Title: 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 (< 20m) and deep (> 20m) ammonium oxidation rates were most strongly predicted by depth and light, surface rates were also regulated by salinity and deep rates by temperature. Surface (< 20m) 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 (> 20m) 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 et al., 1999; Ward, 2005; Grundle and 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 to 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 (Figure 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 ($\sim 100 \text{ m}^3 \text{ s}^{-1}$), and a mean flushing rate of 26 days (Pilson, 1985). Salinity follows a down-bay gradient from ~25 psu 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 micron 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 ($^{15}\text{NH}_4\text{Cl}$, 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 ($\text{Na}^{15}\text{NO}_2$, 98+%, Cambridge Isotope, 100-200 nM) to the two remaining tedlar bags from each depth. As we added tracers in amounts ranging from 3-100% (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 $^{14/15}\text{N}$ analysis from each bag, which was filtered (0.2 micron nylon) and frozen until later analysis. We incubated the tedlar bags at *in situ* temperature in the dark for 24 hours, 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 minutes) through the septa. We gently shook the vials and allowed them to react for 1 hour at 30 °C; during this time, the nitrite was chemically converted to N₂O gas. Finally, we added 0.15 mL of 10 M sodium hydroxide the septa to stop the reaction.

For nitrite oxidation rates, we measured ¹⁵N nitrate production. We first removed *in situ* nitrite by adding 0.25 mL of 0.4 M sulfamic acid to 25 mL of sample, and then, after 10 minutes, neutralized samples with 0.125 mL of 2 M sodium hydroxide (Granger and Sigman, 2009). Next, we added 100 mg of magnesium oxide, 6.6 g of sodium chloride, and 0.75 g of acidified cadmium powder to each 25 mL sample and placed the samples on a shaker table for 17 hours, during which time nitrate was reduced to nitrite (McIlvin and Altabet, 2005; Ryabenko et al., 2009). Finally, we centrifuged samples for 15 minutes at 2000 rpm and treated 7.5 mL of the supernatant liquid with azide as described above.

The isotopic analysis for ^{14/15}N in the resulting N₂O gas was performed on a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany) at the University of California Davis Stable Isotope Facility (N₂O Limit of Quantitation: approximately 150 picomoles, long-term standard deviation ¹⁵N: 0.1 ‰. Following the rate calculations below, these values equate to a limit of detection of 3.4×10⁻⁵ nM d⁻¹ for ammonium oxidation and 4.3×10⁻⁵ nM d⁻¹ for nitrite oxidation). Both ammonium and nitrite oxidation rates were corrected for azide reduction efficiency, and nitrite oxidation rates were also corrected for cadmium reduction efficiency.

We calculated ammonium oxidation rates by determining the atom% enrichment of the nitrite pool after 24 hour incubation and correcting for ammonium source pool tracer dilution (Carini and Joye, 2008):

$$NH_4^+ Ox (nM d^{-1}) = \frac{(atom\% ^{15}N_{24hr} - atom\% ^{15}N_{0hr}) * [NO_2^-]_{24hr}}{\alpha * t}$$

$$\text{Where } \alpha = \frac{[^{15}NH_4^+]}{[^{15}NH_4^+] + [^{14}NH_4^+]}$$

Similarly, we calculated nitrite oxidation rates as:

$$NO_2^- Ox (nM d^{-1}) = \frac{(atom\% ^{15}N_{24hr} - atom\% ^{15}N_{0hr}) * [NO_3^-]_{24hr}}{\alpha * t}$$

$$\text{Where } \alpha = \frac{[^{15}NO_2^-]}{[^{15}NO_2^-] + [^{14}NO_2^-]}$$

2.3 Statistical Analysis

All statistical analysis was performed using JMP Pro 11.0, and significance was determined with an alpha of ≤ 0.05 . If we could not measure a rate of ammonium oxidation or nitrite oxidation, we report the rate as 0. Ammonium and nitrite oxidation rates were not normally distributed. To determine if oxidation rates were significantly different along this estuary to shelf gradient, we used a Wilcoxon/Kruskal-Wallis test followed by a nonparametric comparison for each pair using a Wilcoxon test.

