely specific inhibitor compounds has been extensive and has resulted in a plethora of potentially useful compounds. Many are problematic for reasons not directly related to nitrification. For example, acetylene inhibits both nitrifiers and denitrifiers (Balderston *et al.*, 1976; Berg *et al.*, 1982). Thus its use to measure one process will also inhibit the other, and when one depends on the other (as is the case when denitrification depends upon nitrification for NO$_3^-$), both rates are affected and the independent measurement of one is not possible. It is reported that the length of exposure to acetylene can be optimized to differentiate between its affects on nitrification and denitrification (Kester *et al.*, 1996). N-serve is a commercial preparation that specifically inhibits NH$_3$-oxidizing bacteria (Goring, 1962) and it serves as the basis for the sensitive $^{14}$CO$_2$ method for measurement of nitrification rates. Being chemolithoautotrophs, nitrifiers fix CO$_2$ while oxidizing N. The amount of CO$_2$ fixation due to nitrifiers can be computed by difference between incubations with and without addition of an inhibitor that specifically removes the contribution of nitrifiers (Billen, 1976; Dore and Karl, 1996; Somville, 1978). Then a conversion factor is used to translate the CO$_2$ fixation into NH$_4^+$ and NO$_2^-$ oxidation rates. This conversion factor has been shown to vary in the case of NH$_3$ oxidizers by a factor of five in pure cultures (Billen, 1976; Glover, 1985), and thus its use introduces some uncertainty, since the factor can only be directly determined in field samples if the $^{15}$N methods are performed in parallel (see below).

To complicate matters further, N-serve is not soluble in water, so its addition to samples requires that it be dissolved in an organic solvent. This solvent can affect the other members of the community; e.g., ethanol stimulates dark CO$_2$ incorporation by heterotrophic bacteria (Owens, 1986; Ward, 1986). Thus in systems where heterotrophs are a large part of the overall assemblage, the N-serve plus solvent approach may over estimate the dark CO$_2$ incorporation due to nitrifiers (Priscu and Downes, 1985; Viner, 1990; Ward, 1986).

Inhibitor approaches similar to those described earlier for water samples have been used in sediments (Henricksen *et al.*, 1981; Miller *et al.*, 1993). The methylfluoride and difluoromethane methods (Caffrey and Miller, 1995; Miller *et al.*, 1993) seem particularly promising because the gases can diffuse thoroughly into the core with minimal disturbance of microzones and gradients. These NH$_4^+$ oxidation inhibitors are added to cores and the accumulation of NH$_4^+$ over time is assumed to represent the net rate of nitrification. Other processes that consume NH$_4^+$ would lead to an underestimate of the rate. De Bie *et al.* (2002) found that both
acetylene and methyl fluoride stimulated dark $^{14}$CO$_2$ fixation in estuarine sediments. They concluded that methyl fluoride could not be used to estimate nitrification from this approach in natural samples although NO$_2^-$ production was inhibited by 95% in pure cultures of *Nitrosomonas europaea* by both of these inhibitors. CO$_2$ fixation is an indirect estimate of nitrification and may reflect activities of many other kinds of metabolism. Measurement of NH$_4^+$ accumulation in conjunction with specific inhibitors is more direct, but still subject to interference from other processes that produce and consume NH$_4^+$ in natural samples.

To overcome the bias resulting from uneven dispersal of tracer or inhibitor, sediment rate measurements are often made in slurries, which destroy the gradient structure of sediments, which is essential to the *in situ* fluxes. Slurries may provide useful information on potential rates, but not *in situ* rates. Potential nitrification rates and rates measured in intact cores were not correlated in estuarine sediments (Caffrey *et al.*, 2003). The lack of correlation was explained by the inclusion of variable amounts of anoxic sediments in the slurries from which the potential rates were derived.

### 3.2.2. Isotopic incubation methods

The direct radioisotope tracer method, in which the accumulation of radiolabeled product from added radiolabeled substrate over time yields a rate estimate, is not very practical for measuring rates of nitrification in the environment. Capone *et al.* (1990) were able to quantify nitrification rates using $^{13}$N, but the isotope is so short-lived (10 min half-life) that its use is usually impractical.

The other main approach to measuring nitrification rates directly is to use the stable isotope, $^{15}$N, as a tracer (Olson, 1981a; Ward *et al.*, 1984). The signal of transfer of the tracer from substrate to product pool (e.g., $^{15}$NH$_4^+$ to $^{15}$NO$_2^-$) can be detected regardless of what other processes are occurring in the incubation (*in situ* light conditions can be used) and no assumptions of steady state need be made. The major drawback of this method is the necessity to add a tracer, sometimes in excess of the natural concentration of substrate. In culture, AOB and NOB respond to increased substrate concentrations by increasing oxidation rates (Carlucci and Strickland, 1968; Suzuki *et al.*, 1976; Ward, 1987a), suggesting that addition of non-tracer levels of isotopically labeled substrate should artificially enhance measured oxidation rates. Such an enhancement is not generally observed in the case of NH$_3$ oxidation, however, suggesting that natural nitrifier assemblages are not substrate limited at *in situ* levels (Olson, 1981a, Ward, 1990).

The problem of excess substrate addition in tracer experiments has been largely overcome by the advent of more sensitive mass spectrometers, however, and estimates obtained under conditions approaching *in situ* are possible. Owing to the great sensitivity of isotope ratio mass spectrometry, much shorter incubations (compared to the inhibitor and inventory methods) are possible (a few hours to 24 h are commonly used). Although they have not yet been widely applied for this purpose, the recently introduced sensitive isotope methods for determination of $^{15}$N content of NO$_2^-$ and NO$_3^-$ (McIlvin and Altabet, 2005; Sigman *et al.*, 2001) should make true tracer level incubations possible. Details on the most commonly used isotope tracer methods for
measurement of nitrification can be found in work by Ward and O’Mullan (2005) and in the Chapter by Lipschultz (this volume).

The $^{15}$N approach is most useful in water samples because complete mixing of the tracer is possible. In sediments, rate measurements are constrained by the inhomogeneous nature of the sample and the dependence of rates on the structure of the environment. In this situation, fluxes between overlying water and sediment cores can be analyzed to obtain areal rates. In conjunction with $^{15}$N tracer addition, estimates of nitrification rates can be obtained from the dilution of NO$_2^-$ or NO$_3^-$ in the overlying water due to its production in the sediments (Capone et al., 1992).

The isotope pairing method for measurement of denitrification (Nielsen, 1992; Rysgaard et al., 1993) is essentially an isotope dilution approach from which both nitrification and denitrification rates can be calculated.

Even if rate measurements in sediments are made using whole core incubations, e.g., when the inhibitor is a gas, it is still difficult to obtain a depth distribution of the rate (usually, an areal rate is obtained). A sophisticated measurement and model based system that avoids direct rate measurements has been used to overcome this problem. Microelectrodes, which have very high vertical resolution, are used to measure the fine scale distribution of oxygen and NO$_3^-$ in freshwater sediments. By assuming that the observed vertical gradients represent a steady state condition, reaction-diffusion models can then be used to estimate the rates of nitrification, denitrification and aerobic respiration and to compute the location of the rate processes in relation to the chemical profiles (e.g., Binnerup et al., 1992; Jensen et al., 1994; Meyer et al., 2001; Rysgaard et al., 1994). Recent advances and details of the microelectrode approach can be found in the Chapter by Joye and Anderson (this volume).

Comparison of incubation methods: The inhibitor methods using $^{14}$C have advantages in higher sensitivity, smaller sample volume, easier analysis and therefore greater throughput than the $^{15}$N tracer approaches. Therefore, in spite of the potential artifacts mentioned above, the inhibitor approach remains attractive to some investigators. Incubations of 24 h up to 12 days were used to compare the $^{14}$C inhibitor, $^{15}$NH$_4^+$ oxidation and $^{15}$NO$_3^-$ dilution methods to estimate nitrification rates in the water column of the Baltic Sea (Enoksson, 1986). Artifacts associated with elevated substrate additions and long incubations were identified. The $^{15}$NO$_3^-$ dilution method was not recommended due to sensitivity problems. Andersson et al. (2006) suggested a formal distinction between nitrification activity as measured by $^{15}$N and nitrifier growth as measured by specific inhibition and $^{14}$CO$_2$ incorporation. The ratio of the two rates was found to vary as a function of temperature and oxygen concentration, and the necessity to exclude phytoplankton $^{14}$CO$_2$ uptake by performing incubations in the dark was seen as unacceptably artifactual (Andersson et al., 2006). Gundersen (1966) had long ago reported that the ratio of N oxidized to C fixed decreased at low oxygen concentrations relative to air.

Incubation methods, whether enclosing water in a bottle or bag or sediment in a coring tube, may introduce unavoidable artifacts. Even the act of sampling introduces artifacts; in the case of water sampling, larger sinking particles are usually not included in bottle incubations, so the metabolism associated with such particles is not represented in incubations. Approaches that avoid incubations altogether are therefore attractive, but they are largely limited to measuring changes in chemical
concentrations over time, and therefore can detect net transformation rates only. The compromise made by most investigators is to maximize the size and minimize the length of incubations in order to minimize artifacts associated with wall growth or preferential inclusion or exclusion of grazers.

3.2.3. Geochemical constraints to estimate nitrification rates
Stable isotope signatures and distributions in constituents of the N cycle can be used to identify rate processes and potentially to constrain their rates. Because nitrification is often closely coupled to the processes that produce and consume its substrates and products, nitrification would not be expected to leave a clear signature in many environments. Ammonia oxidizing bacteria in culture, however, exhibit relatively large enrichment factors (Casciotti et al., 2003; Delwiche and Steyn, 1970; Mariotti et al., 1981), leaving behind isotopically enriched NH$_4^+$ and producing isotopically depleted NO$_2^-$. The fractionation associated with NO$_2^-$ oxidation to NO$_3^-$ has not been directly determined in culture, due to the difficulty of separating NO$_2^-$ and NO$_3^-$ for independent isotopic analysis (Delwiche et al., 1970). It is assumed, however, that the $^{15}$N of the NO$_3^-$ produced is depleted relative to the NH$_4^+$ from which it derived, as is the oxygen in NO$_3^-$ relative to that in NO$_2^-$ and water from which it is derived. Because NO$_2^-$ rarely accumulates, most of the fractionation due to nitrification probably happens at the rate limiting NH$_3$ oxidation step (Kendall, 1998). Isotopically light NO$_3^-$ in the surface water of the eastern tropical North Pacific was attributed to nitrification, and direct measurements of nitrification using $^{15}$N tracer techniques substantiated the distribution of the process in the euphotic zone (Sutka et al., 2004).

