Ocean Sci. J. (2016) 51(2):159–167
http://dx.doi.org/10.1007/s12601-016-0014-z

Available online at http://link.springer.com
SpringerLink

Review

Ocean Science Journal
pISSN 1738-5261
eISSN 2005-7172

Review of Inorganic Nitrogen Transformations and Effect of Global Climate Change on Inorganic Nitrogen Cycling in Ocean Ecosystems

Haryun Kim

School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 37673, Korea

Received 26 November 2015; Revised 3 March 2016; Accepted 9 May 2016

Abstract – Inorganic N transformations (nitrification, anaerobic ammonium oxidation, denitrification, and dissimilatory nitrate reduction to ammonium) are regulated by various biogeochemical factors linked either by the supply of electron acceptors and donors or by competition for electron acceptors. This review considers both the microbial community related to each process and the technical methods used to measure each process rate. With this background knowledge, this article summarizes how global climate change through increased pCO_2, ocean acidification, deoxygenation and anthropogenic N deposition will alter oceanic N cycling, and finally emphasizes the need for comprehensive research on inorganic N transformation in marine ecosystems.

Key words – nitrification, denitrification, anammox, DNRA

1. Introduction

Nitrogen (N) is an essential nutrient; its availability can limit primary production. However, most N in ocean water is in the form of N_2, so N_2-fixing microbes must convert it to bioavailable N form for phytoplankton, which is finally removed by denitrification. However, recent findings have reported a significant imbalance in the ocean N budget, with higher output than input (Gruber and Galloway 2008). In addition, a newly discovered process called anaerobic ammonium oxidation (anammox; $NH_4^+ \rightarrow N_2H_4 \rightarrow N_2$) that removes ammonium (NH_4^+) and nitrite (NO_2^-) from ocean water has been observed in anoxic conditions. Contrary to denitrification and anammox, dissimilatory nitrate reduction to ammonium (DNRA; $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) can accumulate NH_4^+ under anaerobic conditions, thereby causing eutrophication in coastal ecosystems (An and Gardner 2002) (Fig. 1). Thus, an integrated description of the N cycle requires comprehensive understanding of inorganic N transformations including nitrification, anammox, denitrification and DNRA. This article provides insights into biogeochemical factors that control their rates, analytical methods to measure their functions, and the microbial community structure that performs them.

Climate change will significantly change N cycling, which will affect ocean ecosystems, for example by altering microbial communities or primary production. In detail, ocean acidification due to increasing CO_2 could decrease nitrification rates ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$), thereby possibly reducing the supply of nitrate (NO_3^-) to ocean ecosystems, reducing emission of nitrous oxide (N_2O) into the atmosphere and a changing of microbial community structures. However, the extension of hypoxic zones due to increasing temperature and the input of anthropogenic N could increase denitrification rates and thereby increase N_2O production. Thus, we need a comprehensive understanding of the effect of global climate change on N cycling in ocean ecosystems. This article summarizes how global climate change affects each inorganic N cycle, how they are interlinked, and highlights where we have significant understanding and sparsity of knowledge in ocean ecosystems.

2. Inorganic N Transformations in Ocean Ecosystems

Nitrification

Nitrification is a biological process that uses oxygen as an electron acceptor and CO_2 as a carbon source to oxidize NH_4^+ to nitrite/nitrate (NO_2^- /NO_3^-) under aerobic conditions. Under anaerobic conditions, the NO_2^- and NO_3^- produced by nitrification

*Corresponding author. E-mail: kharyun@postech.ac.kr

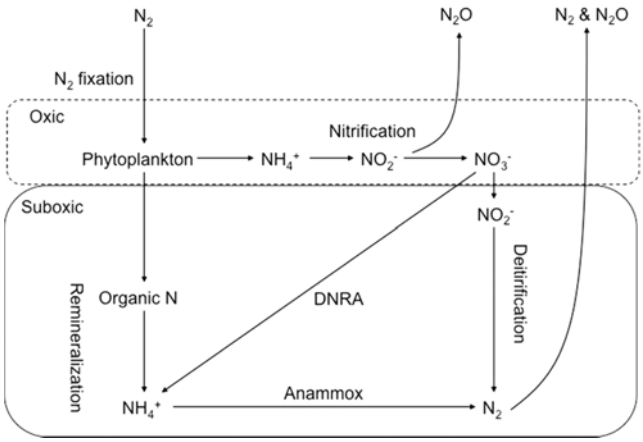

Fig. 1. Inorganic nitrogen transformations in ocean water

are used as electron acceptors in denitrification, anammox, and DNRA; therefore nitrification is an intermediate process that connects aerobic and anaerobic inorganic N cycles.

Nitrification proceeds in two steps: oxidation of NH_4^+ to NO_2^- by an ammonia oxidizer; and oxidation of NO_2^- to NO_3^- by a nitrite oxidizer (Schmidt 1982). The overall process is performed by membrane-bound ammonia monooxygenase (*amo*) and produces hydroxylamine (NH_2OH) and nitroxyl (NOH) as intermediates; these are unstable in aqueous solution and are rapidly oxidized to NO_2^- or N_2O depending on the availability of O_2 (Reddy and DeLaune 2008).

