Estuaries and Coasts (2017) 40:437–446
DOI 10.1007/s12237-016-0161-6

Organic Nitrogen Runoff in Coastal Marshes: Effects on Ecosystem Denitrification

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Received: 10 June 2015 / Revised: 2 August 2016 / Accepted: 27 August 2016 / Published online: 12 September 2016

Abstract Since the 1970s, a shift from inorganic to organic nitrogen-based fertilizer has occurred worldwide, and now urea constitutes greater than 50 % of the global nitrogenous fertilizer usage. As a result, concentrations of urea will likely increase in waterways, facilitating transport to coastal wetland habitats where microbial-mediated transformations have the ability to alleviate excess nitrogen (N) pollution. To assess this biological potential for N removal in a brackish marsh ecosystem, we conducted a 5-day laboratory experiment where we monitored denitrification rate potentials (DNP) in microcosms with intact, vegetated sods, testing treatments of different urea solutions (37.5 and 166.5 mM urea) and a nitrate solution (98.9 mM KNO_3). The addition of urea, regardless of concentration, did not stimulate DNP, while nitrate additions did. Ammonium (NH_4^+) accumulated in the porewater in response to urea treatments, with approximately 80–90 % of urea being hydrolyzed during the experiment. Nitrate concentrations in the nitrate treatment were near zero by the end of the experiment, while measureable amounts of urea were still present in both urea treatments. An increase in DNP followed nitrate additions, but an accumulation of NH_4^+ after urea additions suggests that urea pollution may not be removed by coastal wetlands as efficiently as nitrate pollution, especially when nitrification is limited under anaerobic conditions. Further work exploring the most likely pathways for removal of excess NH_4^+ is necessary to describe the potential impact that increased urea concentrations could have on coastal ecosystems.

Keywords Brackish marsh · Eutrophication · Nitrogen cycling · Urea

Communicated by Charles Simenstad

Electronic supplementary material The online version of this article (doi:10.1007/s12237-016-0161-6) contains supplementary material, which is available to authorized users.

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Introduction

Utilization of urea has increased rapidly over the past few decades (Galloway et al. 1995, 2003; Howarth et al. 2000) and is projected to constitute 70 %, or ~150 million metric tons, of global nitrogen (N)-based fertilizer use by 2020 (Glibert et al. 2006). Originally, it was thought that urea was retained in agricultural soils until it was utilized by plants and soil microbes, but recent studies demonstrate that atmospheric deposition and runoff from agricultural fields and concentrated animal operations have resulted in increased urea concentrations in aquatic ecosystems (Berman 1974; Beman et al. 2005; Switzer 2008; Bogard et al. 2012; Zhao et al. 2014). One of the few urea monitoring efforts to occur since urea production began to rise indicated that increases in urea concentrations over a 5-year period in the Chesapeake Bay were correlated with times of agricultural urea applications in the watershed (Glibert et al. 2005). In a South African estuary, urea concentrations increased tenfold over an annual baseline within 48 h of a storm event, driven primarily by runoff from surrounding pastureland and open pit toilets (Switzer 2008). Although direct runoff of urea fertilizer is likely the major pathway for increasing environmental concentrations of urea, rainfall, dust, and atmospheric aerosols have been

identified as additional sources of urea (Timperley et al. 1985; Cornell et al. 1998; Souza et al. 2015). Overall, these supply routes have been, and continue to be, altered by climate change (e.g., drought) and as industrial processes increasingly utilize urea (e.g., herbicide and insecticide production; Berrada et al. 2003). As urea becomes more affordable, available, and utilized throughout the world, it is likely that concentrations of urea will continue to increase in aquatic ecosystems.

Increased nutrient loading to coastal waters from anthropogenic sources is pervasive and can change the structure and function of coastal ecosystems (Rabalais et al. 2002). In the Gulf of Mexico, the formation of large hypoxic areas (dissolved oxygen levels <2 mg L^{-1}) is common due to N-loading from the Mississippi River basin (Rabalais et al. 2002). Although urea is a natural waste product released by invertebrates and fish, ambient levels (<μM urea-N) of urea have not been shown to affect aquatic ecosystems negatively (Mobley et al. 1995; Remsen 1971). In contrast, excess nutrient additions of urea have been identified as a substantial concern (Glibert et al. 2004, 2014; Cozzi et al. 2014), particularly given the paucity of data on the fate of urea once it enters coastal habitats. Few studies have explored the distribution and transport of urea, making it difficult to predict its spatial and temporal impacts on different ecosystems, particularly those that range from nutrient poor to nutrient rich.