Sutka et al. (2006) investigated the potential for intramolecular isotopic (isotopomer) distributions to identify the source of nitrous oxide in the environment. Ammonia-oxidizing bacteria, methane-oxidizing bacteria (which can also oxidize NH$_4^+$ and hydroxylamine) and denitrifiers can all produce N$_2$O in the laboratory and are all potential sources of N$_2$O in the marine environment. Methanotrophs and AOB had indistinguishable site preferences for $^{15}$N in N$_2$O produced from NH$_3$ or hydroxylamine (Sutka et al., 2003, 2006). AOB can produce N$_2$O by two pathways; one by oxidation of hydroxylamine and one by reduction of NO$_2^-$. The NO$_2^-$ reduction pathway in nitrifiers – nitrifier denitrification – is homologous with that of denitrifiers (Casciotti and Ward, 2001, 2005) and showed the same site preference in N$_2$O as found in denitrifiers (Sutka et al., 2006). Thus the isotopomer approach cannot distinguish between the oxidative production of N$_2$O by methanotrophs vs. AOB, nor can it distinguish between canonical heterotrophic denitrification and nitrifier denitrification. However, the oxidative and reductive pathways to N$_2$O production did have significantly different isotopomer signatures. In order to use isotopomers to identify the source of N$_2$O in the ocean, we need to know which of the two possible pathways AOB use to produce N$_2$O. It may be important that the isotopomer studies mentioned above did not test marine nitrifiers – the observed isotopic fractionation and functional gene sequences indicate that there may be biochemical differences among the genera of AOB, and of course the most abundant AOB and AOA have not been cultured or tested for these pathways. The capacity for nitrifier denitrification may be ubiquitous among AOB (Casciotti and Ward, 2005;
Shaw et al., 2006) and even some of the genome of the crenarchaeal relative of the cultivated AOA possesses the genes that encode the pathway (Hallam et al., 2006).

On a basin or areal scale, the expected rates of nitrification can be estimated from other biogeochemical distributions and rates. Dore and Karl (1996) compared nitrification rates measured using the $^{14}$C inhibitor approach to the estimated fluxes of $N_2O$, assuming a very high $N_2O$ yield from nitrification. Although they reported broad agreement, both rate estimates are very poorly constrained. Ward and Zafiriou (1988) measured $NH_3$ oxidation rates as deep as 3000 m using the $^{15}NH_4^+$ oxidation method and noticed that the rate decayed rapidly with depth (Fig. 5.3). They compared the integrated nitrification rate in the eastern tropical North Pacific to the predicted $N$ mineralization rate derived from sediment trap fluxes in the same area (Martin et al., 1987). The integrated trap flux and nitrification rates differed by about a factor of 2, suggesting broad agreement and implying that nitrification is coupled to mineralization of sinking particles, but that the nitrification process occurs not on the particles themselves, but in the water captured in the incubation.

Berelson used the equation of Martin et al. (1987) to compare mineralization rates at the 17 stations from the regional JGOFS studies and found that the stations varied by only a factor of two in the magnitude of the fitting parameter, b (Berelson, 2001). The greater the value of b, the more rapid the decrease in flux with increasing depth, implying greater mineralization activity at shallower depths. The curvature was correlated with the magnitude of the flux, which varied by a factor of ~20 among the 17 stations. The linear relationship between b and POC flux can be used to constrain mineralization, and thus nitrification, rates, assuming most mineralization occurs in the water column and that the $NH_4^+$ released is completely nitrified. For POC fluxes ranging from 1.3 mmol C m$^{-2}$ day$^{-1}$ (the lowest equatorial Pacific station) to 24.9 mmol C m$^{-2}$ day$^{-1}$ (the North Atlantic Bloom Experiment), PON fluxes of 0.133–0.167 to 2.9–3.6 mmol N m$^{-2}$ day$^{-1}$ can be computed (assuming a C/N ratio of the particles of 8 or 10, respectively; Antia, 2005). The integrated nitrification rates reported by Ward and Zafiriou (1988) for the eastern tropical North Pacific are about the only data suitable for comparison over this vertical extent, and they ranged from 1.1–2.7 mmol N m$^{-2}$ day$^{-1}$.

The fundamental agreement between these estimates of integrated PON flux (i.e., mineralization rates due to PON loss) and observed nitrification rates has important biogeochemical implications. Firstly, nitrification is coupled to mineralization and the rate decreases dramatically with increasing depth. This is consistent with incubation rate measurements that usually detect highest rates within the top 100 m of the water column. Most of the nitrification required to balance mineralization over the water column occurs in the planktonic phase or on particles that can be captured in bottles, rather than on rapidly sinking particles, suggesting that mineralization leads to the break up of particles and the release of dissolved $NH_4^+$ that is subsequently nitrified. Such rate estimates are an important constraint on inferences of microbial metabolic activities: regardless of the phylogenetic identity of the nitrifiers, they can’t be growing or nitrifying very rapidly over most of the deep ocean. Even if 40% of the microbial cells in the deep ocean are crenarchaeota with the capacity for $NH_3$ oxidation, their livelihood, whether it be $NH_3$ oxidation or organoheterotrophy, is fundamentally constrained by substrate supply, which decreases greatly with depth.
The distribution of nitrous oxide may provide a further geochemical constraint on nitrification rates and distributions. Using a global dataset of N₂O distributions, Nevison et al. (2003) derived a parameterization for the instantaneous production by nitrification of N₂O per mole of O₂ consumed as a function of O₂ concentration and depth. Assuming that NH₃ oxidizers produce N₂O in increasing proportion to NO₂⁻ as O₂ concentration decreases (as previously reported; Goreau et al., 1980), an annual N₂O production rate of \( \sim 5.8 \pm 2 \) Tg N year\(^{-1}\) was estimated (Nevison et al., 2003). It is not a simple calculation to derive a total nitrification rate from this result because of the nonlinear relationship between O₂ concentration, nitrification rate and the proportion of NH₄⁺ that is lost as N₂O. Nevertheless, it might be possible to estimate a global nitrification rate with vertical flux data as discussed above, which would also be constrained by the global ocean N₂O flux calculations.

3.3. Distribution of nitrification in water and sediments

When Kaplan reviewed the status of nitrification in the marine environment in 1983, direct measurements of nitrification rates in water column and sediment environments were few and far between. The proliferation of inhibitor approaches, plus increased accessibility and sensitivity of mass and emission spectrometers has allowed great progress in this area. In 1983, Kaplan was unable to make many generalizations about major patterns in the rate and distribution of nitrification. Since that time, advances in experimental data and conceptual frameworks have elucidated the following patterns:

1. Nitrification rates in oxygenated water columns show typical depth distributions with maximum rates near the bottom of the euphotic zone and a rapidly declining rate with increasing depth below that.
2. Nitrification in the water column is tightly coupled with NH₄⁺ regeneration, such that NO₃⁻, not NH₄⁺, accumulates in the deep sea.
3. Nitrification rates are often low in the well lit surface waters of the ocean, probably due to light inhibition and competition for NH₄⁺ with phytoplankton and heterotrophic bacteria.
4. Nitrification is ubiquitous in surface sediments and is often tightly coupled to denitrification in the sediment redox gradient.

3.3.1. Nitrification in the water column

The major N product of organic matter decomposition in seawater is NH₄⁺, but NH₄⁺ is present at trace or undetectable levels in the huge volume of the deep ocean. Rather, deep water contains NO₃⁻ at 20–40 μM concentrations, which would seem to imply that nitrification occurs mainly in the deep ocean. Nitrate concentrations in the surface ocean are usually maintained at low levels because phytoplankton assimilate NO₃⁻ more rapidly than it can be supplied by mixing or diffusion from the deep NO₃⁻ reservoir. Ammonium, which is produced in the photic zone by heterotrophic processes, is also usually immediately assimilated by phytoplankton and heterotrophic bacteria before it can be nitrified. The important physical and biological differences in the source functions of NH₄⁺ and NO₃⁻ are
the basis of the new production paradigm (Dugdale and Goering, 1967; Eppley and Peterson, 1979) as a framework to understand phytoplankton N demand and growth in the surface ocean, and the subsequent flux of N to the deep sea and ocean floor (Fig. 5.1A).

Ammonium is considered a “regenerated” source of N, because it is produced in situ, in the euphotic zone, from ammonification of organic matter. The N in

Figure 5.1 (A) The New Production Paradigm, redrawn from Eppley and Petersen, 1979, representing nitrogen transformations in the surface layer of the ocean. N assimilation by phytoplankton is derived from either “new” (NO₃, N₂) or regenerated (NH₄, amino acids, urea) nitrogen. In the first panel is the original view of Eppley and Petersen, in which nitrification occurs only below the euphotic zone such that nitrate is a “new” nutrient. The two red arrows are equivalent because “new production” is balanced by the export flux from the euphotic zone. (B) In the second panel, nitrification, as well as various production and consumption terms involving dissolved inorganic nitrogen (DON), occurs in the euphotic zone. Nitrate is thus at least partially a “regenerated” nutrient, and the flux through the labile portion of the DON pool can be rapid and linked to other euphotic zone processes.
$\text{NH}_4^+$ is recycled rapidly and repeatedly between living biomass (phytoplankton, the zooplankton that graze on them, protozoans, bacteria and archaea) and the inorganic nutrient form, which is released from heterotrophic metabolism and grazing. Nitrate, on the other hand, is "new" N because it is virtually absent from the euphotic zone most of the time and must be transported into the system by physical means—mixing or upwelling from deep waters or falling in rain—in order for phytoplankton to use it. The rate of NO$_3^-$ supply can be equated with the steady state rate of export production, primary production based on NO$_3^-$ as a N source (Eppley and Peterson, 1979). This equality between supply and export makes it possible to measure "new production," and by inference, the sinking flux of N, by measuring the assimilation of NO$_3^-$ in incubated samples. In this scenario, nitrification is responsible for the production of NO$_3^-$ in the deep water, from whence it is transported to the surface layer; nitrification is explicitly absent from the euphotic zone itself.

Depth distribution of nitrification rates: Actual measurements of the depth distribution and rate of nitrification show, however, that NO$_3^-$ production and consumption are not in fact so completely or conveniently separated in space. The highest nitrification rates, both $\text{NH}_4^+$ oxidation and NO$_2^-$ oxidation, occur not in the deep ocean, but in a region near the bottom of the euphotic zone. In this depth interval, the light intensity is very reduced and phytoplankton are light limited, and their rates of nutrient assimilation are therefore reduced. It is in this interval that nitrifying bacteria can compete with phytoplankton for $\text{NH}_4^+$; a sharp peak is often observed in the nitrification rate at a depth in the water column where the light intensity is 5–10% of surface light intensity or in the vicinity of the deep chlorophyll maximum, when it occurs (Lipschultz et al., 1990; Sutka et al., 2004; Ward, 1987b; Ward et al., 1984) (Fig. 5.2).