The rate of nitrification is limited by low O_2; nitrifiers cease to work at O_2 concentrations of 1 to 6 μM (Henriksen and Kemp 1988). In coastal ecosystems increased rates of growth of phytoplankton can deplete O_2 concentrations and thereby decrease nitrification rates through O_2 deficiency (Jenkins and Kemp 1984). However, certain nitrifying bacteria such as *Nitrosomonas europaea* can survive at the oxic-anoxic interface (Voytek and Ward 1995) whilst the recently discovered *archaeal* nitrifiers have been observed to survive under extremely low NH_4^+ and O_2 (Verhamme et al. 2011); cultures of *Nitrosopumilus maritimus* can be grown at < 10 nM NH_4^+ (Martens-Habbena et al. 2009). Genome studies of the archaeal *amo* (*amoA*) nitrifier, *N. maritimus* revealed that marine nitrifier *archaea* have a 3-hydroxypropionate/4-hydroxybutryate pathway, which is an electron transport system that differs from that of known bacterial *amo* (*amoB*) nitrifiers and consumes organic N instead of NH_4^+ as an electron donor. Thus, the archeal nitrifiers can grow both autotrophically and mixtrophically in harsh conditions such as depleted O_2 and NH_4^+. This diversity means that nitrification is broadly distributed and influences both N and C cycles in ocean ecosystems (Walker et al. 2010).

The nitrification rate can be measured in three ways. The easiest is the direct inorganic N (DIN) inventory method, in which $[NH_4^+]$, $[NO_2^-]$, and $[NO_3^-]$ are analyzed in laboratory and environmental samples (Ferguson et al. 2007). However, the DIN method requires a long incubation time and dark conditions to block phytoplankton growth; these conditions reduce the accuracy of the analysis. Another method is to measure $[NH_4^+]$, $[NO_2^-]$, and $[NO_3^-]$ by using specific nitrification inhibitors (nitrapyrin, acetylene, allylthiourea); this method has been widely used to measure nitrification rates in coastal sediments (Macfarlane and Herbert 1984). However, we do not know whether these chemicals can inhibit *amoA* gene or mineralization. If these inhibitors affect the *amoA* genes or mineralization rates, the nitrification rate may be underestimated. A third method is the ^{15}N-nitrate isotope tracer technique. $^{15}NH_4^+$ is added to a sample, which is then incubated for a short period, and the concentration of $^{15}NO_3^-$ produced from $^{15}NH_4^+$ oxidation is determined (Koike and Hattori 1978). This method has the advantage that it measures a specific nitrification by-product directly, and it is more sensitive than other methods. However, the added $^{15}NH_4^+$ could stimulate nitrification rates; if this happens, nitrification rates can be overestimated.

Anaerobic ammonium oxidation (Anammox)

Anammox bacteria consume NH_4^+ as an electron donor, NO_2^- as an electron acceptor, and CO_2 as a C source, and oxidize NH_4^+ anaerobically to N_2 under anoxic conditions. All of these processes take place in the anammoxosome, an intracytoplasmic compartment bounded by a single membrane that contains ladderane lipids, which are tightly packed to minimize the loss of gaseous intermediates such as nitric oxide (NO) and hydrazine (N_2H_4) (Jetten et al. 2009). Anammox bacteria have a high affinity (K_s < 5 μM) for NH_4^+ and NO_2^-, and can therefore survive under very low NH_4^+ and NO_2^-. However, their metabolic activity is slow (15 to 80 μmol N_2 per g dry weight of cells per min) compared to other processes such as nitrification and denitrification, so cell doubling time is long (approximately11 to 20 days) and population growth rates are low (Strous et al. 1997).

Anammox removes inorganic N from the Black sea, and accounts for up to 40% of total N_2 gas production in anoxic regions (Kuypers et al. 2003). At a depth of 200 m in the anoxic waters of Golfo Dulce (Pacific Coast of Central America), 19 to 35% of total N_2 gas production results from anammox (Dalsgaard et al. 2003), and at Benguela (Atlantic Coast of

Southern Africa) within the oxygen deficient zone (ODZ) anammox removed 1.4 ± 1 Tg of fixed N per year (Kuypers et al. 2005). Anammox has also been detected in the Arctic Ocean, although there the process contributed < 5% to the total N_2 production (Rysgaard et al. 2004). The water chemistry in these ODZ oceanic ecosystems is globally very similar and account for 30 to 50% of the global N_2 removal (Dalsgaard et al. 2003).

Nitrifiers oxidize NH_4^+ to NO_2^- or NO_3^-, which diffuses to anaerobic zones where anammox occurs. *Archaeal* nitrifiers are found in O_2 limited conditions and supply NO_2^- to anammox bacteria; this transfer implies that nitrification is important to the anammox process (Lam et al. 2009; Füssel et al. 2012). However, denitrifiers also consume NO_2^-, so competition for NO_2^- uptake with denitrifiers is a potent regulating factor for anammox activity (Trimmer et al. 2003; Engström et al. 2005).

With respect to NH_4^+ supply, the mineralization of organic matter and subsequent release of NH_4^+ are tightly related to high anammox rates; high anammox rates were observed in the upper ODZs because high ammonification near the surface supplies NH_4^+ to anammox bacteria (Hamersley et al. 2007; Füssel et al. 2012). However, the presence of organic matter can indirectly inhibit anammox rates because it accelerates denitrification, which in turn hinders anammox activity by competing for NO_2^- (Strous et al. 1998). Sediments taken at the transition between the Baltic and North Sea indicate the relative importance of anammox decreases as organic matter content increases (Thamdrup and Dalsgaard 2002).