Potentially negative consequences of elevated urea have been identified as a concern for sensitive coastal areas (Glibert et al. 2006). Previous studies of N loading suggest that the prevalence of chemically reduced forms of N (NH_4^+ and urea) can increase N:P (phosphorus) and thereby contribute to the growth of harmful algal blooms (HABs; Glibert et al. 2014; Berman and Chava 1999; Flores and Herreo 2005; Ginn et al. 2009). In addition, cyanobacteria and dinoflagellates in marine ecosystems often prefer reduced N forms over nitrate, responding quickly to additions of urea and NH_4^+ (Berg et al. 2003; Heil et al. 2007). Blooms of the dinoflagellate, *Prorocentrum minimum*, were observed following elevated urea concentrations in the Chesapeake Bay (Glibert et al. 2001), lending further support to the idea that increasing urea concentrations can lead to HAB formation. While the development of HABs is often associated with a number of different water quality and eutrophic conditions (Heisler et al. 2008), studies suggest that increased urea loading to water bodies can contribute to eutrophication and HABs.

The majority of research on biogeochemical cycling of urea has been completed in agricultural soils, including rice paddies (Liang et al. 2007; De-Xi et al. 2007). However, rice paddies are highly modified physically, chemically, and hydrologically to maximize crop production, making them poor surrogates for understanding the fate of urea in non-agricultural wetlands. Models designed to predict the generation of hypoxic zones and formation of noxious algal blooms in aquatic, estuarine, or marine ecosystems would benefit from a clearer understanding of processes responsible for urea removal. This is especially true in wetland ecosystems, which are often viewed as the last line of defense for adjacent water bodies.

As ecotones between terrestrial and aquatic environments, wetlands may effectively remove urea pollution via pathways such as denitrification. Microbial communities within the wetland soil matrix and overlying waters have the genetic capacity to lower concentrations of N delivered as nitrate or NH_4^+ via cellular assimilation or removal via nitrate reduction to N_2 gas (denitrification). In comparison, urea must first be hydrolyzed by active extracellular or intracellular ureases, which transform urea to NH_4^+ and carbon dioxide (Mobley et al. 1995). This NH_4^+ can then be assimilated (Gribsholt et al. 2006), lost via volatilization (Swensen and Singh 1997), nitrified (Silva et al. 2005), or converted to nitrate and removed by denitrification (Di and Cameron 2008). Of these pathways, denitrification has been identified as a primary N sink in wetlands and has been highlighted as removing excess N in many wetland ecosystems (Seitzinger et al. 2006). Unlike denitrification of nitrate, two individual steps are necessary for urea to be denitrified, and because wetland soils are often oxygen-deficient due to frequent inundation or saturation, rates of nitrification (conversion of NH_4^+ to nitrate) may be depressed, thereby limiting the loss of urea via denitrification. In the absence of coupled nitrification and denitrification, anaerobic NH_4^+ oxidation (anammox) may remove excess NH_4^+ in wetland soils when microorganisms convert NH_4^+ directly to di-nitrogen gas (N_2) under anaerobic conditions (Humbert et al. 2012; Koop-Jakobsen and Giblin 2009). Of these potential pathways of N loss, we focused on urea additions and effects on denitrification in coastal wetlands.

Our primary objective was to examine how concentrations of urea affect rates of denitrification compared to nitrate in coastal wetland soils. We collected intact, vegetated wetland sods from a brackish coastal marsh in Louisiana for use in a controlled microcosm experiment in which nitrate and urea inputs were manipulated. As wetland soils are typically oxygen-poor, we anticipated that denitrification would proceed quickly. In contrast, we predicted that available ureases would hydrolyze urea quickly, but that saturated conditions would not favor nitrification, thereby limiting denitrification. Thus, we expected differences in N removal capacity among microcosms receiving nitrate vs. urea, causing denitrification rates to be lower in urea treatments.

Materials and Methods

Site Description

Sods were collected on July 13, 2013, from a sub-tropical, microtidal (tidal range ~ 0.15 m), mesohaline (porewater

salinity ~7 psu on average) marsh along Bayou Lacombe in Big Branch Marsh National Wildlife Refuge (BBM NWR), which is located on the northern shore of Lake Pontchartrain in Louisiana, USA (N 30° 15.90′, W 89° 57.07′). Big Branch Marsh NWR covers almost 61 km^2 of coastal habitats, including marsh comprised of emergent, herbaceous vegetation. The brackish marsh plant community is dominated by two species, *Spartina patens* (Ait) Muhl. (marsh hay cordgrass), a C_4 grass, and *Schoenoplectus americanus* Volk. Ex: Schinz & R Keller (American bulrush), a C_3 sedge. Percent plant composition, based on number of shoots per microcosm, averaged 52:48 (*S. patens*:*S. americanus*) (Table 1). During 2013, air temperatures at BBM NWR ranged from 21 to 41 °C, with an average of 27 °C in early July. The total monthly precipitation during July 2013 was 22.30 cm with the most extreme daily rain event measured at 5.64 cm. Porewater N varied over time at BBM NWR, particularly for NH_4^+, and compared to the microcosms, contained less N as nitrate (Table 2). Prior to the addition of nutrients, microcosm sods were ~11–13 % organic and slightly acidic.