To explore potential drivers of ammonium and nitrite oxidation, we conducted a multivariate correlation analysis of all rates across all sites (Table 2). We then ran a stepwise multiple regression and selected the best fitting model based on the minimum Akaike's Information Criteria (AIC) (Akaike, 1974; Graham, 2003). To meet the assumptions for parametric statistics, we log-transformed ammonium and nitrate oxidation rates, salinity,

dissolved oxygen, % of surface light, and pH (i.e. calculated $[H^+]$) prior to analysis. We included substrate concentration in the multiple regression for ammonium oxidation (i.e., $[NH_4^+]$), but we could not include the substrate for the nitrite oxidation in the multiple regression due to the non-detectable nitrite concentrations (see Table 1). For each best-fitting model, we present standardized parameter estimates, which normalize the slopes of each term so that the influence of each variable in the best-fitting model can be easily compared to each other. Next, we further explored drivers of ammonium and oxidation rates divided into groups based on depth: surface (< 20 m) and deep (> 20 m). We excluded one outlier from Site 2 in the correlation and multiple linear regression analysis (nitrite oxidation: 4.53 nM d^{-1} , Site 2, June 2012, 6 m) as determined by Dixon's Q-test at the 99% confidence level ($Q=0.79$, $n=14$). Finally, we examined ammonium and nitrite oxidation rates as a function of pH to see if decreasing pH had a negative impact on oxidation rates.

3. Results

3.1 Water Column Oxidation Rates From Shore to Shelf

Water column ammonium oxidation rates ranged from 0 to 20 nM d^{-1} with a median rate of 0.03 nM d^{-1} , while nitrite oxidation rates ranged from 0 to 99 nM d^{-1} , with a median rate of 0.41 nM d^{-1} (Table 1). Ammonium and nitrite oxidation rates were correlated to each other ($r_t=0.32$, $p=0.0084$). On average, however, nitrite oxidation rates outpaced ammonium oxidation rates and accounted for over 66% of total nitrification. Multiple regression analysis found that depth, $[H^+]$ (i.e. pH), and light were the primary drivers of ammonium oxidation rates from all depths combined (Table 3). Nitrite oxidation rates from all sampling depths were also primarily driven by depth and light, as well as temperature (Table 3).

Along the shore to shelf transect, water column ammonium and nitrite oxidation rates increased significantly with distance from shore ($p=0.0096$, $p=0.023$, respectively; Figure 2). This difference was primarily driven by changes in oxidation rates with depth. Specifically, higher rates of ammonium and nitrite oxidation were observed at depths below 20 m when compared to rates above 20 m ($p<0.0001$, $p<0.0001$, respectively; Figure 3). In most cases this depth (20 m) also corresponded to a change in light levels where less than ~1% of surface light was measured. Light was only a significant driver of surface and deep ammonium oxidation rates, and depth was a significant driver of all oxidation rates except for surface nitrate oxidation (Table 3). These data appear to separate into two groups: surface (< 20 m) and deep (> 20 m). Below we discuss environmental drivers of oxidation rates in terms of these two groups.

3.2 Relationship between *in situ* Environmental Conditions and Water Column Oxidation Rates

We examined how ammonium and nitrite oxidation rates correlated with the environmental parameters measured in this study (Table 2). We found that ammonium and nitrite oxidation rates were both negatively correlated to light ($r_{\tau} = -0.39$, $p=0.0027$; $r_{\tau} = -0.35$, $p=0.0086$, respectively). Ammonium oxidation was also negatively correlated to dissolved oxygen concentrations ($r_{\tau} = -0.48$, $p<0.0001$) but positively correlated to depth ($r_{\tau}=0.54$, $p<0.0001$) and salinity ($r_{\tau}=0.42$, $p=0.0006$). Nitrite oxidation also displayed a negative correlation with pH ($r_{\tau} = -0.30$, $p=0.017$) (Table 2).

The multiple regression analysis showed that salinity, light, and depth account for almost 70% of the variation in surface ammonium oxidation rates, while depth, light, and temperature

together account for almost 80% of the variation in deep ammonium oxidation rates (Table 3). For nitrite oxidation, the multiple regression analysis demonstrated that $[H^+]$ alone accounted for 51% of variation in surface rates, while deep nitrite oxidation rates were driven by salinity, $[H^+]$, temperature, and depth (Table 3). Finally, we found significant relationships between oxidation rates and pH (Table 2, Figure 4), and determined that surface nitrite oxidation rates were strongly and inversely correlated to pH ($R^2=0.90$, $p<0.0001$; Figure 4).