Rates of nitrification reported for the open ocean are in the range of a few to a few hundred nanomolar per day, and have been detected as deep as 3000 m (Ward and Zafiriou, 1988). Most of the data discussed here are $\text{NH}_4^+$ oxidation rates. Nitrite oxidation rate methods are more prone to experimental artifacts; many fewer results have been published and many of the high rates reported are probably overestimates. Rates of nitrification reported in various aquatic environments are presented in Table 5.2. Where profiles extending to a depth of several hundred to a few thousand meters are available, the main pattern that emerges is the association of the highest rates of NH$_3$ oxidation with the lower region of the euphotic zone (Fig. 5.3). In the eastern tropical North Pacific, NH$_3$ oxidation rates at the maximum were no more than 20 nM day$^{-1}$ (Ward and Zafiriou, 1988). In the Peru upwelling region, a maximum rate of 747 nM day$^{-1}$ was reported (Lipschultz et al., 1990). In the temperate eastern Pacific Ocean off western North America, maximum rates of 45 nM day$^{-1}$ were reported (Ward, 1987b). Nitrite oxidation shows a less predictable distribution with depth, probably due to methodological problems; in the Peru upwelling system, maximum rates of 600 nM day$^{-1}$ were observed near the lower boundary of the euphotic zone, but high rates (e.g., nearly 300 nM day$^{-1}$) were observed within the oxygen minimum zone (Lipschultz et al., 1990).

Several studies of nitrification rates have focused on the primary NO$_2^-$ maximum, rather than attempting complete depth profiles. Dore and Karl (1996) reported a few
rate measurements based on inhibitor experiments from the central Pacific Ocean and calculated rates up to 137.4 and 138 nM day\(^{-1}\) for \(\text{NH}_4^+\) and \(\text{NO}_2^-\) oxidation, respectively. Rates 10-fold lower were reported for the same station using \(^{15}\text{N}\) tracer methods (Sutka et al., 2004). Ammonium and \(\text{NO}_2^-\) oxidation rates were usually comparable, and were maximal just below the primary \(\text{NO}_2^-\) maximum. Bianchi et al. (1994) reported nitrification rates up to 1–2 \(\mu\text{M day}^{-1}\) in the Rhone River Plume, with rates decreasing to the usual oceanic levels with increasing distance from shore in the Mediterranean Sea. In a recent compilation of nitrification rate measurements, Yool et al. (2007), a great range of rates in the upper 250 m of open ocean environments. Using a specific nitrification rate (d\(^{-1}\)) they did not detect consistent depth patterns, but did document nitrification within the photic zone. Clearly the simple model that distinguishes \(\text{NO}_3^-\) as a new nutrient should be modified (Fig. 5.1B); nitrate produced from nitrification in the euphotic zone is often sufficient to meet phytoplankton demand in the same depth interval.

The rate of nitrification in deep ocean water is minimal, due to the decreasing flux of \(\text{NH}_4^+\) from organic matter decomposition with increasing depth. Thus in many parts of the world ocean, the typical depth distribution of nitrification shows a subsurface maximum which occurs near the bottom of the euphotic zone, and very low rates persisting to great depths. The great accumulation of \(\text{NO}_3^-\) in the deep sea is therefore due to a small production term and a lack of any significant consumption terms. An exception to this deep sea condition is found in the vicinity of

![Figure 5.2](image-url) Depth distribution of ammonia oxidation rate (VNH4) and ammonium assimilation rate (VPN) in the surface layer in Monterey Bay, CA. Data were obtained from \(^{15}\text{N}-\text{NH}_4\) tracer incubations at simulated in situ light intensities. (From Ward 2005)
### Table 5.2  Nitrification Rates Reported from Aquatic Systems

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth (m)</th>
<th>NH$_4^+$ Ox Rate (nmol l$^{-1}$ day$^{-1}$)</th>
<th>NO$_2^-$ Ox Rate (nmol l$^{-1}$ day$^{-1}$)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water column</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sagami Bay, Japan</td>
<td>Photic zone ave, June light</td>
<td>50,000</td>
<td></td>
<td>$^{15}$N tracer</td>
<td>Miyazeki et al., 1973</td>
</tr>
<tr>
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<td>Photic zone ave, June dark</td>
<td>26,000</td>
<td></td>
<td>$^{15}$N tracer</td>
<td>Miyazeki et al., 1973</td>
</tr>
<tr>
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<td>Photic zone ave, October, light</td>
<td>38,000</td>
<td></td>
<td>$^{15}$N tracer</td>
<td>Miyazeki et al., 1973</td>
</tr>
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<td>Sagami Bay, Japan</td>
<td>Photic zone ave, October, dark</td>
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<td></td>
<td>$^{15}$N tracer</td>
<td>Miyazeki et al., 1973</td>
</tr>
<tr>
<td>Mangrove forest, Arabian Sea coast (&lt;3 m depth)</td>
<td>Surface, seasonal study</td>
<td>2.4–2321</td>
<td></td>
<td>$^{15}$N tracer</td>
<td>Dham et al., 2002</td>
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<td>Western North Pacific</td>
<td>10–20 m</td>
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<td>Miyazeki et al., 1975</td>
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<tr>
<td>Western North Pacific</td>
<td>100–125 m</td>
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<td>Subsurface max (approx 70 m)</td>
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<td>Olson, 1981a</td>
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<td>30–60 m</td>
<td>20–30</td>
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<td>$^{15}$N tracer</td>
<td>Ward et al., 1984</td>
</tr>
<tr>
<td>Location</td>
<td>Depth (m)</td>
<td>Nitrification (mm/year)</td>
<td>Subsurface/Maximum Depth (m)</td>
<td>Tracer</td>
<td>Reference</td>
</tr>
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<td>-------------------------------------------</td>
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<td>Ward, 1987b</td>
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<td>NO$_2^-$ Ox Rate (nmol l$^{-1}$ day$^{-1}$)</td>
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<td>$^{15}$N tracer</td>
<td>Clark et al., 2008</td>
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<td>Surface plume summer</td>
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<td>Bianchi et al., 1999</td>
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<td>0 m</td>
<td>24</td>
<td>14.4–36</td>
<td>Inhibitor</td>
<td>Bianchi et al., 1997</td>
</tr>
<tr>
<td>Southern Ocean</td>
<td>50 m</td>
<td>24–43.2</td>
<td>28.8–48</td>
<td>Inhibitor</td>
<td>Bianchi et al., 1997</td>
</tr>
<tr>
<td>Southern Ocean</td>
<td>100 m</td>
<td>24–84</td>
<td>9.6–72</td>
<td>Inhibitor</td>
<td>Bianchi et al., 1997</td>
</tr>
<tr>
<td>Gulf of Mexico</td>
<td>70 m</td>
<td>Up to 91</td>
<td></td>
<td>Δ [NO$_3^-$], drogue</td>
<td>French et al., 1983</td>
</tr>
<tr>
<td>Hydrothermal Plume</td>
<td>1750–2200 m</td>
<td></td>
<td></td>
<td>Inhibitor</td>
<td>Lam et al., 2004</td>
</tr>
<tr>
<td>Marine Sediments</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Coral Reef Sediments,</td>
<td></td>
<td>Areal or Mass Rate</td>
<td>Areal or Mass Rate</td>
<td></td>
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</tr>
<tr>
<td>Australia</td>
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</tr>
<tr>
<td>Location</td>
<td>Environment Type</td>
<td>Flux Type</td>
<td>Flux Units</td>
<td>Authors (Year)</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>---------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Coral Reef, Enewetak Atoll</td>
<td>Surface, integrated</td>
<td>DIN flux, N-serve inhibition</td>
<td>85–1008 mmol N m⁻² day⁻¹</td>
<td>Webb and Weibe, 1975</td>
<td></td>
</tr>
<tr>
<td>Sponges, Caribbean Sea</td>
<td>Surface, integrated</td>
<td>DIN flux</td>
<td>Up to 413 mmol N m⁻² day⁻¹</td>
<td>Diaz et al., 1997</td>
<td></td>
</tr>
<tr>
<td>Sponges, Caribbean Sea</td>
<td>Surface, per g</td>
<td>DIN flux</td>
<td>Up to 620 mmol N g⁻¹ wwt h⁻¹</td>
<td>Corredor et al., 1988</td>
<td></td>
</tr>
<tr>
<td>Sponges, Caribbean Sea</td>
<td>Chondrilla nucula</td>
<td>DIN flux</td>
<td>11.52 mmol N m⁻² day⁻¹</td>
<td>Corredor et al., 1988</td>
<td></td>
</tr>
<tr>
<td>Estuarine sediments, sandy</td>
<td>Surface, per g</td>
<td>DIN flux, difluoromethane inhibition</td>
<td>Up to 40 nmol N g⁻¹ h⁻¹</td>
<td>Magalhaes et al., 2005</td>
<td></td>
</tr>
<tr>
<td>Estuarine sediment, rocky biofilm</td>
<td>Surface, per g</td>
<td>DIN flux, difluoromethane inhibition</td>
<td>up to 190 mmol N g⁻¹ h⁻¹</td>
<td>Magalhaes et al., 2005</td>
<td></td>
</tr>
<tr>
<td>Marine Loch cores 0–2 cm</td>
<td>Surface, per g</td>
<td>¹⁵N tracer</td>
<td>1600 µmol l⁻¹ day⁻¹</td>
<td>Mortimer et al., 2004</td>
<td></td>
</tr>
<tr>
<td>Marine Loch cores 6–8 cm</td>
<td>Surface, per g</td>
<td>¹⁵N tracer</td>
<td>83.8 µmol l⁻¹ day⁻¹</td>
<td>Mortimer et al., 2004</td>
<td></td>
</tr>
<tr>
<td>Marine Loch cores Anoxic layers</td>
<td>Surface, per g</td>
<td>¹⁵N tracer</td>
<td>0.005–1.55 µmol l⁻¹ day⁻¹</td>
<td>Mortimer et al., 2004</td>
<td></td>
</tr>
<tr>
<td>Muddy sediment</td>
<td>Surface, integrated</td>
<td>DIN flux</td>
<td>120–432 mmol N m⁻² day⁻¹</td>
<td>Gilbert et al., 2003</td>
<td></td>
</tr>
<tr>
<td>Mid-Atlantic Bight continental shelf</td>
<td>Surface, integrated</td>
<td>N₂ and NO₃⁻ flux</td>
<td>0.64–2.82 mmol N m⁻² day⁻¹</td>
<td>Laursen and Seitzinger, 2002</td>
<td></td>
</tr>
<tr>
<td>Svalbard shelf</td>
<td>Surface, integrated</td>
<td>DIN flux</td>
<td>16–112 mmol N g⁻¹ wwt h⁻¹</td>
<td>Thamdrup and Fleischer, 1998</td>
<td></td>
</tr>
</tbody>
</table>
hydrothermal vent plumes, where NH$_4^+$ concentrations are elevated to hundreds of nM (Lilley et al., 1993) and oxidation rates (up to 91 nM day$^{-1}$) on the same order as those detected in surface water have been reported (Lam et al., 2004).