Experimental Microcosm Design

Twenty-five intact sods of soil and vegetation were collected at BBM NWR, placed in 13.2-L buckets (hereafter, microcosms), and transported to a greenhouse facility at the University of Alabama. Sods had both a diameter and soil depth of ~30 cm and were partially saturated for transport. Microcosms were randomly assigned to a location in the greenhouse and were not moved during the experiment. Prior to the experiment, all sods were completely saturated (unfiltered field water) and allowed to acclimate to greenhouse conditions for 36 h. Data loggers (Onset HOBO®) were used to monitor air and soil temperatures in the greenhouse. Air temperature ranged from 23 to 38 °C (avg. 28 °C), and soil temperatures measured at a depth of 5 cm ranged from 24 to 32 °C (avg. 27 °C) (Online Resource 1). Water levels were maintained using filtered Bayou Lacombe water pre-filtered with a Whatman GF/D filter (pore size = 2.7 μm), then filtered with Whatman GF/F (pore size = 0.7 μm) that was stored at 4 °C until use. Water levels were visually monitored in the buckets and adjusted manually when necessary to maintain flooding to soil surface. Although this microcosm approach limited in situ hydrologic influences on microbial soil processes, this sacrifice in realism was necessary to create a more controlled environment in which to examine DNP.

Once positioned in the greenhouse, microcosms were randomly assigned to one of four nutrient treatments (n = 5 per treatment), which included controls, a nitrate addition created by dissolving 2.0 g KNO_3 in 200 mL of deionized water (solution 98.9 mM), and two levels of urea additions. Urea groups were designated as low urea and high urea and were created by dissolving 0.45 g (solution 37.5 mM urea) and 2.0 g (solution 166.5 mM urea) urea (Baker ACS, 99.5 % min), respectively, in 200 mL of deionized water and adding the entire 200 mL to each microcosm. A second high urea group of five microcosms included a P amendment to examine potential P limitation (Online Resource 2). Porewater and soil samples were collected from each microcosm prior to enrichment (T0 = pre-treatment) and then daily for 5 days following enrichment (six total time points) to assess transformations of urea and inorganic N concentrations and changes in denitrification rate potentials (DNP).

Other studies have documented urea concentrations elevated to 25.0 μM urea-N in bulk precipitation (Souza et al. 2015); 36.2 μM urea-N after a storm in an estuary (Switzer 2008); or 150 μM urea-N in a lake (Berman 1974). Our maximum average treatment urea-N concentrations ranged from 224.02 μM (low urea after 6 h) to 259.64 μM (high urea after 30 h) urea-N and were higher than those reported in these studies. However, urea use is projected to continue increasing (Glibert et al. 2006), and as such, the goal of our study was to examine denitrification potential when exposed to high levels of nutrient loading during pulse events, not to replicate urea concentrations reported in other studies.

Water Sampling and Analysis

Duplicate porewater samples were collected using aluminum sipper tubes inserted to a depth of 10 cm in each microcosm at

Table 1 Plant species coverage in greenhouse microcosms (area = ~0.07 m^2)

Treatment	Average no. of stems		Percent cover		Avg. total aboveground biomass (g/area)
	S. americanus	*S. patens*	*S. americanus*	*S. Patens*	
Control	63.4 ± *29.4*	33.4 ± *22.8*	65.5	34.5	74.53 ± *13.8*
Nitrate	34.2 ± *29.7*	59.8 ± *61.5*	36.4	63.6	78.08 ± *51.8*
Low urea	37.6 ± *11.6*	83.8 ± *68.1*	31.0	69.0	74.82 ± *15.9*
High urea	60.0 ± *24.3*	38.3 ± *21.7*	61.1	38.9	77.52 ± *26.4*

Mean values represent vegetation collected from replicate microcosms (n = 5). Standard deviations are in italics. Average number of stems and aboveground biomass represent estimates for each treatment over microcosm area

Table 2 Comparisons of porewater N in May and June, 2013, at Big Branch NWR and in the microcosms prior to receiving nutrient treatments

Porewater (μM)			
	NH_4^+	NO_3^-	NO_2^-
May 2013	0.58 ± *0.66*	0.04 ± *0.08*	0.23 ± *0.11*
June 2013	6.17 ± *4.79*	0.11 ± *0.13*	0.21 ± *0.17*
Microcosm	1.83 ± *1.92*	2.96 ± *2.79*	0.09 ± *0.08*