4. Discussion

We found that summer water column ammonium and nitrite oxidation rates were significantly lower closer to shore and this was largely driven by higher oxidation rates in the deeper, offshore waters. In most instances, nitrite oxidation rates were higher than ammonium oxidation rates, with ammonium oxidation rates accounting for on average just ~35% of total nitrification. We also found that ammonium and nitrite oxidation rates were best predicted by different *in situ* environmental characteristics.

4.1 Water Column Oxidation Rates

Reported rates of ammonium and nitrite oxidation range widely both within and across marine ecosystems (Table 4). Water column rates of ammonium and nitrite oxidation we measured within the estuary (Sites 1 and 2) fall at the low end of observed rates for the Atlantic Ocean (Clark et al., 2007, 2008) and are similar to mean rates reported for a temperate, Pacific coastal system (Ward, 1987). Several other estuarine studies, using similar ^{15}N tracer methods, report higher rates ranging up from 120 $nM\ d^{-1}$ in Saanich Inlet, British Columbia (Ward and

Kilpatrick, 1990) to 82,000 nM d⁻¹ in the Chesapeake Bay (McCarthy et al., 1984; Horrigan et al., 1990). Ammonium and nitrite oxidation rates we measured outside of the estuary are more variable and overall significantly higher than the estuarine sites. As we discuss below, the higher offshore rates are driven by high rates deeper in the water column below the 1% light level. In fact, surface water oxidation rates are similar between the estuary and offshore sites. We also observed a clear spatial pattern with the lowest mean oxidation rates found at the head of the estuary (Site 1), increasing down bay, and finally the highest mean rates are observed at the outermost shelf site (Site 4).

Despite differences in the magnitude of oxidation rates between those in the surface waters and those in the deep waters, nitrite oxidation rates were generally higher than ammonium oxidation rates. In studies that investigate both processes, this is not uncommon, and the data presented here add to the growing body of literature that highlights the importance of this understudied, second step of nitrification (Table 4). The large difference between ammonium and nitrite oxidation rates also suggests that these processes may be “uncoupled” more often than we normally assume. Indeed, two recent studies also argue for the independence of these two steps (Clark et al., 2014; Bristow et al., 2015).

A “strong decoupling” between ammonium and nitrite oxidation was reported by Bristow et al. (2015) in the Gulf of Mexico hypoxic zone, with ammonium oxidation rates proceeding up to 30 times higher than nitrite oxidation rates under hypoxic conditions. Bristow et al. (2015) attributed this decoupling of nitrification to a variety of factors, including temperature and substrate availability, and bottom water oxygen concentrations. Additionally, “the two stages of nitrification were uncoupled,” in the Northwest European shelf waters where rates of ammonium oxidation were much slower than nitrite oxidation (average ratio of 0.22:1) (Clark et al., 2014).

While the specific environmental factors controlling the difference between the two steps of nitrification on the Northwest European shelf could not be defined, Clark et al. (2014) reported that most of the variation in rates was associated with differences among location. We find a similar pattern here, as the mean ammonium to nitrite oxidation ratio varied by site with Site 1, 2, and 4 being low (0.21:1, 0.20:1, and 0.43:1, respectively) and Site 3 being 1:1. Overall, the mean ammonium to nitrite oxidation ratio was 0.43:1 (± 0.16). The 1:1 ratio observed at Site 3 is driven by three ammonium oxidation rates that were $\geq 20\%$ the concurrently measured nitrite oxidation rates.