The first known anammox bacterium, *Brocadia anammoxidans* (phylum Planctomycetes*)* was detected in biofilm by using 16S rRNA gene sequence analysis and Fluorescence In-Situ Hybridization (FISH) with specific oligonucleotide probes (Strous et al. 1998). Using anammox-specific 16S rRNA gene primers (mainly Pla 46F) and anammox-specific oligonucleotide probes, researches have reported the presence of four "*candidatus*" genera of anammox bacteria: *Brocade* (Kartal et al. 2004), *Kuenenia* (Schmid et al. 2000), *Jettenia* (Quan et al. 2008) and *Scalindua* (Kuypers et al. 2003). Penton et al. (2006) developed a new primer that is 100% specific in the recovery of 700-bp 16S rRNA gene sequences that have ≥ 96% homology to the *Scalindua* group of anammox bacteria. This new primer detected anammox bacteria in 11 geographically and biogeochemically diverse freshwater and marine sediments.

Analytical quantification of the rate of anammox involves the isotope pairing method, which is an addition of $^{15}NH_4^+$ and $^{14}NO_2^-$ to environmental samples. After addition of $^{15}NH_4^+$ and and a period of incubation the ratio of $^{14}N^{15}N$ to $^{14}N^{14}N$ is analyzed using a gas chromatography-isotope ratio mass spectrometry (GC-IRMS) and expressed as $\delta^{14}N^{15}N$ values versus air (Kuypers et al. 2003). N_2H_4 and NH_2OH are intermediate products in the anammox process, so the rates at which they are produced are related to the overall rates of anammox. NH_4^+, N_2H_4, and NH_2OH are all measured colorimetrically over certain time intervals during the anoxic incubation of anammox bacteria. However, this method requires a large number of anammox cells, so measuring anammox activity *in situ* is a difficult task (Jetten et al. 2005). In anammox bacteria, ladderane lipids surround the anammoxosome. This unique lipid can be used as a biomarker for an estimation of anammox activity. After filtering the samples, the lipid is extracted using Soxhlet apparatus, saponified and separated into fatty acid and neutral-lipid fractions. These fractions are analyzed by gas chromatography-mass spectrometry (GC-MS) to obtain the concentrations of ladderane lipids (Kuypers et al. 2003).

Denitrification

Denitrification is the conversion of NO_2^-/NO_3^- to N_2 gas and is one of the processes by which fixed N moves from ocean to the atmosphere. Denitrifiers are heterotrophs and use NO_2^-/NO_3^- as an electron acceptor and organic matter as an electron donor and C source. Most denitrifiers are facultative anaerobes that use O_2 as an electron acceptor when it is available, but use NO_2^-/NO_3^- as an electron acceptor and reduce it to N_2 gas when O_2 is low.

The anaerobic conditions required for denitrification occur in coastal and shelf sediments and in ocean water columns in which ODZs are created by high O_2 consumption rates driven by organic matter decomposition coupled with low ventilation rates (e.g., Arabian sea, Black sea). The amount of N removal by ocean denitrification ranges from 200 to 300 Tg N y^{-1}, which is twice the rate of removal by estuarine and shelf sediment denitrification (100 to 250 Tg N y^{-1}) and significantly higher than those of terrestrial denitrification (4 to 8 Tg N y^{-1}) (Voss et al. 2013). The ratio of the permanent removal of fixed N by denitrification and anammox is 71 to 29 (Ward 2013); this observation emphasizes the importance of N removal by denitrification in ocean ecosystems.

Because most denitrifiers are heterotrophs that use organic C as an energy source, denitrification rates can be limited by organic C contents. Incubation experiments showed that an input of organic C increased the denitrification rates in the

ODZs of the Eastern Tropical North and South Pacific oceans (Ward et al. 2008) and central Baltic Sea (Btrettar and Rheinheimer 1992). However, under C-rich conditions such as benthic sediments and shelf regions, denitrification rates were limited by O_2 and NO_3^-, and C quality rather than C quantity (Laursen and Seitzinger 2001).

Denitrifiers are classified into three groups based on their energy source: organotrophs use organic matter and comprise the most common group of denitrifiers; lithotrophs use inorganic matter (e.g., hydrogen or reduced sulfur compounds); phototrophs use light as their energy source (Tate 2000). Numerous bacterial and archeal genera include strains capable of denitrification. All molecular research on the denitrifying microbial structures use functional gene coding to search for nitrate reductases such as *nirS* and *nirK*, and coding for nitrous oxide reductases such as *nosZ* (Braker 2000).

To measure denitrification activity, several methods have been used. An indirect method, 'acetylene blocking', is most extensively used because of its low cost, simplicity, and high sensitivity. The principle of this method is to use acetylene to inhibit the reduction of N_2O to N_2, and then to use gas chromatography equipped with an electron capture detector (GC-ECD) to measure the amount of N_2O produced by denitrification (Tiedje 1988). However, acetylene gas can inhibit nitrification, so this method underestimates denitrification rates when nitrification is coupled to denitrification (Seitzinger et al 1993). Another method is to measure the rates of NO_2^- / NO_3^- consumption for a certain time interval. However, immobilization and anammox bacteria can consume NO_2^-, so this method has low sensitivity and can underestimate the rate (Alef et al. 1993). The isotope method can improve the sensitivity and specificity of detection of denitrification rates. This method assumes that N_2 gas that includes ^{15}N ($^{15}N^{14}N$ and $^{15}N^{15}N$ gas) forms by random association of ^{15}N and ^{14}N, and becomes homogenously distributed in the denitrification zone. A sample is provided with $^{15}NO_3^-$ and incubated, and the isotopic composition of the N_2 gas produced is analyzed using GC-IRMS (Groffman et al. 2006). However, enrichment with $^{15}NO_3^-$ can stimulate denitrification rates, resulting in overestimation, and mixing $^{15}NO_3^-$ homogeneously into a heterogeneous environment such as sediment is a difficult task (Groffman et al. 2006). To directly quantify the end product (N_2), membrane inlet mass spectrometry (MIMS) has been broadly used in ocean ecosystems; this method detects the N_2 to Ar ratios with high precision (~0.03%) and small samples (< 7 mL) (An et al. 2001). Recently a combination of direct N_2 measurements with isotope ($^{15}N_2$) signal detection has been used as a method to distinguish denitrification from N_2 fixation where the two processes co-exist (An et al. 2001).