Based partly on anecdotal evidence from culture work, observations in waste water treatment systems with very high particulate loads, the tendency of nitrifiers to grow in aggregates in bioreactor biofilms, and the prevalence of small particles in natural waters, it has been suggested that nitrification occurs mainly on particles and is mediated by particle-attached bacteria (Hagopian and Riley, 1998). Nitrifier sequences were found both associated with particles and in the bulk seawater phase in the northwestern Mediterranean Sea. In the clone library of 16S rRNA sequences, *Nitrosomonas*-like sequences were preferentially associated with particles and *Nitrosospira*-like sequences dominated in clones from the planktonic phase (Phillips et al., 1999). This may indicate niche preference by the different groups on the basis of attachment to particles, substrate concentration or other physical/

**Figure 5.3** Depth distribution of ammonia oxidation rate from four stations in the Eastern Tropical North Pacific. Data obtained from ±N-NH$_4$ tracer incubations at simulated in situ light intensities. (From Ward and Zafiriou 1988)
chemical parameters of particle surfaces (Prosser and Embley, 2002). Karl et al. (1984) reported high numbers of AOB associated with particles caught in sediment traps and attributed high rates of chemoautotrophy to the particle-associated assemblages. The relative contribution to nitrification rates by particle-associated vs. truly planktonic nitrifying bacteria or those associated with smaller suspended particles is difficult to assess. The distribution of rates as a function of depth indicates that rapidly sinking, i.e., large, particles cannot be the main site of the process (see above, geochemical constraints); nitrification associated with suspended small particles could be consistent with the typical subsurface rate maximum and depth profile that is characteristic of oceanic waters. Nitrifiers appear to escape predation by protozoans by aggregating into particles that are too large to be grazed (Lavrentyev et al., 1997). Thus a trophic effect on nitrification is implied, which also has ramifications for the planktonic vs. particle-associated question.

3.3.2. Nitrification in sediments
The magnitude of nitrification rates in sediments can be much higher, and is certainly more variable, than that reported from water column measurements (Table 5.2). The variability arises not only from the small scale heterogeneity inherent in sediments (partly due also to bioturbation and association of nitrification with the walls of faunal tubes in the sediments), but from the wide range in the level of organic matter input to sediments in shallow water. Very high NO\textsubscript{2}\textsuperscript{−} and NO\textsubscript{3}\textsuperscript{−} fluxes to the water column from sediments, including coral reefs (Capone et al., 1990; Webb and Weibe, 1975) and sponge dominated reef surfaces (Diaz and Ward, 1997), have been attributed to nitrification associated with invertebrates and biotic sediments. Welsh and Castadelli (2004) showed that nitrification activity was associated with the tissues, as well as the external shell surfaces, of filter feeding bivalves. They suggested that nitrifying bacteria may colonize invertebrates and establish specific biological relationships with them (Welsh and Castadelli, 2004), as was suggested by the high nitrification rates observed in sponges (Diaz and Ward, 1997) and the detection of AOB sequences in sponge tissues. High rates have also been reported from the shallow waters of sediment dominated systems such as mangrove forests (Dham et al., 2002) Nitrification in the shallow water column of the mangrove forest was highly seasonal and strongly coupled with NH\textsubscript{4}\textsuperscript{+} regeneration and NO\textsubscript{2}\textsuperscript{−} assimilation rates (Dham et al., 2002).

In contrast, nitrification rates were very low in the sediments of a coastal lagoon where uptake of DIN by seagrass dominated the N cycle (Rysgaard et al., 1996).

Irrigation by worm tubes and sediment infauna has been shown to increase nitrification rates by increasing oxygen availability (Pelegri et al., 1994; Pelegri and Blackburn, 1996; Svensson, 1998). The presence of worm tubes, which are actively irrigated by the invertebrate inhabitants, introduces small scale spatial variability into the oxygen distribution in sediments. Nielsen et al. (2004) used microelectrodes to measure small scale distributions of oxygen and NO\textsubscript{3}\textsuperscript{−} around individual worm tubes and found that nitrification and NO\textsubscript{3}\textsuperscript{−} reduction (denitrification plus NO\textsubscript{3}\textsuperscript{−} reduction to NH\textsubscript{4}\textsuperscript{+}) were spatially separated: nitrification was restricted to the oxic zone (up to 1.5 mm) around the tubes, while NO\textsubscript{3}\textsuperscript{−} reduction occurred in the adjacent anoxic zone. Both processes were stimulated by the addition of NH\textsubscript{4}\textsuperscript{+} indicating that NO\textsubscript{3}\textsuperscript{−} produced by nitrification limited the rate of denitrification. Webb and Eyre
(2004) found that the presence of burrowing shrimp (*Trypaea australiensis*) increased denitrification rates by fivefold compared to unoccupied sediments, while oxygen consumption increased by 81%. Most of the increased oxygen demand was attributed to microbial respiration and oxidation processes (rather than to respiration by the shrimp itself), implying that the increased denitrification was coupled to increased nitrification resulting from increased organic matter decomposition (Webb and Eyre, 2004). Gilbert *et al.* (2003) investigated the relationship between coupled nitrification/denitrification rates and the density of macrofaunal burrow spacing using simulated burrows and reaction–diffusion models. Nitrification rates were maximal at an intermediate tube density, and denitrification varied in direct proportion to nitrification, indicating the importance of the coupling of the two processes through *in situ* NO$_3^-$ production. The main controls on nitrification rate were the supply of oxygen and NH$_4^+$, and competition for oxygen by other reductants. Dollhopf *et al.* (2005) found that coupled potential rates of nitrification and denitrification in salt marsh sediments were positively correlated with each other and with macrofaunal burrow abundance and attributed this effect to enhanced oxygen availability due to bioirrigation. Nitrification potential, measured using chlorate inhibition, was positively correlated with the occurrence of macrofaunal structures in estuarine sediments (Mayer *et al.*, 1995). Local sediment conditions are likely to be important in determining the degree of coupling and net rates of nitrification and denitrification. In a bay in southern Australia, denitrification rates were not related to bioirrigation and it was suggested that high irrigation rates removed NH$_4^+$ that was regenerated in anoxic regions of the sediments too fast for it to be nitrified and recycled (Berelson *et al.*, 1998). Any variability among nitrifier community composition between aerated and non-aerated sites would introduce complexity into the community response, perhaps shifting the optimum to slightly different length and packing scales, but probably not changing the nature of the relationships and the coupling between nitrification and denitrification.

Nitrifiers living in marine sediments that are periodically or persistently exposed to anoxic conditions would need to be able to survive periods of inactivity or even serious inhibition in order to recover when conditions improved. The physiological basis of this survival or tolerance of anoxic conditions is unknown, and the degree to which nitrifiers can recover from serious anoxia is questionable. In microcosm studies with estuarine sediments, nitrification was almost completely inhibited by sulfide, the end product of bacterial sulfate reduction that occurs widely in anoxic marine sediments (Joye and Hollibaugh, 1995). A pulse of sulfide, which was detectable in the sediments for only a few hours, inhibited nitrification for at least 24 h. Thus in marine sediments where sulfate reduction occurs, the ability of nitrifiers to respond to daily oxygen fluctuations may be impeded. Even when the oxygen–sulfide interface deepens during the day, nitrifiers may be unable to recover from the sulfide poisoning. In that case, both nitrification and denitrification (which is partially dependent upon NO$_3^-$ supply from nitrification) may occur at slower rates than would be predicted for similar environments in freshwater sediments (where sulfate, and therefore sulfide release from sulfate reduction, is much less prevalent). In contrast, Jensen *et al.* (1993) reported that nitrification could be stimulated essentially instantaneously by addition of oxygen to sulfidic freshwater sediments. Using microelectrodes to measure oxygen and NO$_3^-$...
profiles in a sediment core, Jensen et al. (1993) detected an almost immediate increase in nitrification rate when anaerobic sediments were aerated, implying that nitrifiers are inhibited but not poisoned by sulfide. When sediments were allowed to equilibrate with overlying water of differing oxygen concentrations, the zones of nitrification and denitrification stabilized at greater depths with increasing oxygen concentration, reflecting oxygen penetration into the core (Fig. 6, Jensen et al., 1994). This difference may reflect the different community composition of freshwater and marine nitrifying bacteria, or it may be related somehow to salinity and its effect on nitrifiers.

In both deep and shallow sediments, nitrification can be one of the main sinks for oxygen (Blackburn and Blackburn, 1993; Grundmanis and Murray, 1977). Nitrification accounted for up to 50% of the oxygen demand in the Mississippi River plume at intermediate salinities (Pakulska et al., 1995) but was much less important in the nearby Atchafalaya River, which is dominated by heterotrophic processes (Pakulska et al., 2000). In continental shelf sediments, nitrification and denitrification are often closely linked. Coupled nitrification/denitrification is invoked to explain the observation that the rate of N2 flux out of sediments can greatly exceed the diffusive flux of NO3− into the sediments (Devol and Christensen, 1993). Ammonium, produced during aerobic and anaerobic organic matter mineralization, is oxidized to NO3− and subsequently reduced to N2. Anaerobic oxidation of the regenerated NH4+ could also be a factor in the otherwise lack balance between NO3− supply and consumption. Nitrification can supply up to 100% of the NO3− consumed by denitrification (Laursen and Seitzinger, 2002; Lehmann et al., 2004). In deep sea sediments, denitrification may not be detected in the upper 30–50 cm, and NO3− accumulates in near Redfield stoichiometry due to nitrification of regenerated NH4+ during organic matter diagenesis (Grundmanis and Murray, 1982).

