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Coastal water column ammonium and nitrite oxidation are decoupled in summer

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ABSTRACT

Water column nitrification is a key process in the nitrogen cycle as it links reduced and oxidized forms of nitrogen and also provides the substrate (nitrate) needed for reactive nitrogen removal by denitrification. We measured potential water column ammonium and nitrite oxidation rates at four sites along an estuary to continental shelf gradient over two summers. In most cases, nitrite oxidation rates outpaced ammonium oxidation rates. Overall, ammonium and nitrite oxidation rates were higher outside of the estuary, and this trend was primarily driven by higher oxidation rates in deeper waters. Additionally, both ammonium and nitrite oxidation rates were impacted by different *in situ* variables. Ammonium oxidation rates throughout the water column as a whole were most positively correlated to depth and salinity and negatively correlated to dissolved oxygen and light. In contrast, nitrite oxidation rates throughout the water column were negatively correlated with light and pH. Multivariate regression analysis revealed that while both surface (<20 m) and deep (>20 m) ammonium oxidation rates were most strongly predicted by depth and light, surface rates were also regulated by salinity and deep rates by temperature. Surface (<20 m) nitrite oxidation rates were best explained by $[H^+]$ (i.e. pH) alone, while salinity, $[H^+]$, temperature, and depth all played a role in predicting deep (>20 m) nitrite oxidation rates. These results support the growing body of evidence that ammonium oxidation and nitrite oxidation are not always coupled, should be measured separately, and are influenced by different environmental conditions.

1. Introduction

Coastal ecosystems are key environments for the recycling and filtering of nutrients (Hopkinson et al., 1999; Testa et al., 2008; Eyre and Ferguson, 2009; Perez-Villalona et al., 2015). In particular, these ecosystems process nitrogen (N) by transforming the element through various oxidation states (Owens, 1986). Rapid changes in physical and chemical conditions are observed within coastal ecosystems across both space and time. As such, coastal ecosystems are often considered a natural laboratory, where we can take advantage of *in situ* fluctuating conditions to better understand how N cycling processes respond to changes in the environment (e.g., salinity, pH, inorganic N concentrations, etc.). Here we focus on one N cycling process, nitrification, as it has important implications for water column productivity as well as ecosystem fixed nitrogen loss (Heiss et al., 2012; Morse et al., 2014; Smith et al., 2014).

Typically nitrification is a two-step process that links reduced and oxidized portions of the N cycle, although recently complete nitrification was found in one bacteria (van Kessel et al., 2015; Daims et al., 2015). The first step, ammonium (NH_4^+) oxidation, converts NH_4^+ to nitrite (NO_2^-) and is generally considered the rate-limiting step of nitrification (Ward, 2008). Ammonium oxidizers compete with phytoplankton and heterotrophic bacteria for substrate (Martens-Habbena et al., 2009; Smith et al., 2014), and in turn ammonium availability can influence the distribution and activity of ammonium oxidizing bacteria and archaea in marine environments (Urakawa et al., 2014). Overall, relationships between ammonium oxidation rates and *in situ* environmental conditions (e.g. temperature, salinity, dissolved oxygen, substrate availability) have been well documented in a variety of marine environments (Bianchi and Lefevre, 1999; Ward, 2005; Grundle and

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Juniper, 2011).

While recent work has highlighted the importance of nitrite oxidation (e.g., Clark et al., 2008; Fussel et al., 2012), we know much less about this second step of nitrification. In fact, reports of nitrite oxidation rates in the literature are limited and very few studies have examined the impact of environmental conditions on this process. Nitrite oxidation converts NO_2^- to nitrate (NO_3^-) providing the substrate (nitrate) needed for denitrification – a critical filtering mechanism which removes between 30 and 50% of N loads to coastal systems (Seitzinger and Kroeze, 1998; Seitzinger et al., 2006). Denitrification is important, as excess N can lead to a variety of negative consequences in coastal waters, including eutrophication, hypoxia, and decreases in biodiversity (Nixon, 1995; Galloway et al., 2003; Diaz and Rosenberg, 2008).

The two steps of nitrification are generally considered to occur as separate, but tightly coupled, processes in marine waters, with nitrite oxidizers relying on ammonium oxidizers for substrate (Ward, 2008). However, in a recent study, nitrite oxidizing bacteria were found to be able to reduce cyanate to ammonium, which could in turn be used by ammonium oxidizers to form nitrite in a "reciprocal feeding" pattern (Palatinszky et al., 2015). And, for the first time, both steps of nitrification were recently described in two species of *Nitrospira* bacteria (van Kessel et al., 2015; Daims et al., 2015). Although the widespread environmental importance of these recent finding remains to be seen, they highlight how much more there is to learn about nitrification.

Additional evidence also suggests that ammonium and nitrite oxidation may not always be tightly coupled within marine water columns. The presence of a "primary nitrite maximum" in ocean water columns is often attributed to a strong ammonium oxidizing community and nitrite oxidation rates that cannot keep pace with nitrite production (Lomas and Lipschultz, 2006; Beman et al., 2013; Santoro et al., 2013). Yet, in other marine locations ranging from the coast to the open ocean, much higher nitrite oxidation rates have been measured compared to rates of ammonium oxidization (e.g., Olson, 1981; Ward and Kilpatrick, 1991; Grundle and Juniper, 2011; Fussel et al., 2012; among many others).