Dissimilatory nitrate reduction to ammonium

DNRA bacteria reducing NO_3^- to NH_4^+ under anoxic conditions are obligate anaerobes that live in highly-reduced conditions such as found in lake sediments, permanently waterlogged wetlands, ocean ODZs, or river plumes that receive particulate organic matter and NO_3^- from groundwater (Reddy and DeLaune 2008).

Estuarine and coastal soils have a mixture of freshwater and salt water, relatively high amounts of organic matter and nutrients, and low O_2 due to decaying organic matter; these are favorable conditions for DNRA. High rates of DNRA have been observed in Laguna Madre Baffin (coastal lagoons, Gulf of Mexico), and Concepcion (Chile, South Pacific Ocean) Bays (0.66 to 32.94 mM N m^{-2} d^{-1}), and are considered the primary cause of eutrophication by supplying NH_4^+ to phytoplankton (An and Gardner 2002).

Adding C sources such as glucose, carbohydrates or organic matter increases DNRA rates because microbes that perform this activity are heterotrophs that use organic C as an energy source (Kelso et al. 1999; Tobias et al. 2001). Previous research observed high DNRA rates in C-rich conditions such as estuarine sediments that receive their organic matter from sea cage trout farms (Christian et al. 2000). Thus, Bonin et al. (1998) suggested that DNRA could occur in the coastal sediments, but because C is limiting, DNRA is not an important process in the water column.

Reduced sulfur compounds (hydrogen sulfide, iron sulfide, and thiosulfate) can be used as an electron donor instead of organic matter for DNRA. Addition of sulfide increased the oxidation of sulfide, whilst simultaneously reducing NO_3^- to NH_4^+ (Brunet and Garcia-Gil 1996). In Laguna Madre and Baffin Bay, sulfide-induced DNRA contributed to the accumulation of NH_4^+, whereas sulfate reduction with DNRA inhibited denitrification; this observation emphasizes that sulfide oxidation is an important component of DNRA (An and Gardner 2002).

The functional gene for DNRA is the cytochrome *c* nitrite reductase gene *nrfA*, which was significantly expressed in the ODZs in the Peruvian and Omani Shelves (Lam et al. 2009; Jensen et al. 2011) and in estuarine sediments (Smith et al. 2007). However, the use of genetic techniques to identify *nrf*A has limitations because very little sequence information

related to the *nrfA* gene is available in databases, which mostly consider pathogens relevant to humans. Therefore, to enable investigation of DNRA communities for ocean ecosystems, specific functional primers and probes to detect DNRA bacteria should be developed for ocean ecosystems, and additional sequence data should be accumulated.

Generally, ^{15}N-tracing techniques are used to measure DNRA rates. When $^{15}NO_3^-$ is added to environmental samples, DNRA bacteria reduce it to $^{15}NH_4^+$. After incubation for a designated time, the $^{15}NH_4^+$ ratios and concentrations are measured using an isotope ratio mass spectrophotometer equipped with an elemental analyzer (EA-IRMS). To preclude the possibility of $^{15}NO_3^-$ immobilization by microbes, some studies added NH_4^+ simultaneously to samples to inhibit microbial uptake of NO_3^- (Rütting et al. 2011).

3. Effects of Global Climate Change on Inorganic N Cycling in Ocean Ecosystems

Effects of increasing pCO_2

Increasing pCO_2 in ocean surface waters can increase N_2 fixation rates because N_2-fixing organisms use CO_2 as a C source. One of the major N_2 fixing species is *Trichodesmium*, which is responsible for up to 50% of total marine N_2 fixation (Mahaffey et al. 2005) and is widely used in experiments to test the effects of pCO_2. An increase in pCO_2 to that projected for the year 2100 (atmospheric CO_2 750 ppm) enhanced N_2 fixation of *Trichodesmium* by 35 to 65% (Hutchins et al. 2007; Kranz et al. 2009); this increase amplifies the influx of organic N and nutrients to oligotrophic ocean ecosystems and can possibly support other inorganic N transformation rates by supplying energy sources. However, responses of other *Trichodesmium* species, diatoms/diazotrophs (e.g., *Crocosphaera watsonii*), and unicellular cyanobacteria to increasing pCO_2 are not known. In addition, the long-term response of N_2 fixers to elevated pCO_2 is not known; nor are interactions of the CO_2 effects coupled with the influence of other trace nutrients (e.g., iron (Fe) and phosphate (P)) or other potentially limiting factors, such as light and temperature, therefore conclusions about the general trends should be treated with caution (Hutchins et al. 2009).

In addition, an increase in pCO_2 can increase C-fixation rates of nitrifiers and anammox bacteria because they consume CO_2 as a C source and exploit the low affinity of the RuBisCO enzyme for CO_2. However, as of yet no experimental results have demonstrated that elevated pCO_2 influences nitrification or anammox rates and interactions between N_2 fixation and nitrification and anammox processes. Thus, research should be conducted to determine the effect of increasing pCO_2 on N cycling, and this should consider the various factors, including microbial composition and structure, the role of limiting factors, and interactions across the processes.