Although oxygen and NH4+ conditions likely differ between planktonic and sediment environments, there is no clear evidence from clone libraries that water column and sediment nitrifying communities are significantly different in composition and regulation. The most extensive comparisons are possible on the basis of amoA clone libraries from Chesapeake Bay sediments (Francis et al., 2003) and the Monterey Bay water column (O’Mullan and Ward, 2005). Although unique sequences were found in both environments, it was more common to find the sequences from different environments clustering together, and with representatives from both planktonic and sediment sequences from other studies. Thus on the basis of functional genes alone, it is not possible to distinguish major sediment vs planktonic strains. 16S rRNA sequences that were 96–98% identical with known betaproteobacterial nitrifying isolates were obtained in clone libraries from anoxic sediments in a marine Loch (Freitag and Prosser, 2003). The sedimentation rate at this site implied that the sediments from which the sequences originated had been buried for about 100 years, implying the long term viability of nitrifying bacteria in anoxic environments. The same authors did not detect gammaproteobacterial or anammox type planctomycetes-like sequences, and concluded that some groups of betaproteobacterial AOB might be capable of growing and perhaps nitrifying under anoxic conditions. Mortimer et al. (2002, 2004)
detected a sharp peak of NO$_3^-$ in pore water profiles from the sediment environment, and were able to detect low NH$_3$ oxidation rates at the depth (20 cm) of the NO$_3^-$ peak, and also documented the presence of both *Nitrosospira*– and *Nitrosomonas*–like 16S rRNA sequences in the sediments. It was concluded that the buried NO$_3^-$ peak was due to slumping of the coastal sediments and represented a non steady state feature, rather than a long term result of anoxic NH$_3$ oxidation. The ability of AOB to persist for long periods in unfavorable conditions is well known, but the continued activity of conventional aerobic AOB in anoxic sediments is doubtful.

### 4. Environmental Variables Affecting Nitrification Rates and Distributions

It is clear from the discussion of the depth distribution of nitrification rates that variables such as light intensity and substrate concentration are important determinants of the magnitude and location of nitrification in the water column, as they are for much of the biogeochemical cycling in the environment. Their effects have been studied in both laboratory culture experiments and in field samples, using incubations and measurements of natural assemblages. Culture studies have so far been restricted to bacterial nitrifiers, so regulation inferred from studies of AOB may not apply to archaeal ammonia oxidizers. On the other hand, rate measurements from environmental samples reflect the ecological properties of the natural assemblages, regardless of their phylogenetic composition. Thus environmental effects inferred from field samples may provide insight into the physiology of uncultivated organisms involved in the processes.

#### 4.1. Temperature

The effect of temperature is of potential importance in wastewater systems where nitrifying bacteria are cultured under artificial conditions, or in terrestrial environments or shallow marine systems where temperature changes on short time scales. Temperature was a significant regulator of nitrification rates in the shallow water column of mangrove forests (Dham *et al*., 2002), although oxygen and NH$_4^+$ concentrations were more important. Different temperature optima are reported for strains isolated from different kinds of terrestrial environments (Golovacheva, 1976; Jiang and Bakken, 1999). A marine betaproteobacterial AOB species with a temperature tolerance between $-5^\circ$C and nearly $29^\circ$C was isolated from Alaskan shelf sediments (Jones *et al*., 1988). In the oceanic environment, however, temperature is not generally considered to be an important variable for nitrification because bacterial populations are generally adapted to the temperature of their environments. Thus, one can demonstrate a classical dependence of the rate of nitrification on temperature in any particular environment, but temperature is not generally the limiting factor *in situ*. Nitrifiers adapted to low temperature can nitrify under low temperature conditions at rates comparable to the rates attained by nitrifiers adapted to higher
temperatures living under high temperature conditions. Nitrification rate was highly adapted to temperature in arctic sediments, with temperature optima ranging from 14 to 40°C, depending on the temperature of the environment (Thamdrup and Fleischer, 1998). While temperature is an important master variable for biological processes, nitrification is if anything less sensitive to regulation by temperature than other processes, and is usually regulated in the environment by some other variable.

4.2. Salinity

Because salinity in the open ocean does not vary on scales that are important to physiological response in bacteria, sensitivity to salinity changes is not an important variable in determining nitrification rates or nitrifying bacterial community composition in the ocean. It appears, however, that salinity is a very important, perhaps dominant, variable in regulating nitrification in riverine and estuarine environments. Highest nitrification rates were associated with low to moderate salinities in the Mississippi (7 psu) and Atchafalaya (8 psu) River plumes (Pakulski et al., 2000), but turbidity and competition with phytoplankton and other bacteria also played a role in determining the distribution of nitrification. An earlier study in the Mississippi River plume had detected highest nitrification rates at higher salinities (27 psu) (Pakulski et al., 1995).

Using the N-serve inhibition method with $^{14}\text{CO}_2$ uptake to estimate nitrification, Somville (1984) showed that salinity was an important variable in determining nitrification rates in the Schelde estuary in The Netherlands. She concluded that nitrifier populations were adapted to their in situ salinities and suggested that community composition – and salt tolerance – varied along the estuarine gradient. Large shifts in the composition of 16S rRNA clone libraries representing AOB communities were subsequently found along the salinity gradient from 0 to 15 psu in the Schelde estuary (Bollman and Laanbroek, 2002; de Bie et al., 2001). The Schelde libraries were dominated by Nitrosomonas-like sequences, but two different groups switched dominance in the clone libraries between about 3 and 5 psu. Nitrosospira-like sequences were detected only rarely and mostly at the highest salinity station. Sequences representing both the uncultivated marine Nitrosospira clade and the marine Nitrosomonas clade were found in clone libraries from Chesapeake Bay sediments (Francis et al., 2003). The level of amoA sequence diversity within samples from Chesapeake Bay was compared to the pairwise differences in environmental parameters (e.g., salinity, oxygen, temperature, NO$_3^-$ and NH$_4^+$ concentrations) between stations. Difference in salinity was the dominant variable and accounted for 62% of the variation in pairwise sequence dissimilarity between stations. Ammonia concentration, the next most important variable, explained only an additional 2.7%. The greatest diversity in AOB clone libraries occurred in the freshwater end member stations, and these libraries were also most different in composition from all the other samples, indicating that different organisms perform the nitrification function in different parts of the bay, depending on salinity. Members of the uncultivated Nitrosospira clade dominated the clone libraries from higher salinity stations. In a study that included three estuarine stations with salinities ranging from 8.7 to 31.7, different and temporally stable
AOB communities were detected by terminal restriction fragment analysis of amoA genes (Bernhard et al., 2005). Nitrosospira-like sequences were also identified as the seawater dominants in this environment.

Using sediments collected from the middle of the estuarine salinity gradient in the Douro estuary (Portugal), manipulation of salinity in the overlying water was used to test the effect of salinity on nitrification and denitrification (Magalhaes et al., 2005). Denitrification rates did not vary in response to salinities of 0 and 35 psu, but nitrification was optimal at 15 psu. This may indicate the presence of a halotolerant denitrifier assemblage, while the nitrifier assemblage was adapted to its in situ salinity and was inhibited by both lower and higher salinity. In the Rhone river estuary, highest nitrification rates were found in the surface low salinity layer, but both NH$_3$ and NO$_2^-$ oxidation rates were correlated primarily with NH$_4^+$ concentration, and influenced by temperature, rather than salinity directly (Bianchi et al., 1999).

Rysgaard et al. (1999) tested the effect of salinity on nitrification in a Danish estuary to determine whether the increased desorption of NH$_4^+$ from sediments was responsible for the decreasing nitrification rates at high salinity. They concluded that salinity influenced nitrification rates independently of NH$_4^+$ concentrations and suggested that some physiological factor must be involved.

Many authors have described the salinity tolerances of nitrifying bacterial cultures (Macfarlane and Herbert, 1984; Stehr et al., 1995) and it is clear that halotolerance is not the rule among AOB. Seven strains of Nitrosomonas isolated from different regions of the Elbe showed different ranges of salinity tolerance (Stehr et al., 1995). Nitrosococcus halophilus, one of two known species in the gammaproteobacterial AOB, has a higher salinity optimum and upper limit than does N. oceani, the strain detected widely in the open ocean (Koops et al., 1990). The salt requirements of cultivated AOB are summarized by Koops et al. (2003). Salinity may restrict the distribution of nitrifying strains and help determine nitrifier community composition, but as with temperature, appropriately adapted communities minimize the variability in nitrification rates as a function of salinity. The apparent dominance of AOA over AOB abundance in seawater is consistent with strong selection by salinity in favor of AOA. AOA have also been detected in terrestrial (Leininger et al., 2006) and wastewater (Park et al., 2006) environments, however, and salinity as a variable in the overall ecology of AOA remains to be investigated.

### 4.3. Inhibitory compounds

Both NH$_3$ oxidizers and NO$_2^-$ oxidizers, but especially the former, are susceptible to inhibition by a wide range of compounds, and several different modes of action have been documented (Bedard and Knowles, 1989). The two most common modes of inhibition are: (1) interference with the active site of the primary enzyme (i.e., NH$_3$ monooxygenase in AOB; NO$_2^-$ oxidoreductase in NOB) by compounds that share structural homology with NH$_3$ or NO$_2^-$ and (2) metal binding compounds, which interfere with the action or availability of copper in the NH$_3$-oxidizing enzymes. In both NH$_3$- and NO$_2^-$-oxidizers, the susceptibility to inhibitors by key enzymes forms the basis of some methods used to measure the rate of nitrification in the environment (see above).
In terrestrial systems, the presence of certain organic compounds (e.g., monoterpenes produced by plants) has been proposed to limit the rate of nitrification and the inhibition of nitrification in acid soils has long been of concern. The potential of naturally occurring organic compounds to inhibit nitrification in seawater has not been considered very much. Inhibition by organosulfur compounds has been demonstrated in cultured marine NH$_3$ oxidizers (Ly et al., 1993) and the inhibitory effect of sulfide on nitrification is thought to limit nitrification and coupled nitrification/denitrification in marine sediments (see below; Joye and Hollibaugh, 1995). While naturally occurring organic compounds have not been investigated as potential inhibitors in seawater, the product of their photodecomposition, carbon monoxide, has been implicated (Jones and Morita, 1984). While CO, like methane, acts as a substrate analog or a suicide inhibitor for NH$_3$ oxidizers, the direct inhibitory effect of light on nitrifiers (see below) is considered to outweigh the potential effect of CO inhibition in surface waters. As a substrate analog, CO can be oxidized by NH$_3$-oxidizing bacteria, and Jones (1992) proposed to exploit this capability as a method for quantification of NH$_3$ oxidation rates in natural systems. Like methane oxidation (Ward, 1987b), however, the rate of CO oxidation by nitrifiers depends on the relative concentration of alternative substrates, which may cause artifacts in relating overall rates of CO oxidation to nitrification. The susceptibility of AOA to the known suite of nitrification inhibitors remains to be investigated.