To understand the overall uncoupling of nitrification we observe in this study, we determined how much substrate ammonium oxidation provides for the second step of nitrification. On average, ammonium oxidation rates in the surface waters could provide $\sim 20\%$ of the nitrite consumed by nitrite oxidation, while ammonium oxidation rates in deep water (> 20 m) could provide almost 90%. Of course, this is an average and spatial and temporal variability (e.g., Site 3, July and August mid-depth ammonium oxidation rates) highlight that dynamic nature of nitrification. Nonetheless, the majority of the rates observed here suggest that other nitrite sources were needed to drive the observed surface nitrite oxidation rates. A potential nitrite source is sediment dissimilatory nitrate reduction to ammonia (DNRA), which can provide a potential nitrite source to overlaying water columns (Kelso et al., 1997). On several occasions, as part of a different project, dissolved inorganic nitrogen fluxes from the sediments of the same study sites were measured (Fields, 2013). We can use these DIN fluxes to investigate the potential role of DNRA as a nitrite source in this study. To do so, we prorated the mean sediment nitrite efflux from estuarine Site 2 ($\sim 1.6 \mu\text{mol m}^{-2} \text{ hr}^{-1}$) by depth (8 m), and calculated that the benthos could provide over 9 times the substrate needed for the average observed water

column nitrite oxidation rates at Site 2. This benthic nitrogen could easily reach the surface waters of Site 2 as this estuary (Narragansett Bay) is characterized as being generally well mixed (Nixon et al. 2009). We hypothesize that excess nitrite in these, and perhaps other estuarine waters, may fuel nitrite oxidation throughout the water column. Of course, this does not explain the surface water decoupling in the sites outside of the estuary. If we apply the same logic to the deeper, offshore sites, we determined that benthos does not appear to play such an important role in providing water column substrate, as the sediment nitrite release at Site 4 ($\sim 0.5 \mu\text{mol m}^{-2} \text{hr}^{-1}$) when prorated for the water column depth accounted for less than 1% of the nitrite oxidized below the thermocline. In this case, other sources of nitrite must be driving the observed nitrite oxidation rates. A natural nitrite source may include degradation of cyanate, as the ammonia oxidizing strain *Nitrosophaera gargensis* has been shown capable of using cyanate as an energy source by reducing cyanate to ammonium and then nitrite (Palatinszky et al., 2015). These organisms were shown to be capable of degrading cyanate in a 50 nM concentration culture, which is within the range of natural concentrations 1.4 – 41 nM in the nearby Chesapeake Bay and North Atlantic Ocean (Widner et al., 2013). Additional natural nitrite sources to the marine environment can also include incomplete reduction of nitrate to ammonia by phytoplankton (Lomas and Lipschultz, 2006; Mackey et al., 2011) and dark phytoplankton release of nitrite (Collos, 1998; Grundle and Juniper, 2011).

4.2 Relationship between *in situ* Environmental Conditions and Water Column Oxidation Rates

Across all sites and sampling depths, ammonium and nitrite oxidation rates appear to be regulated by different *in situ* variables. Water column ammonium oxidation rates from all

sampling depths and sites were positively correlated to depth and salinity, and negatively correlated to light and dissolved oxygen (Table 2). In contrast, nitrite oxidation rates across all sampling sites and depths displayed significant negative correlations light and pH (Table 2). These correlations with light along with the significant ($p < 0.0001$) exponential increase of ammonium and nitrite oxidation rates with depth (Figure 3) suggest it is best to separate oxidation rates by depth when exploring environmental drivers. As such, the *in situ* controls on ammonium and nitrite oxidation rates from surface waters ($< 20\text{m}$) and deep waters ($> 20\text{m}$) will be addressed separately.

Surface ammonium oxidation rates were best described by salinity, light, and depth (Table 3). The positive relationship between ammonium oxidation rates and salinity we observe is different from previous coastal studies. Trends in other coastal systems with higher rates of nitrification at lower salinities has been attributed to increased human nutrient loading and the release of inorganic nitrogen from particles as fresh and salt waters mix (Somville, 1984; Bianchi et al., 1999). However, freshwater input is low in Narragansett Bay and thus inorganic N released from freshwater mixing is not likely to provide substrate for ammonium oxidation. Instead, we hypothesize that our observed trend of increasing surface ammonium oxidation rates with salinity may be driven by competition for substrate (NH_4^+). In marine systems, competition for ammonium with phytoplankton can regulate ammonium oxidation rates (Smith et al., 2014). Water column primary production is highest in Narragansett Bay in the Providence River Estuary (where Site 1 is located) and decreases down bay (Nixon et al. 2009) as salinity increases. While we did not measure primary production in this study, we propose that the low rates of ammonium oxidation at the head of the estuary are driven in large part by the pattern of productivity in this system.