Ocean acidification

Some 30 to 40% of anthropogenic CO_2 has dissolved in the ocean, and has caused ocean pH to drop by ~0.1 compared to preindustrial levels; an additional 0.3–0.4 decline is anticipated by the year 2100 (Calderia and Wickett 2003). Dissolved CO_2 reacts with water molecules; this reaction releases H^+ which combines with carbonate ions CO_3^{2-} to form bicarbonate ions (HCO_3^-), thereby decreasing CO_3^{2-} concentration and seawater pH. The ocean acidification (OA) caused by dissolution of CO_2 in seawater inhibits N cycling, especially process such as nitrification that involve pH-dependent redox reactions. Because the pH determines the NH_3/NH_4^+ equilibrium in seawater ($NH_3 + H^+ \rightleftharpoons NH_4^+$; $pK_a = 9.3$) (Zeebe and Wolf-Gladrow 2001), the reduction of pH decreases the ratio of NH_3 to NH_4^+ (Fig. 2). Previous research showed that cultures of *Nitrosococcus oceani*, a widespread ammonia oxidizer, decreased their rates of nitrification by 20–36% with a 0.4–0.5 pH decrease because the bacteria preferred to use NH_3 rather than NH_4^+ (Ward 1987).

A pH reduction of 8.1 to 7.84 will decrease the proportion of $[NH_3]$ to the total $[NH_x]$ from 6.3 to 3.5% (Fig. 2) and this

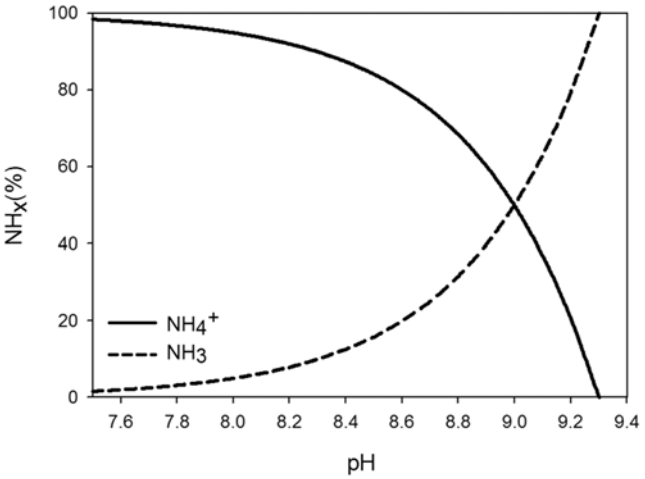

Fig. 2. The percentage of $[NH_3]$ and $[NH_4^+]$ in total $[NH_x]$ vs. pH. This figure was modified from the supplementary material published by Beman et al. (2010). The values were calculated using $[NH_3] = [NH_4^+] \times 10^{(pH-pKa)}$, where $pK_a = 9.3$ (Zeebe and Wolf-Gladrow 2001)

pH decline will lead to a 3–44% reduction in nitrification rates in the Atlantic and Pacific Oceans (Beman et al. 2011). A pH decrease of 0.1 in ocean waters could decrease its emission of N_2O by up to our current annual anthropogenic N_2O emissions from both fossil fuel combustion and industrial activity (Beman et al. 2011). In addition, decreased nitrification rates could reduce the supply of NO_3^- to ocean ecosystems; this decline could drive a change from NO_3^--supported to NH_4^+-supported primary production. Thus, smaller organisms, pico and nano-phytoplankton, that mainly consumes NH_4^+ will have an advantage in nutrient uptake, but large phytoplankton such as diatoms that mainly uptake NO_3^- will be at a disadvantage (Yool et al. 2007). Contrary to these expectations, Piontek et al. (2013) showed that OA increased the activity of bacterial extracellular hydrolytic enzymes. This increased activity was because of an enhanced nutrient regeneration under OA conditions, though this did not cause any change in nitrification rates or N_2O production in shelf regions (Clark et al. 2014). These results imply that nitrifiers in coastal zones might already be adapted to acidic conditions; if this is true, then the responses of nitrifiers to OA conditions could vary depending on the site characteristics.

Deoxygenation

One consequence of global warming is ocean deoxygenation, O_2 solubility in water decreases as temperature increases, also the increased temperature of surface seawater enhances upper-ocean stratification, thereby reducing the O_2 supply to the ocean interior (Bopp et al. 2002). Ocean models predict that the global ocean oxygen inventory will be reduced by 1 to 7% over the next century due to global warming; this decline will continue and expand the ODZs and thereby influence biogeochemical N cycling (Keeling et al. 2010).

At $O_2 < 5$ μM, heterotrophic bacteria use NO_3^- as an electron acceptor instead of O_2, and by this process ultimately create anoxic conditions and influence inorganic N transformations. For example, denitrification and anammox processes are enhanced due to an increase in NO_3^- respiratory processes, but nitrification is inhibited by O_2 limitation; as a result, NO_3^- is removed, but NH_4^+ accumulates (Codispoti et al. 2001; Deutsch et al. 2007). However, we do not yet know how the combined effects of OA and deoxygenation will affect nitrification and denitrification rates and N_2O production on global ocean scales. In addition, we do not know how the reduction in the supply of NO_3^- because of OA will affect denitrification rates under deoxygenated conditions.

Anthropogenic N deposition

Approximately 67 Tg of anthropogenic N per year is directly deposited to the open ocean from the atmosphere (Duce et al. 2008). Nitrification and denitrification rates can be enhanced by atmospheric N deposition due to the supply of bioavailable N to nitrifiers and denitrifiers (Tyrrell 2011). This atmospheric N deposition increases surface ocean primary production (up to ~0.3 Pg C y^{-1}) from where bioavailable N is transported through the surface waters to the deep ocean. This enhanced nitrification and denitrification driven by N deposition can drive an increase in emission of N_2O by up to 1.6 Tg N_2O y^{-1}, which is about 1/3 of total oceanic N_2O emissions (Duce et al. 2008). However, OA and deoxygenation also influence the N_2O emission so that we do not know yet how the combined effect of increasing N deposition, OA and deoxygenation controls inorganic N cycling and N_2O emission.