In this study, we quantified potential rates of summer water column ammonium and nitrite oxidation at four sites from the head of an estuary to the continental shelf. We had three primary questions: 1) Do rates of ammonium and nitrite oxidation vary along this gradient? 2) Do rates of ammonium and nitrite oxidation rates vary with depth? 3) What environmental conditions best predict ammonium and nitrite oxidation rates along this gradient?

2. Materials and methods

2.1. Site description

We collected water column samples at four sites off the Southern New England coast in the summers of 2012 and 2013 (Fig. 1). We sampled Site 1 twice (June 2012, August 2012), Site 2 four times (June 2012, August 2012, July 2013, August 2013), Site 3 twice (July 2012, August 2012), and Site 4 on one occasion (July 2012).

Two of the sites (Sites 1 and 2) are located within Narragansett Bay proper, a phytoplankton-based temperate estuary with a mean depth of ~8 m (Nixon et al., 1995), low freshwater input low (~100 $m^3\ s^{-1}$), and a mean flushing rate of 26 days (Pilson, 1985). Salinity follows a down-bay gradient from ~25 psμ at the head to ~32 at the mouth (Fulweiler and Nixon, 2009). Dissolved inorganic nitrogen concentrations as well as primary production rates are highest at the head of the estuary (Site 1) and decrease down bay (Site 2; Nixon et al., 2009).

Sites 3 and 4 are located outside of the estuary on the Southern New England continental shelf. Site 3 is located in Rhode Island Sound, a phytoplankton-based system that typically experiences strong vertical stratification in the summer (Shonting and Cook, 1970; Ullman et al., 2014), which leads to summer nutrient limitation (Fields et al., 2014). Site 4 is the site located furthest from shore in area known as the "Mud Patch." This was our deepest site, approximately 110 km south of Cape Cod with fine-grained sediments winnowed from Georges Bank (Twichell et al., 1981).

2.2. Water column ammonium and nitrite oxidation rates

Water samples were collected with a 5-L Niskin bottle at various depths according to the station and sampling event (Table 1). Light levels were measured at most sampling events and depths using A Li-Cor LI-190 terrestrial and LI-193 underwater quantum PAR sensors with an LI1400 Data Logger (Table 1). We immediately collected water samples from the Niskin bottle for *in situ* dissolved inorganic nitrogen concentrations (DIN: NH_4^+, NO_2^-, NO_3^-). Each water sample was filtered with 0.2 μm nylon filters and frozen for later analysis via standard colorimetric techniques (Grasshoff, 1976) using a Varian Cary 100 Bio UV-Vis (detection limits: 0.09, 0.02, 0.20 μM NH_4^+, NO_2^-, NO_3^- respectively in 2012; 0.3, 0.3, 0.2 μM in 2013). We also measured temperature, dissolved oxygen, salinity, and pH of the water from the Niskin bottles using a HACH HQ40d meter. Ammonium and nitrite oxidation rates were measured separately using methods similar to Newell et al. (2011). We transferred site water from each depth directly from the Niskin bottle into four 1-L tedlar bags, and started incubations immediately on board after air bubbles were removed and tedlar bags were weighed. We added enriched ammonium tracer ($^{15}NH_4Cl$, 99%, Cambridge Isotope, 100–200 nM) (Ward, 2005) to two of the tedlar bags from each depth to measure ammonium oxidation rates. Similarly, we determined nitrite oxidation rates by adding enriched nitrite as a tracer ($Na^{15}NO_2$, 98+%, Cambridge Isotope, 100–200 nM) to the two remaining tedlar bags from each depth. As we added tracers in amounts ranging from 3 to 100% (mean 33%) of *in situ* NH_4^+ and 13–100% (mean 79%) of *in situ* NO_2^- pools, our rates should be considered as potential rates. Importantly, there was no relationship between ammonium oxidation rate ($p = 0.18$) and percentage of tracer added. There was a significant relationship between nitrite oxidation and percentage of tracer added ($p < 0.0001$) however, it was opposite of what we would expect with lower rates of nitrite oxidation at the highest tracer additions. Thus, we think that the addition of tracer did not artificially increase rates of nitrite oxidation.

We also added ^{14}N carriers, sodium nitrite ($NaNO_2$) and potassium nitrate (KNO_3), to the ammonium oxidation and nitrite oxidation sets, respectively, in a concentration twice as high as the tracer (Ward, 2005). We added tracers and carriers to the gas-tight tedlar bags through septa injection, then gently shook the bags by hand to mix tracer/carrier solutions throughout the sample. We collected an initial aliquot for $^{14/15}N$ analysis from each bag, which was filtered (0.2 μm nylon) and frozen until later analysis. We incubated the tedlar bags at *in situ* temperature in the dark for 24 h, after which we collected a final aliquot which was filtered and frozen for later laboratory analysis.

We determined ammonium and nitrite oxidation rates by measuring the accumulation of ^{15}N in nitrite or nitrate pools, respectively, by converting nitrite to nitrous oxide (N_2O) gas using a sodium azide reduction method (McIlvin and Altabet, 2005; Mackey et al., 2011; Newell et al., 2011). For ammonium oxidation rates, we placed 7.5 mL of sample into 12 mL Exetainer vials (Labco, UK) and capped them. Then, we added 0.25 mL of 1:1 (v:v) 2 M sodium azide:20% acetic acid solution (purged with He gas for 30 min) through the septa. We gently shook the vials and allowed