360 Additionally, ammonium availability can also shape the activity and distribution of
361 ammonia oxidizing bacteria (AOB) and archaea (AOA) (Urakawa et al., 2014). AOB are known
362 to be light limited (Hyman and Arp, 1992; Guerrero and Jones, 1996a,b), which may also
363 contribute to the negative relationship we observe between surface ammonium oxidation rates
364 and light. Further, the increase in ammonium oxidation rates at low light levels could also be
365 explained by a general decrease in substrate competition between AOA and AOB and
366 phytoplankton. Thus, this relationship with light also fits well with our hypothesis that surface
367 ammonium oxidation rates may be regulated by primary production in our study region.

368 Surface ammonium oxidation rates also showed a negative relationship with depth – a
369 trend which appears contradictory to the relationship with light discussed above. However,
370 based on standardized parameter estimates, the other variables (salinity and light) were between
371 1.5 - 2.5 times more predictive than depth in the multiple regression model (Table 3). The depth
372 range covered by the surface ammonium oxidation rates was only ~10 m, while light levels
373 varied from ~1-80%. Thus, we suggest that the primary drivers of surface ammonium oxidation
374 at our study sites are light and salinity.

375 However, in deeper waters (> 20m), depth was a main predictor of ammonium oxidation
376 rates (Table 3). Here, ammonium oxidation rates again display contradictory trends between
377 depth and light. Depth is more predictive in the regression model for rates > 20m, and the depth
378 range (~30 m) far exceeds the differences in light levels ($\leq 5\%$). Therefore, depth is likely a
379 better descriptor of ammonium oxidation rates at our deep sampling sites. Deep ammonium
380 oxidation rates are also related to temperature (Table 3). This positive relationship can simply be
381 explained by increased metabolic rates at higher temperature. Previous experiments in the
382 Narragansett Bay region (Sites 1 and 2), showed a positive, exponential relationship between

temperature and water column total nitrification rates, consistent with metabolic rates increasing with temperature (Berounksy 1990).

Like deep ammonium oxidation, nitrite oxidation rates at > 20m sampling depth also showed positive relationships with temperature and depth in multiple regression analysis. Thus, at least at depth, ammonium and nitrite oxidation rates may respond similarly to these two environmental controls. Deep nitrite oxidation rates also show a positive relationship with salinity in the multiple regression. However, salinity only ranged by 3 psu in deep waters. It is likely that the positive impact of salinity for deep nitrite oxidation rates was heavily influenced by a single sampling occasion at Site 3 at a salinity of 29.1, while the remaining deep nitrite oxidation rates were measured when salinity ranged from 31.6 – 32.2. Site 3 is located within Rhode Island Sound, where salinity anomalies (Shonting and Cook, 1970) and intrusions of slope water across the continental shelf (Draxler et al. 1985) have been previously reported. We suggest that temperature, depth, and $[H^+]$ (discussed below) are in fact the dominant variables influencing deep nitrite oxidation rates, and the occurrence of a low salinity intrusion event may be masking these trends.

The other *in situ* variable that largely influences deep nitrite oxidation rates is $[H^+]$ (Table 3). This positive relationship with $[H^+]$ corresponds to a negative relationship with pH, as $pH = -\log[H^+]$. Additionally, $[H^+]$ alone is the best predictor of surface nitrite oxidation rates (Table 3), and surface nitrite oxidation rates are significantly, negatively related to pH (Figure 4). While this negative relationship with pH may at first seem contradictory to current studies investigating ocean acidification, our results here are similar to trends we reported between total nitrification and pH from the 1980s in Narragansett Bay (Fulweiler et al., 2011), and suggest that the two steps of nitrification may respond dissimilarly to pH shifts.

4.3 pH impacts on nitrification

There is a growing body of literature highlighting the negative impacts of ocean acidification on nitrification rates. Specifically, these studies have found that nitrification rates decline under lower pH conditions (Huesemann et al., 2002; Beman et al., 2011; Kitidis et al., 2011). These studies however are often only looking at the impact of decreasing pH on ammonium oxidation (e.g., Beman et al. 2011) and do not include nitrite oxidation. The negative impact on ammonium oxidation is driven by the almost 50% reduction in the availability of ammonia molecules because of the pKa of approximately 9.19 of the ammonium/ammonia buffer system in seawater (Millero 2007). If ammonia is the critical substrate in nitrification, then decreases in pH will lead to decreased ammonia concentrations and thus reductions in total nitrification.