4. Conclusion

Inorganic N transformation rates vary depending on biogeochemical properties (e.g. concentrations of NO_2^-, NO_3^-, NH_4^+, organic C, and O_2; pH and the intensity of reducing conditions). In particular, the supply of electron acceptors and donors mainly control each inorganic N transformation rate so that interactions or competition among processes (e.g. coupled nitrification-denitrification or ammonification-nitrification; or competition between denitrification and anammox for nitrite uptake) has an important effect on their rates. Using mesocosm experimental work as a tool for understanding global climate change's effect on ocean inorganic N cycling it has been found that N_2 fixation rates increased in response to increased pCO_2. However, the responses of N_2 fixers varies depending on the community structures, and with little long-term experimental and field research conducted conclusions about the general trends of pCO_2 effect on N_2 fixation should be treated cautiously. Other effects of global climate change such as increased atmospheric N deposition and surface ocean deoxygenation can enhance denitrification rates due to an increase in the supply of NO_3^- to denitrifiers and formation of anoxic conditions favorable to denitrification, thereby increasing the emission of N_2O from ocean to atmosphere. However, deoxygenation and acidification could reduce the supply of O_2 and NH_4^+ to nitrifiers and thereby suppress nitrification which would, in turn, reduce the supply of NO_3^- to denitrifiers and consequently reduce emissions of N_2O (Table 1). Thus, we do not know yet whether atmospheric N

Table 1. The effect of global warming on inorganic nitrogen transformations in ocean ecosystems

Factors	Consequence	Limitation	Reference
Increased pCO_2	· Stimulate activity of N_2 fixing cyanobacteria and more supply of organic N to oligotrophic ocean · Possibly increase in anammox and nitrification rates	· Responses to increased pCO_2 differ depending on the microbial species · Few mesocosm and field experiments	Calderia and Wickett 2003; Mahaffey et al. 2005; Hutchins et al. 2007; Kranz et al. 2009
Ocean acidification (OA)	· Decrease in nitrification due to the decrease in the ratio of $[NH_3]$ to $[NH_4^+]$. · Change in microbial structure from nitrate-supported to ammonium-supported composition. · Possibly decrease in N_2O emission due to decrease in nitrification activity	· Shelf and coastal regions could be not affected by OA because of adaptation of nitrifiers to acidic conditions. · Few mesocosm and field experiments	Piontek et al. 2013; Clark et al. 2014
Deoxygenation	· Enhance denitrification and increase in N_2O emission · Accumulation of ammonium due to decrease in nitrification, resulting in change of microbial structures. · Limited light availability	· Less clear the how much deoxygenation produce the N_2O emission	Codispoti et al. 2001; Deutsch et al. 2007; Keeling et al. 2010
Atmospheric N deposition	· Enhanced nitrification and denitrification · Increase in N_2O emission in coastal zones	· No mesocosm and field experiments	Duce et al. 2008; Tyrrell 2011

deposition will offset the deoxygenation and acidification effects of decreased nitrate supply to denitrifiers. In addition, research on the combined influences of deoxygenation, acidification and N addition on inorganic N cycling has not yet been conducted. Therefore, the effect of global climate change on the environmentally significant biogeochemical cycling of inorganic N coupled to N_2O emission is far from being understood and any apparently meaningful conclusions should be considered with caution; comprehensive research on these complex situations and how they are controlled by the ocean ecosystem is needed.

Acknowledgements

I thank Professor Il-Nam Kim (Incheon National University), Professor Andrew Ogram (University of Florida) and Dr. Matthew R. Jones (Pohang University of Science and Technology) for their valuable comments. I thank the two reviewers for their constructive comments and time.

References

Alef K, Nannipieri P, Trasar-Cepeda C (1993) Methods in applied soil microbiology and biochemistry. Academic Press, London, 608 p

An S, Gardner WS (2002) Dissimilatory nitrate reduction to ammonium as a nitrogen link, versus denitrification as a sink in a shallow estuary (Languna Madre/Baffin Bay, Texas). Mar Ecol-Prog Ser **237**:41–50

An S, Gardner WS, Kana TM (2001) Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometer (MIMS) analysis. Appl Environ Microb **67**:1171–1178

Beman JM, Chow CE, King AL, Feng Y, Fuhrman JA, Andersson A, Bates NR, Popp BN, Hutchins DA (2011) Global declines in oceanic nitrification rates as a consequence of ocean acidification. P Natl Acad Sci USA **108**:208–213

Bonin P, Omnes P, Chalamet A (1998) Simultaneous occurrence of denitrification and nitrate ammonification in sediments of the French Mediterranean Coast. Hydrobiologia **389**:169–182

Bopp L, Quéré CL, Heimann M, Manning AC, Monfray P (2002) Climate-induced oceanic oxygen fluxes: implications for the contemporary carbon budget. Global Biogeochem Cy **16**. doi:10.1029/2001GB001445

Braker G, Zhou J, Wu L, Devol AH, Tiedje JM (2000) Nitrite reductase genes (*nirK* and *nirS*) as functional markers to investigate diversity of denitrifying bacteria in pacific northwest marine sediment communities. Appl Environ Microb **66**:2096–2104

Brettar I, Rheinheimer G (1992) Influence of carbon availability on denitrification in the Central Baltic Sea. Limnol Oceanogr **37**:1146–1163