Mean values are present along with standard deviations in italics

each sampling time period. Pre-treatment water samples were collected 1 h before nutrient additions were made and then 6 h post-treatment application. Following the 6-h sampling, water was collected at 30, 54, 78, and 102 h. All water samples were filtered using ashed (500 °C for 4 h), 0.7 μm Whatman GF/F filters. Urea, nitrate, nitrite, and NH_4^+ samples were stored in 20-mL scintillation vials at −20 °C until analysis. Dissolved urea (hereafter, units = μM urea-N) was quantified via a direct measurement method (Goeyens et al. 1998), with modifications detailed in Revilla et al. (2005). Nitrate, nitrite, and NH_4^+ concentrations were determined using standard wet chemical techniques modified for the Skalar SAN Autoanalyzer (Pinckney et al. 2001). Dissolved organic carbon (DOC) and soluble reactive phosphorus (SRP) samples were stored at 4 °C and analyzed within 24 h of collection (Online Resources 2 and 3). SRP was analyzed using spectrophotometric determination by the NH_4^+-molybdate method (Parsons et al. 1984). For DOC analysis, samples first were acidified with ultrapure HCl to a pH of 2 and then measured on a TOC Shimadzu TOC/TNM-1 analyzer (Hansell 1993).

Potential Denitrification Rates

To measure denitrification rates, we followed the acetylene inhibition technique (Sørensen 1978) as modified by Dollhopf et al. (2005), which estimates potential denitrification rates (DNP) by maximizing conditions conducive for the process. Nitrification is inhibited by acetylene; therefore, rates determined with this approach underestimate denitrification that would be supported by coupled nitrification–denitrification, if you assume ambient nitrate concentrations in the 4-h incubation dropped below the level limiting to denitrifiers. Three soil cores (diameter ~2 cm; 5 cm deep) containing soil and fine roots were collected from each microcosm during the experiment. Upon collection, all cores were homogenized and immediately prepared for DNP analysis. Approximately 20 g of soil and 50 mL of filtered site water were added to a 120-mL Wheaton bottle and allowed to equilibrate at room temperature. Final DNP estimates were the average of five replicates, where an individual sample consisted of three cores taken and homogenized at each time point. Bottles were sealed with airtight septae and flushed with 90 % N_2/10 % CO_2 gas mixture for 10 min to create an anoxic environment. Once each bottle was anoxic, 10 mL of acetylene gas was added to each bottle to stimulate the accumulation of nitrous oxide (N_2O) during denitrification, and samples were incubated in the dark for 4 h at 23 °C (room temperature) on a shaker table. Nitrous oxide from the headspace gas was sampled, stored in exetainer vials and analyzed within 7 days of collection using gas chromatography. A gas chromatographer fitted with an electron capture detector (Shimadzu ECD-GC-2014; Shimadzu, Canby, OR, USA) was used to measure nitrous oxide concentrations, the detection limit of which was 50 μg/L with a precision of 5 μg/L. Preliminary work with replicate marsh soils allowed us to confirm that our 4-h incubation treatment with 20 g of wet soil would produce a linear relationship. Preliminary work compared soil depth (0–5 and 5–10 cm) and soil mass (20 and 40 g) over time (2, 4, and 6 h). Denitrification rate potentials were calculated as both micromoles N_2O per gram per hour dry soil and per gram ash-free dry mass (μmol N_2O [g $AFDM^{-1}$] h^{-1}). Previous work by Wall et al. (2005) highlights that AFDM may better reflect microbial activity as it accounts for differences in soil particle size and texture.

Statistical Analysis

Treatments and the control group were monitored using a total of 20 microcosms in replicates of five. Urea, NH_4^+, nitrate, and nitrite concentrations, DNP, and pH, which were measured for three treatments and controls over time, were compared by two-way repeated-measures ANOVAs (nutrient treatment and time as factors) using the statistical program SigmaPlot 12.0 (Systat Software Inc., San Jose, CA). Tukey's HSD was used to test for interactions within and between each treatment and time point. Data that did not meet normality assumptions were transformed using natural log (ln) prior to analysis.

Results

DNP was consistently lower in both urea treatments as compared to the nitrate-enriched treatment (Fig. 1), indicating that after urea hydrolysis, coupled nitrification and denitrification was unable to remove N as efficiently as when nitrate was added directly to saturated microcosm soils. In the nitrate treatment, maximum DNP occurred mid-way through the study (54 h, 256.76 ± 49.25 μmol N_2O [g $AFDM^{-1}$] h^{-1}; $p < 0.001$) before declining to a level similar to the pre-treatment level (0.47 ± 0.29 and 3.84 ± 1.94 μmol N_2O [g $AFDM^{-1}$] h^{-1} at 0 and 102 h, respectively; Fig. 1; $p > 0.05$). In contrast, DNP in the urea treatments and controls were