Here we found that nitrite oxidation rates were significantly negatively correlated to pH (Table 2) and that surface nitrite oxidation rates decreased as pH increased (Figure 4). The multiple regression analysis demonstrated that surface and deep nitrite oxidation rates were significantly, positively correlated with $[H^+]$ (i.e. negatively related to pH). Our data show no relationship, positive or negative, between pH and surface or deep ammonium oxidation. We previously reported that water column total nitrification rates increased as pH decreased in Narragansett Bay, close to Sites 1 and 2 in this study (Fulweiler et al., 2011). These new data suggest that even then over two decades ago nitrification in this system was driven by nitrite oxidation. They also suggest that there might be a mechanism that increases rates of nitrite oxidation under lower pH. Perhaps, like has been observed for cyanobacteria water column nitrogen fixation (e.g., Hutchins et al. 2007, Levitan et al. 2007), increased carbon dioxide concentrations have a fertilizing impact on nitrite oxidation. Regardless of the mechanism, we

propose that changes in pH may not be deleterious to total nitrification rates and these results argue for the need to study nitrification as two separate processes that are influenced by environmental parameters in different ways.

5. Conclusions

From an estuary to the continental shelf break, high potential rates of summer water column nitrite oxidation highlight this necessary half of nitrification and also suggest a decoupling from ammonium oxidation. Additionally, ammonium and nitrite oxidation rates are related in different ways to *in situ* water column conditions, and these relationships appear to change with depth. Thus, we suggest that *in situ* environmental controls on nitrification as a whole cannot be determined by evaluating ammonium oxidation alone.

Environmental conditions are continuing to change as a result of human activity, including conditions that this study indicates as important drivers of ammonium and nitrite oxidation. As we consider future changes in water column nitrogen cycling, this study underscores the idea that we can no longer apply the lessons learned from ammonia oxidation to also predict changes in nitrite oxidation. Instead, we must consider both ammonium and nitrite oxidation separately, and evaluate potential impacts on these two processes on varying spatial and temporal scales.

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Figure 1.

Locations off the Southern New England coast where water column samples were collected.

Isobath depths are in meters.

Figure 2.

Average potential rates of summer water column ammonium (A, $R^2=0.98$, $p=0.0096$) and nitrite (B, $R^2=0.96$, $p=0.023$) oxidation rates as a function of distance from Site 1 in this study.

Figure 3.

Potential rates of summer water column ammonium (A, $R^2=0.69$, $p<0.0001$) and nitrite (B, $R^2=0.56$, $p<0.0001$) oxidation rates as a function of depth at four sites along an estuary to continental shelf gradient (Site 1: circles, Site 2: squares, Site 3: triangles, Site 4: diamonds). Open symbols represent surface (< 20 m) rates, closed symbols represent deep (> 20 m) rates.

Figure 4.

Potential rates of surface (< 20 m) summer water column nitrite oxidation are negatively, exponentially related to pH ($R^2=0.90$, $p<0.0001$).

Individual water column ammonium oxidation (NH_4^+ Ox.) and nitrite oxidation (NO_2^- Ox.) rates from all four sampling sites. Environmental variables are also presented, including sampling depth, temperature, salinity, percent of surface light, dissolved oxygen concentrations (DO), pH, and *in situ* dissolved inorganic nitrogen concentrations (ammonium, $[\text{NH}_4^+]$; nitrite, $[\text{NO}_2^-]$; nitrate, $[\text{NO}_3^-]$).