Brunet RC, Garcia-Gil LJ (1996) Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. FEMS Microbiol Ecol **21**:131–138

Calderia K, Wickett ME (2003) Oceanography: anthropogenic carbon and ocean pH. Nature **425**:365. doi:10.1038/425365a

Christensen PB, Rysgaard S, Sloth NP, Dalsgaard T, Schwaerter S (2000) Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. Aquat Microb Ecol **21**:73–84

Clark DR, Brown IJ, Rees AP, Somerfield PJ, Miller PI (2014) The influence of ocean acidification on nitrogen regeneration and nitrous oxide production in the northwest European shelf sea. Biogeosciences **11**:4985–5005

Codispoti LA, Brandes JA, Christensen JP, Devol AH, Naqvi SWA,

Paerl HW, Yoshinari T (2001) The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene? Sci Mar **65**:85–105

Dalsgaard T, Canfield DE, Petersen J, Thamdrup B, Acuña-González J (2003) N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature **422**:606–608

Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP (2007) Spatial coupling of nitrogen inputs and losses in the ocean. Nature **445**:163–167

Duce RA, LaRoche J, Altieri K, Arrigo KR, Baker AR, Capone DG, Cornell S, Dentener F, Galloway J, Ganeshram RS, Geider RJ, Jickells T, Kuypers MM, Langlois R, Liss PS, Liu SM, Middelburg JJ, Moore CM, Nickovic S, Oschlies A, Pedersen T, Prospero J, Schlitzer R, Seitzinger S, Sorensen LL, Uematsu M, Ulloa O, Voss M, Ward B, Zamora L (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science **320**:893–897

Engström P, Dalsgaard T, Hulth S, Aller RC (2005) Anaerobic NH_4^+ oxidation by NO_2^- (anammox): implication for N_2 production in coastal marine sediments. Geochim Cosmochim Ac **69**: 2057–2065

Ferguson A, Eyre B, Gay J, Emtage N, Brooks L (2007) Benthic metabolism and nitrogen cycling in a sub-tropical coastal embayment: spatial and seasonal variation and controlling factors. Aquat Microb Ecol **48**:175–195

Füssel J, Lam P, Lavik G, Jensen MM, Holtappels M, Günter M, Kuypers MMM (2012) Nitrite oxidation in the Nambian oxygen minimum zone. ISME J **6**:1200–1209

Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA (2006) Methods for measuring denitrification: driverse approaches to a difficult problem. Ecol Appl **16**:2091–2122

Gruber N, Galloway NJ (2008) An Earth-system perspective of the global nitrogen clcye. Nature **451**:293–296

Hamersley MR, Lavik G, Woebken D, Rattray JE, Lam P, Hopmans EC, Damsté JSS, Krüger S, Graco M, Gutiérrez D (2007) Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. Limnol Oceanogr **52**:923–933

Henriksen K, Kemp WM (1988) Nitrification in estuarine and coastal marine sediments. In: Blackburn TH (ed) Nitrogen cycling in coastal marine environments. John Wiley and Sons, New York, pp 207.

Hutchins DA, Fu FX, Zhang Y, Warner ME, Feng Y, Portune K, Bernhardt PW, Mulholland MR (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr **52**:1293–1304

Hutchins DA, Mulholland MR, Fu F (2009) Nutrient cycles and marine microbes in a CO_2-enriched ocean. Oceanography **22**:128–145

Jenkins MC, Kemp WM (1984) The coupling of nitrification and denitrification in two estuarine sediments. Limnol Oceanogr **29**:609–619

Jensen MM, Lam P, Revsbech NP, Nagel B, Gaye B, Jetten MS, Kuypers MM (2011) Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory nitrite reduction to ammonium. ISME J **5**:1660–1670

Jetten M, Schmid M, van de Pas-Schoonen K, Damsté JS, Strous M (2005) Anammox organisms: enrichment, cultivation, and environmental analysis. In: Jared RL (ed) Methods in enzymology. Academic Press, Salt Lake, pp 34–57.

Jetten MSM, Niftrik LV, Strous M, Kartal B, Keltjens JT, Op den Camp HJM (2009) Biochemistry and molecular biology of anammox bacteria. Crit Rev Biochem Mol **44**:65–84

Kartal B, van Niftrik L, Sliekers O, Schmid MC, Schmidt I, van de Pas-Schoonen K, Cirpus I, van der Star W, van Loosdrecht M, Abma W, Kuenen JG, Mulder JW, Jetten MSM, den Camp HO, Strous M, van de Vossenberg J (2004) Application, eco-physiology and biodiversity of anaerobic ammonium-oxidizing bacteria. Rev Environ Sci Bio **3**:255–264

Keeling RF, Körtzinger A, Gruber N (2010) Ocean deoxygenation in a warming world. Ann Rev Mar Sci **2**:199–229

Kelso BHL, Smith RV, Laughlin RJ (1999) Effects of carbon substrates on nitrite accumulation in freshwater sediments. Appl Environ Microbiol **65**:61–66

Koike I, Hattori A (1978) Denitrification and ammonia formation in anaerobic coastal sediments. Appl Environ Microb **35**:278–282

Kranz SA, Sültemeyer D, Richter KU, Rost B (2009) Carbon acquisition by *Trichodesmium*: the effect of pCO_2 and diurnal changes. Limnol Oceanogr **54**:548–559

Kuypers MMM, Lavik G, Woebken D, Schmid M, Fuchs BM, Amann R, Jørgensen BB, Jetten MSM (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. P Natl Acad Sci USA **102**:6478–6483

Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jørgensen BB, Kuenen JG, Sinninghe Damsté JS, Strous M, Jetten MS (2003) Anaerobic NH_4^+ oxidation by anammox bacteria in the Black Sea. Nature **422**:608–611

Lam P, Lavik G, Jensen MM, van de Vossenberg J, Schmid M, Woebken D, Gutiérrez D, Amann R, Jetten MSM, Kuypers MMM (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. P Natl Acad Sci USA **106**:4752–4757

Laursen AE, Seitzinger SP (2001) The role of denitrification in nitrogen removal and carbon mineralization in Mid-Atlantic Bight sediments. Cont Shelf Res **22**:1397–1416

Macfarlane GT, Herbert RA (1984) Effect of oxygen tension, salinity, temperature and organic matter concentration on the growth and nitrifying activity of an estuarine strain of *Nitrosomonas*. FEMS Microbiol Lett **23**:107–111

Mahaffey C, Michaels AF, Capone DG (2005) The conundrum of marine N_2 fixation. Am J Sci **305**:546–595

Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation

of nitrifying Archaea and Bacteria. Nature **461**:976–979

Penton CR, Devol AH, Tiedje JM (2006) Molecular evidence for the broad distribution of anaerobic ammonium oxidizing bacteria in freshwater and marine sediments. Appl Environ Microb **72**:6829–6832

Piontek J, Borchard C, Sperlling M, Schulz KG, Riebesell U, Engel A (2013) Reponse of baterioplankton activity in an Arctic fjord system eo elevated pCO_2: results from a mesocosm pertubation study. Biogeosciences **10**:297–314

Quan ZX, Rhee SK, Zuo JE, Yang Y, Bae JW, Park JR, Lee ST, Park YH (2008) Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environ Microbiol **10**:3130–3139

Reddy KR, DeLaune RD (2008) Biogeochemistry of wetlands. CRC press, Boca Raton, 800 p

Rysgaard S, Glud RN, Risgaard-Petersen N, Dalsgaard T (2004) Denitrification and anammox activity in Arctic marine sediments. Limnol Oceanogr **49**:1493–1502

Rütting T, Boeckx P, Müller C, Klemedtsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. Biogeosciences **8**:1779–1791

Schmid M, Twachtmann U, Klein M, Strous M, Juretschko S, Jetten M, Metzger JW, Schleifer KH, Wagner M (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Syst Appl Microbiol **23**:93–106

Schmidt EL (1982) Nitrification in soil. In: Stevenson FJ (ed) Nitrogen in agricultural soils. Agronomy Monogrph, ASA, CSSA and SSSA, Madison, pp 253–288

Seitzinger S, Nielsen L, Caffrey J, Christensen P (1993) Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry **23**:147–167

Smith CJ, Nedwell DB, Dong LF, Osborn AM (2007) Diversity and abundance of nitrate reductase genes (*narG* and *napA*), nitrite reductase genes (*nirS* and *nrfA*), and their transcripts in estuarine sediments. Appl Environ Microb **73**:3612–3622

Strous M, Heijnen HH, Kuenen JG, Jetten MSM (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl Microbiol Biot **50**:589–596

Strous M, Van Gerven E, Zheng P, Kuenen JG, Jetten MSM (1997) Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. Water Res **31**:1955–1962

Tate RL (2000) Soil microbiology. John Wiley and Sons, New York, 536 p

Thamdrup B, Dalsgaard T (2002) Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl Environ Microb **68**:1312–1318

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. John Wiley and Sons, New York, pp 179–244

Tobias CR, Anderson IC, Canuel EA, Macko SA (2001) Nitrogen transformations through a fringing marsh-aquifer ecotone. Mar Ecol-Prog Ser **210**:25–39

Trimmer M, Nicholls JC, Deflandre B (2003) Anaerobic NH_4^+ oxidation measured in sediment along the Thames estuary, United Kingdom. Appl Environ Microb **69**:6447–6454

Tyrrell T (2011) Anthropogenic modification of the oceans. Philos T Roy Soc A **369**:887–908

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determine differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J **5**:1067–1071

Voss M, Bange HW, Dippner JW, Middelburg JJ, Montoya JP, Ward BB (2013) The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Philos T Roy Soc B **368**(1621). doi:10.1098/rstb.2013.0121

Voytek MA, Ward BB (1995) Detection of ammonium-oxidizing bacteria of the beta-subclass of the class *Proteobacteria* in aquatic samples with the PCR. Appl Environ Microb **61**:1444–1450

Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, Arp DJ, Brochier-Armanet C, Chain PSG, Chan PP, Gollabgir A, Hemp J, Hügler M, Karr EA, Könneke M, Shin M, Lawton TJ, Lowe T, Martens-Habbena W, Sayavedra-Soto LA, Lang D, Sievert SM, Rosenzweig AC, Manning G, Stahl DA (2010) Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. P Natl Acad Sci USA **107**:8818–8823

Ward BB (1987) Kinetic studies on ammonia and methane oxidation by Nitrosococcus oceanus. Arch Microbiol **147**:126–133.

Ward BB (2013) How nitrogen is lost. Science **341**:352–353

Ward BB, Tuit CB, Jayakumar A, Rich JJ, Moffett J, Naqvi SWA (2008) Organic carbon, and not copper, controls denitrification in oxygen minimum zones of the ocean. Deep-Sea Res Pt I **55**:1672–1683

Yool A, Martin AP, Fernádez C, Clark DR (2007) The significance of nitrification for oceanic new production. Nature **447**:999–1002

Zeebe RE, Wolf-Gladrow D (2001) CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier, Amsterdam, 360 p