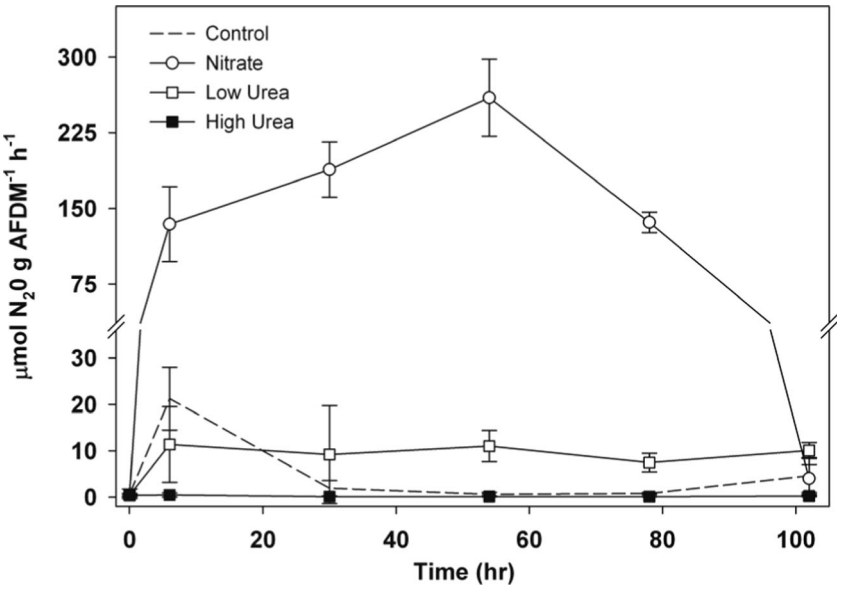

Fig. 1 Changes in denitrification rate potential measurements at ~5 cm soil depth in microcosms. The four treatments were control (no additions), plus nitrate, and plus low or high urea. *Points* are means of denitrification rate measurements collected from replicate microcosms ($n = 5$) for each treatment. *Error bars* (where visible) are standard deviation

statistically similar over time (Fig. 1; $p > 0.05$), with the greatest DNP observed at 6 h in the control. In the low urea treatment, DNP peaked at 6 h (11.34 ± 8.15 μmol N_2O [g $AFDM^{-1}$] h^{-1}) and remained relatively stable thereafter (range = 0.48 ± 0.36 to 11.34 ± 8.15 μmol N_2O [g $AFDM^{-1}$] h^{-1}), while DNP was below 1 μM μmol N_2O [g $AFDM^{-1}$] h^{-1} in the high urea treatment for the entire experiment. Despite the lack of DNP stimulation with urea enrichment, the nitrate treatment confirmed that microcosm conditions in the greenhouse were primed for denitrification to occur, with removal of almost all nitrate in porewater after approximately 4 days.

Urea concentrations increased by 22.72 ± 19.8 μM urea-N in the low treatment and 56.18 ± 15.94 μM urea-N in the high treatment after 102 h. Urea was not significantly produced in the control or nitrate treatments throughout the study (4.06 ± 3.48 and 7.86 ± 4.56 μM urea-N; Fig. 2; $p < 0.05$). Urea concentrations peaked at 6 and 30 h (224.02 ± 44.50 and 259.64 ± 139.94 μM urea-N) in the low and high urea treatments and were an order of magnitude higher than control and nitrate groups (14.68 ± 8.78 and 3.60 ± 2.46 μM urea-N, respectively; $p < 0.001$; Fig. 2). In addition to differences in urea, those microcosms with urea additions also had increased DOC concentrations (low urea range = 21.7 ± 7.1 to 38.1 ± 2.3; high urea range = 29.1 ± 10.6 to 69.0 ± 29.5), but only in the high urea treatment did DOC concentrations remain elevated above pre-treatment levels ($p < 0.001$; Online Resource 3). No change in DOC over time was observed in control and nitrate treatments ($p > 0.05$; Online Resource 3).

Prior to nutrient additions, the average nitrate concentration across treatments was 3.75 ± 1.51 μM. In the control and urea treatments, porewater nitrate concentrations remained low, with maximum nitrate concentrations of only 5.12 ± 3.12 and 4.89 ± 6.24 μM, respectively. By the end of the study, nitrate was depleted in the urea treatments, as concentrations fell below the detection limit. In the nitrate treatment, porewater nitrate increased initially (Fig. 2; $p < 0.001$), with the highest concentration of nitrate (83.95 ± 2.75 μM) occurring at 30 h. By the end of the study, nitrate had decreased significantly below pre-treatment levels (Fig. 2; 0 vs. 102 h: $p = 0.002$) and was similarly low among all treatments (Fig. 2; $p > 0.05$).

Patterns of porewater nitrite concentrations in the nitrate treatment were similar to, but lower than, those for nitrate (Online Resource 4). Like nitrate concentrations, nitrite concentrations increased initially, reaching a maximum concentration of 6.90 ± 2.72 μM at 30 h before declining to pre-treatment levels (Online Resource 4; $p < 0.001$). In the urea treatments, nitrite concentrations remained low, with concentrations remaining relatively stable over time (0.22 ± 0.07 to 0.30 ± 0.18 μM; $p > 0.05$).