4.4. Light

The inhibitory effect of light was reported by German researchers in the 1960s (Muller-Neugluck and Engel, 1961; Muller-Nugluck and Engle, 1961; Schon and Engel, 1962), verified in enrichment cultures in seawater (Horrigan et al., 1981) and later described in more detail (e.g., Vanzella et al., 1989). Even in enrichment cultures of nitrifiers derived from the sea surface film, nitrification was severely inhibited by light, such that periods of more than 12 h darkness were necessary to allow net nitrification to occur over a 24 h period (Horrigan et al., 1981). Vanzella et al. (1989) found evidence that NO$_2^-$ oxidizers were more sensitive to sunlight than were NH$_3$ oxidizers, based on single culture studies, but Guerrero and Jones (1996a) showed that species specific responses may obscure any generalizations among major groups. Light at near UV wavelengths of 300–375 nm, as well as in the visible range (<500 nm), was inhibitory for the cultures tested, and sunlight caused almost complete inhibition within 2 hr of the AOB tested, and 67% and 17% inhibition for the two NOB tested (Guerrero and Jones, 1996a). Many biological molecules such as DNA and proteins, quinones, flavins, NADH and other molecules involved in electron transport activity absorb primarily in the near UV wavelengths. Bock (1965) showed that light caused the oxidation of cytochrome c in both AOB and NOB, and suggested that photoinhibition resulted from damage to the electron transport system. The specific phototargets involved in light inhibition have not been thoroughly investigated, but photosensitivity is consistent with the potential for photodamage of these essential molecules in microbes living in near surface waters. Horrigan and Springer (1990) reported that oceanic strains of NH$_3$ oxidizers were generally more sensitive to light inhibition than were estuarine strains. Degree
of inhibition was inversely correlated with NH₃ oxidation rate, but the mechanism of this relationship could not be determined. Horrigan and Springer (1990) argued that the greater sensitivity of oceanic NH₃ oxidizers might be responsible for the generally lower rates of NH₃ oxidation observed in oceanic vs. river or estuarine environments, but the complex of other factors such as overall rates of material processing could not be independently evaluated.

There is abundant evidence from culture studies that both AOB and NOB are photosensitive. It is a high priority to investigate the photosensitivity of AOA. Even if all nitrifiers exhibit photoinhibition in some form, however, the direct and indirect ecological implications of this physiology for the rates and distributions of nitrification in the environment are not easily predicted. Dissolved organic matter in seawater, as well as turbidity due to sediments or phytoplankton, might all provide photoprotection in surface waters, and regulation of nitrification by other factors discussed in this section may be much more important in many environments.

Ammonia and NO₂⁻ oxidation rates are often reported to be highly coupled and to occur at about the same rate. This coupling would be consistent with the observation that neither NH₄⁺ nor NO₂⁻ accumulates to high levels in most natural waters. For NO₂⁻, the exceptions to this rule are the primary NO₂⁻ maximum of near surface waters and the secondary NO₂⁻ maximum characteristic of oxygen minimum zones. The surface water feature has been attributed to two main processes. Because the primary NO₂⁻ maximum is usually associated with low light intensities at the bottom of the euphotic zone, some interaction between biological processes and light is suspected. In one scenario, phytoplankton are responsible: assimilation of NO₃⁻ (via reduction to NO₂⁻ and then to NH₄⁺) requires energy, especially the step at which NO₂⁻ is reduced to NH₄⁺. Under low light intensity, phytoplankton might not have enough energy to reduce the NO₃⁻ completely, and some of the intermediate NO₂⁻ is allowed to leak out of the cell. In the alternative scenario (Olson, 1981b), nitrifying bacteria are responsible: on the basis of information then available (Bock, 1965; Muller-Neugluck and Engel, 1961; Schon and Engel, 1962), Olson hypothesized that NO₂⁻ oxidizers are more sensitive to light inhibition than are NH₃ oxidizers. Therefore, NH₃ oxidizers are active at slightly shallower depths in the water column than are NO₂⁻ oxidizers. This leads to an accumulation of NO₂⁻ in the interval between the depths at which NH₃- and NO₂⁻-oxidizers are released from light inhibition. Guerrero and Jones (1996a) had reported that AOB were more sensitive to light inhibition than were NOB, but found that NOB required longer recovery times than AOB (Guerrero and Jones, 1996b). Thus differential recovery time, rather than differential light inhibition, could explain the formation of the primary NO₂⁻ maximum by nitrification.

Several studies of nitrification rates in surface seawaters from various geographical regions show profiles that are consistent with light inhibition of both NH₃ and NO₂⁻ oxidation (Lipschultz et al., 1990; Ward and Carlucci, 1985; Ward and Kilpatrick, 1991; Ward, 1987a; Ward et al., 1984). Simulated in situ rate measurements (i.e., measured under realistic light conditions) show a clear negative relationship with ambient light intensity (Ward et al., 1984). Nitrifiers may be somewhat protected from light inhibition in surface waters by the presence of absorbant organic compounds in seawater and by mixing, which prevents long intense
exposure at the surface. However, the data from simulated in situ rate measurements are consistent with light inhibition in surface waters, and the well verified sensitivity of nitrifiers in culture strongly suggests an influence of light in the environment. In a two year study of NH$_4^+$ oxidation in the surface layer the highest rates were usually associated with lowest light intensity, but low rates occurred at all intensities (Fig. 5.4), implying a role for additional factors. Dore and Karl (1996) did not directly assess the effect of light on nitrification rates (all rates were measured in the dark) but they attributed two distinct features within the primary NO$_2^-$ maximum to phytoplankton (the upper primary NO$_2^-$ maximum) and nitrifiers (the lower primary NO$_2^-$ maximum). After a comprehensive review of previous models for the formation of the primary NO$_2^-$ maximum, and the distributions of chlorophyll, NO$_2^-$ and NO$_3^-$ in the Sargasso Sea, Lomas and Lipschultz (2006) concluded that phytoplankton NO$_2^-$ release was the dominant factor in creating and maintaining the primary NO$_2^-$ maximum, with a secondary role in some seasons for nitrification.

Lipschultz et al. (1985) documented the light inhibition of NH$_3$ oxidation in the Delaware River and concluded that this effect influenced the spatial distribution of nitrification in the estuary. Depending on their depth, light is not usually a problem for nitrification in sediments. In shallow sediments, light may have an indirect positive effect on nitrification rates by increasing photosynthesis, and thus increasing oxygen supply to the sediments (Lorenzen et al., 1998).

The fact that nitrification rates are often maximal in the vicinity of the bottom of the euphotic zone has important implications for our understanding of N supply for primary production. The important distinction made in the new production paradigm (see above), between NH$_4^+$, which is the “regenerated” nutrient and NO$_3^-$, the “new” nutrient, is the basis of $^{15}$N-tracer methods to assess new and regenerated primary production. However, if nitrification occurs in the same depth interval where nitrate assimilation occurs, then nitrate too is a “regenerated” nutrient.

![Figure 5.4](image-url) Relationship between ammonia oxidation rate and simulated in situ incubation light intensity. Data are shown for approximately 220 $^{15}$N-NH$_4$ tracer incubations performed over the course of 2 years in Monterey Bay, CA. (From Ward, 2005)
Then, total NO$_3^-$ assimilation would depend on a combination of NO$_3^-$ supplied by nitrification, plus that supplied by mixing from the deep reservoir. Several studies have addressed the question of whether significant nitrification occurs in the euphotic zone, and have concluded that in situ nitrification could supply 100% or more of the phytoplankton NO$_3^-$ demand (Bianchi et al., 1997; Dore and Karl, 1996; Ward et al., 1989b). A large role for in situ nitrification to supply the phytoplankton NO$_3^-$ demand is also supported by biogeochemical models (Mongin et al., 2003). Estimated vertical fluxes of NO$_3^-$ via eddy pumping were also not sufficient to support the observed primary production in the Sargasso Sea (Martin and Pondhaven, 2003). Thus nitrification and NO$_3^-$ assimilation by phytoplankton can be closely coupled, even though the two processes are favored by quite different environmental conditions. Nitrate must be considered a regenerated nutrient in some cases, and estimates of new primary production in the euphotic zone on the assumption that NO$_3^-$ is supplied only as a new nutrient from outside the system is not valid (Yool et al., 2007).

Even in coastal systems where allochthonous nutrient supply can be very large, nitrification and be an important source of NO$_3^-$. Dham et al. (2002) measured very high nitrification rates in the summer in the shallow water of mangrove forests. The rates were highly correlated with both NH$_4^+$ and NO$_3^-$ concentrations and could supply the entire phytoplankton NO$_3^-$ demand in summer.

### 4.5. Substrate concentration

The influence of light may be compounded by the necessity for NH$_3$ oxidizers to utilize NH$_4^+$, which is in short supply in those depth intervals in the water column where light is most intense. When NH$_4^+$ assimilation and NH$_4^+$ oxidation are measured in the same incubation, it is seen that assimilation is more important in the upper portion of the euphotic zone and nitrification in the lower portion (Fig. 5.3 and Ward, 1987a). This pattern suggests that in the well lit upper waters, phytoplankton are able to assimilate NH$_4^+$ but nitrifiers are either unable to compete for NH$_4^+$ in the presence of phytoplankton or else, light inhibition prevents them from utilizing NH$_4^+$ in that environment. In the lower portion of the euphotic zone, phytoplankton may be light limited and less able to assimilate NH$_4^+$, whereas nitrifiers are released from light inhibition and better able to utilize the NH$_4^+$ being released by mineralization or N$_2$ fixation in that interval. There is no direct evidence that nitrifiers are poor competitors with phytoplankton in the lower euphotic zone, but direct tests of this hypothesis in shallow sediments confirm that benthic algae are superior competitors for NH$_4^+$ relative to benthic AOB communities (Risgaard-Petersen et al., 2004b). The analogous situation in the surface ocean is certainly consistent with observed rate and biomass distributions. Incubation experiments under various conditions of light and NH$_4^+$ additions could be used to differentiate the effects of light and substrate competition. It would be especially important to impose true tracer level substrate additions and very sensitive isotope determinations to avoid even minor perturbations caused by additions of substrate and product.