Site	Date	NH ₄ ⁺ Ox. (nM d ⁻¹)	NO ₂ ⁻ Ox. (nM d ⁻¹)	Depth (m)	Temp (°C)	Salinity	Light (% surface)	DO (mg L ⁻¹)	pH	[NH ₄ ⁺] (μM)	[NO ₂] (μM)	[NO ₃] (μM)
1	Jun. 2012	0.003 0.002	1.14 1.04	0.7	21.2	24.2	n.m.	13.62	n.m.	0.60	0.02	0.34
	Aug. 2012	0.004 0.007	0.02 0.01	1.5	28.1	22.4	n.m.	8.88	8.16	0.36	n.d.	0.43
2	Jun. 2012	0.01 0.01 0.05 0.04	1.14 1.09 4.53 1.12	0.7 6.0	20.0 19.4	29.0 30.2	76.5 9.0	8.25 7.98	7.74 7.81	0.45 0.43	0.02 n.d.	0.34 0.39
	Aug. 2012	0.14 0.20 0.15 0.22	0.25 0.34 0.32 0.24	1.7 7.7	25.1 25.3	33.5 33.3	35.8 1.0	6.08 6.02	7.89 7.94	1.51 1.38	0.04 0.04	0.47 0.47
	Jul. 2013	0.00 0.03 0.01	0.97 0.84 0.53	4.6	23.8	27.3	5.4	7.23	7.85	0.64	n.d.	0.27
	Aug. 2013	0.03 0.00 0.02	0.30 0.29 0.46	4.6	21.7	29.4	9.0	7.73	7.88	n.d.	n.d.	0.23
3	Jul. 2012	0.01 0.02 0.01 0.00 0.08 0.72	0.00 0.25 0.00 0.00 1.33 0.56	1.0 21.7 35.7	22.5 19.4 17.1	30.7 30.8 29.1	80.1 0.8 0.0	8.50 9.26 7.91	7.84 7.85 7.62	0.26 0.14 3.01	n.d. n.d. 0.21	0.46 0.40 1.73
	Aug. 2012	0.02 0.03 0.43 0.67 15.67 9.20	0.00 0.00 0.36 0.15 99.09 95.53	4.2 22.5 40.3	16.6 20.4 22.5	32.2 31.6 31.7	47.4 1.8 0.1	7.42 7.05 5.59	8.15 8.11 7.96	0.75 0.38 1.00	n.d. n.d. 0.55	0.72 0.65 7.89
4	Jul. 2012	0.03 0.01 16.07 16.84 20.15 5.39	0.00 0.00 23.07 24.09 76.41 87.20	10.0 30.0 50.0	21.3 17.9 16.3	32.2 32.1 32.1	37.2 5.2 0.7	8.16 7.90 7.61	8.03 7.90 7.84	n.d. 0.78 0.19	n.d. 0.67 0.57	0.59 1.99 4.40

Table 2.

Details of the multivariate correlation analysis. Statistically significant ($p \leq 0.05$) Kendall rank correlation coefficients (Kendall's τ) are bold and shaded in gray.

Ammonium and nitrate oxidation rates (NH_4^+ Ox., NO_2^- Ox.) across all sites were compared to depth (m), percent of surface light (Light), temperature (Temp., $^{\circ}\text{C}$), salinity, dissolved oxygen (DO, mg L^{-1}), pH, and ammonium concentration ($[\text{NH}_4^+]$, μM).

		Depth	Light	Temp.	Salinity	DO	pH	$[\text{NH}_4^+]$
NH_4^+ Ox.	Kendall's τ	0.54	-0.39	-0.19	0.42	-0.48	0.03	0.22
	p-value	<0.0001	0.0027	0.11	0.0006	<0.0001	0.82	0.088
NO_2^- Ox.	Kendall's τ	0.22	-0.35	-0.20	-0.12	-0.15	-0.30	0.19
	p-value	0.069	0.0086	0.10	0.32	0.21	0.017	0.15

Table 3.

Details of the best-fitting multiple regression models as determined by stepwise regression.

Ammonium and nitrate oxidation rates (NH_4^+ Ox., NO_2^- Ox.), salinity, dissolved oxygen, percent of surface light (Light), and pH (i.e. $[\text{H}^+]$) were all log-transformed prior to analysis in order to meet the assumptions for parametric analysis.

The slope term (Estimate) of each variable in the best-fitting model along with the standardized parameter estimate (Std. Est., which normalizes the slopes of each term for ease of comparison) are presented.