Regardless of treatment group, NH_4^+ accumulated in all microcosms over the course of the experiment; however, the timing and extent to which this occurred differed among treatments. In both urea treatments, concentrations of NH_4^+ increased over the first 6 h and remained elevated relative to pre-treatment levels throughout the remainder of the experiment (Fig. 2; $p < 0.001$), despite declines over time in the low urea treatment ($p < 0.001$). Similarly, NH_4^+ concentrations increased initially in the nitrate treatment until 30 h ($p < 0.001$) before declining thereafter ($p < 0.001$). NH_4^+ concentrations in the nitrate treatment were consistently lower than those of the two urea treatments and, although initially higher than the control, were similar between the nitrate and control groups by the end of the study ($p = 0.332$).

Prior to nutrient additions, soil porewater pH was similar for all microcosms, at an average of 6.36 ± 0.35 (Fig. 3). After nutrients were added, however, pH increased above 8.0 for

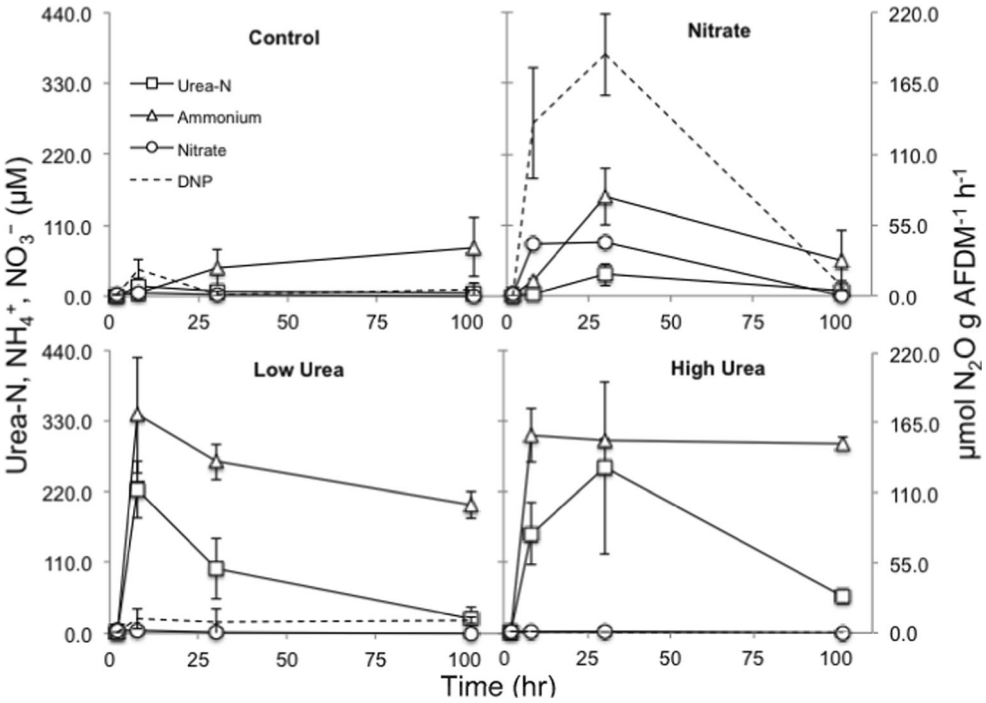

Fig. 2 Changes in porewater nutrient concentrations between treatments, control, nitrate, low urea, and high urea. Left *y-axis* depicts change in concentration of urea-N, NH_4^+, and nitrate. Right *y-axis* depicts change in denitrification rate potentials. *Points* represent mean porewater nutrients and denitrification rates collected from replicate microcosms (n = 5) at each time point. *Error bars* (where visible) are standard deviation

both urea treatments (Fig. 3). In contrast, pH initially dropped to 6.05 ± 0.70 after 6 h in microcosms receiving nitrate before rising thereafter (Fig. 3). By 30 h, all nutrient-amended treatments had an average pH above 8.0, while pH in the controls increased more slowly by comparison.

Discussion

Based on current trends of urea production and use, it is likely that urea loading to aquatic ecosystems will continue to increase. As filters in the landscape, wetlands have the potential to minimize the impact of excess nutrients. Unlike inorganic N pollution, however, our study tested the capacity of coastal wetlands to deal with concentrated pulses of urea, underscoring potential problems associated with the removal of excess urea in saturated wetland soils. Under these conditions, NH_4^+ accumulated in soils and denitrification was lower than in soils amended with nitrate. As predicted, nitrification was unable to generate enough nitrate to stimulate denitrification in urea treatments above rates found in the control. If urea continues to replace nitrate as a fertilizer, and loading of urea from animal husbandry and wet/dry deposition continues to intensify, the ability for coastal wetlands to mitigate increased urea concentrations may be reduced. Collectively, our results demonstrate the potential for limited urea removal in coastal wetland ecosystems and highlight the need to better understand the fate of excess urea.