In cultures of nitrifying bacteria, the dependence of nitrification rate on substrate concentration is first order at low concentrations (10–100 µM; these
concentrations are low for culture conditions and some sediments, but very high compared to surface seawater). The instantaneous rate of NH$_4^+$ oxidation or NO$_2^-$ oxidation increases predictably with increasing NH$_4^+$ or NO$_2^-$ concentration until some, often very high, inhibitory level is reached. However, researchers often have been unable to demonstrate consistent substrate dependence for NH$_3$ oxidizers in natural assemblages. A general relationship between measured NH$_4^+$ oxidation rate and ambient NH$_4^+$ concentration supports the importance of substrate concentration. Early studies (Vaccaro, 1962) showed that net NO$_2^-$ and NO$_3^-$ accumulation in samples from the Gulf of Mexico was stimulated by the addition of NH$_4^+$. In $^{15}$N tracer experiments on water samples when substrate additions are made at the micromolar level representative of the environment, NH$_4^+$ oxidation is usually independent of substrate concentration. Lack of response to substrate perturbation (e.g., Olson, 1981a; Ward and Kilpatrick, 1990) has led to speculation that the affinity of NH$_3$-oxidizers for NH$_4^+$ may be so great that current experimental methods cannot detect a response to enhanced substrate concentrations. In contrast to NH$_3$ oxidation, NO$_2^-$ oxidation in natural samples usually shows a more conventional response to added substrate (e.g., Olson, 1981a), but this second step in the nitrification process has received less attention. If AOA dominate the NH$_3$ oxidizing assemblage of seawater, then the substrate affinity arguments above apply to AOA, but their substrate affinity and kinetics in culture have not been studied.

Nitrification in sandy sediments and in biofilms on rocky substrates in a Portuguese estuary was stimulated by increased NH$_4^+$ concentration, up to thresholds greater than 100 μM, above which higher concentrations became inhibitory (Magalhaes et al., 2005). Considering measurements from several marine and estuarine environments, Caffrey et al. (2003) found that potential nitrification rates were positively correlated with ambient NH$_4^+$ concentration when oxygen was available. Very high NH$_4^+$ concentrations were associated with anoxic sediments, however, where no nitrification occurred. Availability of NH$_4^+$ in sediments is expected to be influenced by ionic strength, because NH$_4^+$ ions are displaced from sediment adsorption sites by other cations at high salinity. The idea that the salinity effect on nitrification could be explained by the interaction with NH$_4^+$ availability (Gardner et al., 1991; Seitzinger et al., 1991) does not appear to be the case (see salinity section above).

4.6. Oxygen concentration

Nitrifying bacteria are traditionally considered to be obligate aerobes; they require molecular oxygen for reactions in the N oxidation pathways and for respiration. They are reputed to be microaerophiles, however, who thrive best under relatively low oxygen conditions. Microaerophily may be important in interface environments such as the sediment water interface and in the oxygen minimum zones of the ocean. The role of oxygen in sedimentary nitrification and coupled nitrification/denitrification is discussed above in the section on nitrification in sediments.

While net nitrification and growth at the expense of inorganic N occurs only under aerobic conditions in autotrophic nitrifiers, both NH$_3^-$ and NO$_2^-$-oxidizing
nitrifiers are apparently capable of partial or even complete denitrification. Loss of fixed N has been observed in cultures of nitrifying bacteria growing at reduced oxygen tension. *Nitrosomonas* sp. marine in a lithotrophic medium (no added organics) grew best under an oxygen concentration of 1% in the headspace (compared to 20% in air) (Goreau *et al.*, 1980) and they also produced the greatest amount of nitrous oxide relative to NO$_2^-$ under those conditions. Production of nitrous oxide, nitric oxide and N$_2$ was reported for *Nitrosomonas* growing in the presence of organic compounds in the absence of oxygen (Stuven *et al.*, 1992). *Nitrosomonas* could also grow using hydrogen as an electron donor and NO$_2^-$ as its electron acceptor (Bock *et al.*, 1995). NO$_2^-$ oxidizers can grow via dissimilatory NO$_3^-$ reduction in the presence of organic matter and the absence of oxygen (Freitag *et al.*, 1987).

The potential ecological impact of this physiological versatility in nitrifying bacteria has not been widely investigated in natural systems. The anaerobic capabilities of nitrifiers have received much more attention in connection with sewage and wastewater treatment, where there is economic incentive to enhance the conversion of NH$_4^+$ to N$_2$ under totally anaerobic or totally aerobic conditions. The conditions that are conducive to denitrification by nitrifying bacteria are the same ones that induce denitrification in classical denitrifying bacteria. It is conceivable that both metabolic types are involved in the production and consumption of trace gases in the same environment. Both N$_2$O and NO are involved in important atmospheric processes; they contribute to greenhouse warming and to catalytic destruction of stratospheric ozone. Thus, understanding which processes are responsible for their production could prove to be important for understanding or potentially regulating their fluxes.

A significant positive correlation between apparent oxygen utilization (AOU) and N$_2$O accumulation is often observed in marine systems (Cohen and Gordon, 1978; Nevison *et al.*, 2003; Yoshinari, 1976). The relationship implies that nitrification is responsible for N$_2$O accumulation in oxic waters where it is released as a byproduct of mineralization going to completion via nitrification. The relationship breaks down at very low oxygen concentrations (~6 μM, Nevison *et al.*, 2003), where N$_2$O is usually below atmospheric saturation due to consumption by denitrification. The lack of correlation between N$_2$O accumulation and N-star (an indicator of N cycle stoichiometry; Deutsch *et al.*, 2001) is additional support for the conclusion that most of the N$_2$O in the ocean is derived from nitrification rather than denitrification (Nevison *et al.*, 2003). In a few special places in the open ocean, oxygen concentration is depleted to a level low enough to allow net denitrification to occur in the water column. These regions, referred to as oxygen deficient zones, occur in the eastern tropical South Pacific (off the coast of Peru), in the Arabian Sea and the eastern tropical North Pacific Ocean (off the west coast of Mexico). The coupling between nitrification and denitrification has also been studied in these systems, which are essentially analogous to the sediment environments described above, except that the oxygen and NO$_3^-$ gradients extend over tens to hundreds of meters. Suboxic and anoxic waters and sediments tend to have large fluxes, and sometimes large accumulations, of the gaseous intermediates of nitrification and denitrification. This is probably due to the sensitivity of the various organisms and enzymes involved in their production and consumption to oxygen concentration in the local environment of the microorganism.
In studies of N cycling in oxygen deficient zones, nitrification and denitrification appear to be linked, as might be expected from analogy with sediment systems. Ammonium oxidation typically is maximal near the bottom of the euphotic zone close to the upper boundary of the oxygen minimum zone, but NO$_2^-$ oxidation is detected within the oxygen minimum zone itself (Lipschultz et al., 1990). This finding is consistent with the ability of NO$_2^-$ oxidizers to persist and metabolize at very low or zero oxygen conditions, but their metabolism under these conditions would be expected to be dissimilatory rather than oxidative. The nitrous oxide that typically accumulates in the suboxic (low but not zero oxygen concentrations) regions of the oxygen minimum zones is thought to be due to nitrification (via NO$_2^-$ reduction by AOB under low oxygen conditions), because nitrous oxide is depleted in the core of the oxygen minimum zone where denitrification rates are thought to be greatest. A direct tracer confirmation of this pathway in nature has not yet been accomplished. Stable isotope measurement of nitrous oxide from oxygen depleted waters in the Arabian Sea imply that both nitrification and denitrification may contribute to the signal (Naqvi et al., 1998; Ward and Zafiriou, 1988). Ward and Zafiriou (1988) attributed the nitric oxide production they observed in the oxygen minimum zone of the eastern tropical North Pacific to nitrification and found that it was equivalent to 18% of the total NH$_3$ oxidation rate.

5. NITRIFICATION AND METHANE OXIDATION

As mentioned above in connection with the physiology of nitrifying bacteria, the NH$_3$ oxidizers and the methanotrophs have important biochemical similarities. These similarities extend to the nature of the primary enzyme in the NH$_3$ and methane oxidation pathways, the sensitivity of the enzymes to a wide range of metabolic inhibitors, the metabolic capabilities of the cell and to the ultrastructure of the cell (Bedard and Knowles, 1989). Methanotrophs, like NH$_3$ oxidizers, depend on two substrates with generally opposite sources. Methane, like NH$_4^+$, accumulates in anoxic habitats where it is produced by strictly anaerobic methanogens. Oxygen diffuses into surface waters or surface sediments from the overlying oxygenated habitats. The classical environment for significant contributions by methanotrophs to system-wide carbon cycling is stratified lakes, in which a large fraction of the annual carbon fixation is cycled through methanogenesis and methane oxidation. The methanotrophic activity is highest at the interface where oxygen and methane coincide. As described above for sediments, such an interface would also be an interface for NH$_4^+$ and oxygen and might be expected to harbor high nitrification activity as well.

Owing to the presence of high sulfate concentrations in seawater, methanogenesis is not as important in marine sediments and seawater as it is in freshwater systems. Nevertheless, there are a few marine environments where methane is found. The situation in Scan Bay or Cape Lookout Bight sediments, or in the water column of the Black Sea or the Cariaco Basin, is largely analogous to that in stratified lakes.

The observation from culture studies that methane oxidizers are capable of oxidizing NH$_3$ (Dalton, 1977) and that NH$_3$ oxidizers are capable of oxidizing
methane (Jones and Morita, 1983; Ward, 1987a), has led to uncertainty about which organisms are responsible for observed methane and NH$_4^+$ fluxes in nature. Although both groups of microorganisms show similar regulation by environmental variables and similar sensitivities to a variety of inhibitors (Bedard and Knowles, 1989), the possibility for differential regulation based on substrate affinity or competition suggested that “cross oxidation” might have important implications for the rate of NH$_4^+$ or methane oxidation in nature. Based on a combination of simulated in situ rate measurements, inhibitor studies and kinetic experiments with natural assemblages, it has been concluded largely that methanotrophs are mostly responsible for methane oxidation and nitrifiers for NH$_4^+$ oxidation in both freshwater and marine environments (Ward and Kilpatrick, 1990; Bedard and Knowles, 1997; Carini et al., 2003). These conclusions do not rule out a role for cross oxidation or participation by both groups in some environments, and the two processes remain problematic to separate entirely in the environment.

6. Future Directions

Our understanding of the biogeochemistry of nitrification has advanced greatly in the past two decades. The basic patterns of distributions and rates have been discovered and are largely understood in terms of the characteristics of the organisms believed to be responsible for the process and their interactions with other components of the ocean’s physical and biological systems. Unpredictable surprise discoveries that change our view of the N cycle are likely to appear, as they have done in recent years. Nevertheless, there are some avenues of future discovery that are more easily predictable on the basis of current research.