Process	Location	Variable	Parameter			Model		
			Estimate	Std. Est.	<i>p</i>	Adj R^2	<i>F</i>	<i>p</i>
NH_4^+ Ox.	All	Depth	0.0085	0.89	<0.0001	0.56	15.02	<0.0001
		$[\text{H}^+]$	-8.0×10^6	-0.22	0.071			
		Light	0.083	0.19	0.19			
	Surface	Salinity	0.095	1.34	<0.0001	0.67	16.91	<0.0001
		Light	-0.0070	-0.90	<0.0001			
		Depth	-0.0005	-0.56	0.0028			
	Deep	Depth	0.018	0.96	0.0004	0.79	14.42	0.0014
		Light	2.47	0.80	0.0009			
		Temp.	0.038	0.42	0.028			
NO_2^- Ox.	All	Depth	0.022	1.06	<0.0001	0.66	23.32	<0.0001
		Temp.	0.030	0.29	0.033			
		Light	0.22	0.23	0.081			
	Surface	$[\text{H}^+]$	3.6×10^6	0.73	0.0001	0.51	22.83	0.0001
	Deep	Salinity	46	1.2	<0.0001	0.99	1866	<0.0001
		$[\text{H}^+]$	8.4×10^7	0.96	<0.0001			
		Temp.	0.13	0.61	<0.0001			
		Depth	0.024	0.55	<0.0001			

Table 4.

Summary of global, concurrently-measured water column ammonium and nitrite oxidation rates.

Locations where nitrite oxidation rates were higher than ammonium oxidation are shown in bold.

Location	NH_4^+ Ox. (nM d^{-1})	NO_2^- Ox. (nM d^{-1})	Method	Reference
Black Sea	5 - 50	48 - 240	^{15}N tracer	Ward & Kilpatrick 1991
Eastern Tropical S Pacific	0 - 280	0 - 600	^{15}N tracer	Lipschultz et al. 1990
Eastern Tropical S Pacific	0 - 89	0 - 928	^{15}N tracer	Kalvelage et al. 2013
Gulf of Mexico	9 - 494	1 - 411	^{15}N tracer	Bristow et al. 2015
Mediterranean Sea	60 - 275	60 - 170	^{14}C incorp.	Bianchi et al. 1999
N Atlantic Gyre	1.3 - 5.5	0.3 - 12	^{15}N dilution	Clark et al. 2007
N Pacific Ocean, ALOHA	1 - 123	n.d.	^{14}C incorp.	Dore & Karl 1996
N Pacific Ocean, ALOHA	1 - 137	0 - 138.0	Assay	Dore & Karl 1996
NE Pacific (British Columbia)	0 - 319	0 - 478	Inhibitor	Grundle & Juniper 2011
NE Pacific (S. California Coast)	0 - 14	0 - 79	^{15}N tracer	Olson 1981
NE Pacific (S. California)	0 - 45	0 - 110	^{15}N tracer	Ward 1987
NW African Upwelling (1% PAR)	1.2 - 2.4	0 - 8.3	^{15}N tracer	Clark et al. 2008
NW African Upwelling (55% PAR)	2.0 - 5.5	0.3 - 4.1	^{15}N tracer	Clark et al. 2008
NW Atlantic (Chesapeake Bay)	70 - 83000	380 - 35000	^{15}N tracer	Horrigan et al. 1990
NW Atlantic (New England)	0 - 20	0 - 99	^{15}N tracer	This study
NW European Shelf	4.8 - 44.4	1.2 - 695	^{15}N dilution	Clark et al. 2014
NW Mediterranean	230 - 570	140 - 350	Inhibitor	Feliatra & Bianchi 1993
NW Mediterranean	230 - 2200	140 - 1430	Inhibitor	Bianchi et al. 1994
NW Mediterranean	120 - 4200	120 - 2304	Inhibitor	Bianchi & Feliatra 1999
Oligo./Equat. Atlantic (1% PAR)	0 - 9.8	1 - 31	^{15}N tracer	Clark et al. 2008
Oligo./Equat. Atlantic (55% PAR)	0.1 - 9.2	0.4 - 12.3	^{15}N tracer	Clark et al. 2008
SE Atlantic (Namibian Coast)	10 - 125	14 - 330	^{15}N tracer	Fussel et al. 2012
Southern Ocean	20 - 80	6 - 53	^{14}C incorp.	Bianchi et al. 1997