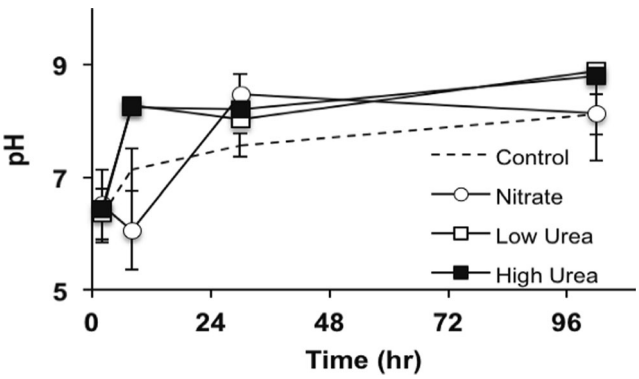

Fig. 3 Change in porewater pH in microcosm treatments over 5 days. *Points* are means of measurements taken from microcosm replicates (n = 5). *Error bars* are standard deviation

Similar to previous studies (e.g., Rivera-Monroy et al. 2010; Palta et al. 2014), we found nitrate additions increased DNP in soils and that final nitrate concentrations in all microcosms were reduced to near zero by the end of the experiment. These results suggest that DNP was limited by the supply of nitrate and that the uptake kinetics of the denitrifying community were not saturated with regard to nitrate. This pattern, along with DNP measurements, suggests that conditions in the current microcosm study favored denitrification when nitrate was available. This is not surprising given our experimental design in which microcosms remained saturated, thereby limiting pore space available to oxygen and restricting the diffusion of atmospheric air into the soil. Unlike many terrestrial ecosystems, wetlands are characterized by anaerobic, hydric soils that favor denitrification (e.g., Mulvaney et al. 1997; Morrissey and Franklin 2014; Seitzinger et al. 2006). Tidal wetlands, which otherwise experience fluctuating water levels, can remain fully submerged for a number of days during and after storm events (Stark et al. 2015), which is when

we would expect the greatest nutrient runoff to occur. Under conditions that favor high, urea-rich runoff, DNP will likely be limited through suppression of the coupling of nitrification and denitrification while soils are fully saturated (Hefting et al. 2004, Palta et al. 2014).

With an emphasis on denitrification as the major pathway for N removal, our results support a conceptual model (Fig. 4) that highlights the accumulation of NH_4^+ and minimal potential for denitrification in saturated wetland soils. While studies have shown that wetland plants can promote coupled nitrification-denitrification by creating an oxidized rhizosphere (e.g., Reddy et al. 1989; Hamersley and Howes 2005; Revsbech et al. 2005), in highly reduced conditions, oxygen transport to the rhizosphere may be inadequate to overcome root oxygen deficiencies and can result in NH_4^+ accumulation (Mendelssohn et al. 1981; Mendelssohn and McKee 1988). In our experiment, coupled nitrification-denitrification was not alleviated by oxygen released by plants in the rhizosphere, as indicated by the accumulation of NH_4^+ and reduced DNP. Instead, our results support reported patterns of declining nitrification with increasing soil saturation, where maximum rates of nitrification are expected at water potentials near −10 kPa or with a water table 10–30 cm below the surface (Saby 1969; Schjønning et al. 2003; Hefting et al. 2004). In the fully saturated soils of our study, water potential was near −33 kPa and produced conditions that were unfavorable for nitrification. We acknowledge that by keeping microcosm soils saturated, our study did not capture the potential for tidal fluctuations to enhance nitrification during low tides, and we would expect the rates of nitrification to vary under normal field conditions in which water levels fluctuate over time. Even so, it is likely that denitrification rates would be lower in wetlands receiving urea compared to those receiving nitrate because demand for nitrate for denitrification and biological assimilation is high.

In addition to a decoupling of nitrification and denitrification, short circuits to N cycling in our experiment could have been created by slow rates of urea decomposition. The initial hydrolysis of urea is dependent on the availability of urease, which has not been well studied in wetlands and could be limiting in some ecosystems. Urease activity can vary on seasonal and spatial scales. For example, over an elevation gradient in a Gulf of Mexico tidal marsh, urease rates were found to be highest at lower elevation sites, but changes in edaphic conditions and temperature were found to significantly affect urease activity over time (Lee, unpublished data). In the waterlogged soils of our study, the majority of urea transformations were likely the result of soil extracellular enzymes, microorganism-associated membrane enzymes, and organism uptake (Mobley and Hausinger 1989; Jørgensen 2006; Solomon et al. 2010). However, when soils become saturated with NH_4^+, it is possible that urease production ceases (Mobley and Hausinger 1989; Pedersen and Borum 1993), which would explain why urea concentrations remained elevated compared to pre-treatment levels in the urea treatments. Based on observations from our experiment, removal of urea pollution could be impeded by the buildup of excess NH_4^+ in saturated soils. Similarly, organisms such as ammonia-oxidizing bacteria and archaea are also known to be sensitive to high levels of NH_4^+ (Bollmann and Laanbroek 2001; Tourna et al. 2010), with substrate (NH_4^+/NH_3) inhibition of nitrification starting at ~1.0 mM in a variety of soils (Koper et al. 2010; Shi and Norton 2000; Norton and Stark 2011), levels that may have occurred in our study, but were not captured in our experimental design.