1. Two recent discoveries are presently causing major reevaluation of our understanding of the N cycle. The anammox process was first described in 1995 (Mulder et al., 1995; van de Graaf et al., 1995) and is reviewed elsewhere in this volume (chapter by Devol). In the time between the first draft of this review and its final revision, the NH$_3$ oxidizing archaea have been cultivated (Konnecke et al., 2005), shown to be both ubiquitous (Francis et al., 2005; Leininger et al., 2006) and abundant (Wuchter et al., 2006) in both aquatic and terrestrial environments. At this point, it is entirely unclear how extensively the discovery of the AOA will change our understanding of nitrification in the ocean. There are several obvious high priority questions that should be addressed immediately:
   i. What fraction of the ubiquitous Crenarchaeota in the ocean are AOA?
   ii. Are the marine AOA predominantly autotrophic or do they have a facultative or mixotrophic metabolism that allows them to utilize alternative energy generation modes?
   iii. What are the pathways of NH$_3$ oxidation in AOA? Are they homologous with those of AOB? Do AOA possess a microaerophilic or anaerobic metabolism similar to the nitrifier denitrification pathway of AOB?
iv. How do the physiological characteristics of the AOA compare with those of the AOB in terms of substrate affinity, light sensitivity, growth rates, oxygen requirements, $\text{N}_2\text{O}$ production, etc.? I.e., are AOA and AOB regulated in the environment by similar factors?

v. What fraction of $\text{NH}_3$ oxidation in the ocean is due to AOA vs. AOB?

It would be very surprising if AOA turn out to have metabolisms and physiological characteristics quite different from those of the AOB, because the knowledge summarized above about nitrification in the environment obviously includes the influence of AOA. That is, conclusions about the distribution and environmental regulation of nitrification rates are derived from samples that contained the natural assemblage of AOA as well as AOB and NOB. What is still missing is the thorough characterization of organismal biochemistry from culture work, or from *in situ* characterizations using molecular methodology such as qPCR to evaluate gene expression and activities in response to environmental variables.

The discovery of AOA does not change the magnitude or distribution of nitrification rates. It may that AOA, rather than AOB, are predominantly responsible for the measured rates, but that does not alter the rates. Thus the *in situ* abundance and activities of AOA, when determined, must be compatible with the biogeochemical constraints discussed above.

Are AOA involved in $\text{N}_2\text{O}$ production? Preliminary information from soil Archaeal metagenomic fragments reveal a *nirK* homolog similar to that found in *Nitrosomonas europaea* (Treusch *et al.*, 2005). The *nirK* of *N. europaea* is quite distinct from that found in conventional denitrifiers and from the closely related marine clades of AOB (Casciotti and Ward, 2001). The protein encoded by this type of *nirK* apparently is not involved in NO production leading to $\text{N}_2\text{O}$ from $\text{NO}_2^-$ (Beaumont *et al.*, 2002), but may play a role in $\text{NO}_2^-$/C0 reduction or some more general electron transport function. The genome of *C. symbiosum* also contains elements of the $\text{NH}_3$ oxidation pathway, but it appears to be incomplete (Hallam *et al.*, 2006). There is no homolog for the second critical enzyme, hydroxylamine oxidoreductase, and again, the *nirK* homolog is not the type usually recognized as involved in $\text{NO}_2^-$/C0 reduction to NO. The pathways involved in $\text{NH}_3$ oxidation and the potential for nitrifier denitrification in AOA are likely to be resolved soon, and they will provide much insight into the role of AOA in marine N cycling.

Why have AOA never been cultivated before? Although AOB were never thought to be abundant in seawater, extrapolation from *in situ* numbers and rates estimated from cultures usually resulted in a rough match such that no huge missing nitrification flux was identified. Such estimates and extrapolations are very poorly constrained, however, because even the most important/abundant AOB in seawater (the marine *Nitrosospira* type) have not been cultivated and therefore extrapolations from culture to environment could be wildly incorrect. Abundance estimates based on FISH indicate that AOA outnumber AOB in the open ocean by as much as 1000-fold (Wuchter *et al.*, 2006). Nevertheless, AOB have been cultivated from locations all over the world, even though they are notoriously difficult to cultivate and fastidious in culture. The evidence that AOA have only once been obtained in culture, and that from a marine aquarium (Konnecke *et al.*, 2005) suggests that AOA
have some fundamentally different metabolic requirements than AOB. The expla-
nation could be the same that applied to *Pelagibacter ubique*, identified in 16S rRNA
clone libraries as SAR11. Although ubiquitous in clone libraries, *P. ubique* was never
cultivated until very low substrate clean culture techniques were applied (Rappe
*et al.*, 2002). *P. ubique* and its close relatives are estimated to contribute up to 50% of
the surface ocean microbial communities, yet it does not grow under standard rich
media conditions. Perhaps AOB are the weeds of the marine nitrifiers and AOA are
the ever present dominant assemblage that is important in normal—low substrate
clean—environmental conditions, the *P. ubique* of NH₃ oxidizers. Alternatively,
perhaps most AOA use NH₃ oxidation only as a background or support metabolism
and therefore do not compete well in the obligately autotrophic conditions usually
employed to enrich for nitrifiers. The only cultivated AOA appears to be obligately
autotrophic, but the uncultivated marine Crenarchaeota assimilate organic com-
 pounds (Ouverney and Fuhrman, 2000), indicating that a more versatile metabolism
may prevail in the AOA that dominate the marine environment.

Are NOA waiting to be discovered? Even less is known about the abundance and
growth characteristics of important marine NOB than the AOB; it is entirely
possible that NOA are also present. On the other hand, NO₂⁻ does not accumulate
in most of the world's ocean, specifically in the deep ocean where the Crenarch-
aeota, suspected to be AOA, constitute up to 40% of the microbial cells. If AOA are
abundant and active, the resident NO₂⁻ oxidizing assemblage is clearly capable of
keeping up with them. On balance, this argues that most of that 40% of cells is not
very active in NH₃ oxidation, or another 40% of the assemblage would be required
to consume the NO₂⁻.

2. Molecular ecological research has arisen as a powerful research tool and has led
to major new ecological insights in recent years. As is the case for every process
that has been investigated, this approach has led to the discovery of immense
diversity at the genetic level among organisms involved in nitrification. The
degree to which this genetic diversity in relation to distribution or regulation
of biogeochemical reaction rates is the focus of much current research. Cultivated
nitrifiers and rates measured in natural samples make it clear that
different strains of nitrifiers have different physiological optima and are thus
likely to inhabit different ecological niches. Although so far untested, this
statement probably applies to AOA as well as AOB and NOB. Does the
variety of physiological types ensure that ecosystem function is maintained in
widely different environmental conditions by the activity of different assem-
blages under different conditions? What is the time scale for change in the
microbial assemblage in response to environmental change? Which environ-
mental changes are most likely to affect nitrification rates? A combination of
biogeochemical and molecular methods can address these questions.

3. Our understanding of the biochemistry of nitrification is based largely on
insights from cultivated strains. Molecular data obtained from clone libraries
indicate that the most important AOB in the ocean are not represented in the
culture collection. The same is probably true for NOB, but much less molec-
ular information is available for these organisms. At this stage, the most
important AOA have not been identified even in clone libraries, but they
certainly are not in culture. Because pure cultures are such a powerful way to
investigate the biochemistry and regulation of microbial biogeochemistry,
every effort should be made to cultivate both AOA and the so far uncultivated
important AOB clades. It is clear that innovative cultivation techniques will be
required, perhaps on the *P. ubique* model.

4. Rising CO$_2$ in the atmosphere or purposeful deep ocean CO$_2$ disposal may
lead to decreases in oceanic pH. pH was not even considered as a factor that
influences nitrification rate in the list of potential factors mentioned earlier,
because ocean pH is reliably invariant over most of the ocean in today’s world.
But changes of 1–2 pH units in response to CO$_2$ increases could inhibit
nitrification rates by up to 90% (Huesemann *et al.*, 2002). Long term inhibition
of nitrification may lead to the accumulation of NH$_4^+$ in the oxic waters,
decreases in NO$_3^-$ available for denitrification, and changes in phytoplankton
community composition as a result of nutrient preferences and competition.

5. Nitrifiers are responsible for the accumulations of N$_2$O that typically occur at
the upper boundaries of OMZ regions. It has been suggested (Fuhrman and
Capone, 1991; Jin and Gruber, 2003; Law and Ling, 2001) that increased
productivity in the surface ocean, leading to increased organic flux to deeper
waters, could increase the total volume of suboxic water, and thence to
enhanced N$_2$O fluxes to the atmosphere. Global warming due to increased
atmospheric inventories of greenhouse gases may cause increasingly stable
thermal stratification, which could in turn lead to further enlargement of the
major oxygen minimum zones of the world ocean. Increased stratification due
to atmospheric warming might lead to increased N$_2$O fluxes to the atmosphere
from this source, thus compounding the greenhouse warming effect of CO$_2$.
These possibilities for changes in N$_2$O dynamics point to the necessity to
understand factors that regulate trace gas metabolism by nitrifiers, as well as
denitrifiers. The most likely avenues of progress in this area are through stable
isotope analyses, including isotopomer approaches (*Popp et al.*, 2002; Sutka
*et al.*, 2004), and through genomics. The former can elucidate the biochemical
pathways responsible for the formation of compounds with multiple potential
sources. Genomics can provide insights into the genetic capabilities, pathways
and regulation of metabolic processes that result in trace gas production
consumption. Genetic data provide the information necessary to develop
specific probes and assays to identify which organisms are involved in particular
transformations in particular times and places.

6. Better rate measurements: Although the currently available methods for direct
assessment of nitrification rates and the geochemical constraints provide inter-
nally consistent estimates of the overall rates and distributions of nitrification,
improvements in direct rate methods are sorely needed to investigate the
effects of environmental variables on rates under realistic conditions. These
improvements will likely come with the application of even greater sensitivity
isotope tracer methods, which require less substrate perturbation for detection
of signal in the product pool. In addition, stable isotope methods that do not
require tracer addition may possess the sensitivity to detect small fractionation
changes in incubated samples. It is particularly important to apply these efforts to the measurement of NO\textsubscript{$\text{2}^{-}$} oxidation rates. Nitrite oxidation rates have only rarely been measured and have suffered from methodological artifacts. Direct rate measurements would be useful in investigating the actual degree of coupling between the two steps in nitrification.

7. Nitrite oxidation and NO\textsubscript{$\text{2}^{-}$} oxidizing bacteria have not received the attention accorded to the first half of the nitrification process. The discovery of AOA raises even more questions about the abundance and activity of NO\textsubscript{$\text{2}^{-}$} oxidizers. With the publication of several NOB genomes (Starkenburg et al., 2006; 2008), new tools are also at hand for the molecular ecological study of NOB to address the parallel questions of diversity and abundance raised by recent findings on the AOB and AOA. Finally, are the NOB responsible for the consumption of NO\textsubscript{$\text{2}^{-}$} generated by AOB and AOA or are NOA awaiting discovery?

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