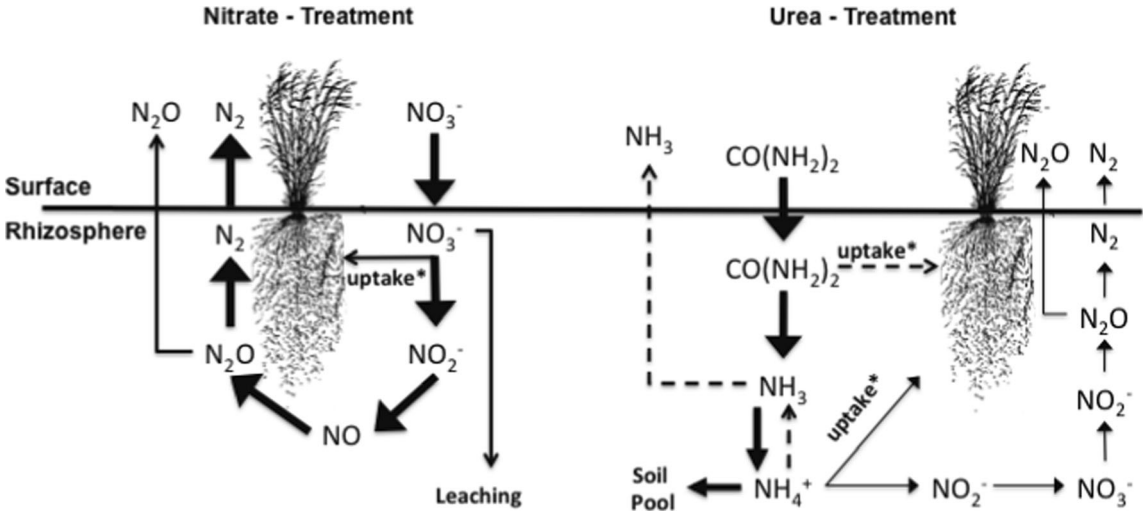

Fig. 4 Conceptual model illustrating microcosm differences in removal pathways for N treatments in saturated soils. *Arrow weight* represents the likelihood of each N removal pathway based on our experimental results. *Thicker lines/arrows* identify transformations that were occurring at high rates and *thinner lines/arrows* identify transformations that were occurring more slowly or not at all. *Dashed lines/arrows* represent pathways in which data were not available to support, but are potentially important pathways for urea removal. *uptake** represents microbial and plant assimilation

Increases in pH have been previously documented following urea additions, as was observed in our study (Singh and Nye 1984). Porewater pH increased from 6.40 ± 0.05, a pH similar to that observed at the field site, to 8.84 ± 0.06 in our urea treatments over time, which should have favored nitrification. However, this pH shift could have affected the rates at which nitrification would occur and may have caused shifts in microbial community structure (Sahrawat 1982; Hartman et al. 2008). Ammonia-oxidizing bacteria and archaea both contribute to nitrification (Lam et al. 2007; Nugroho et al. 2005), but evidence suggests that pH plays an important role in selecting the ammonia-oxidizing community present under different environmental conditions (e.g., Di and Cameron 2008; Wessén et al. 2010). As a consequence of pH shifts in microcosms, not only could a change in distribution of ammonia-oxidizing bacteria and archaea have occurred, previous research has highlighted how pH shifts can affect their NH_4^+ oxidizing activity (Avrahami et al. 2003; Nicol et al. 2008). If we had monitored our microcosms longer, it is possible a community of ammonia-oxidizing organisms would have established under these higher pH conditions and been more effective at oxidizing the NH_4^+ present. It is uncertain how long such a transition or recovery in prokaryotic community and activity may take.

NH_4^+ concentrations leveled off in our study after ~30 h, which could also be the result of the increase in pH. Typically, NH_4^+ (ions) and ammonia (gas) exist at equilibrium, but this relationship is dependent on pH. As pH becomes more basic (7.5–8.0), the equilibrium will shift towards ammonia. Given our pH measurements, it is possible the ammonia concentrations increased considerably. Thus, the chance for ammonia (gas) to be lost during the latter half of our experiment could have increased as well. Previous work by Saggar et al. (2013) highlighted the loss of ammonia gas after the hydrolysis of urea in soil systems. Ammonia volatilization at a higher pH may represent an alternate pathway for N loss from wetlands, but it is unclear to what extent this pathway is capable of removing urea pollution under different hydro-edaphic conditions. Also, wetlands have been recognized for their ability to buffer a variety of polluting effluents (Faulkner and Richardson 1989; Dunbabin and Bowmer 1992); thus, further studies will be necessary to understand potential ramifications of increasing urea and pH shifts in wetland ecosystems. By examining shifts in porewater chemistry, we demonstrated that N removal via denitrification was higher in microcosms amended with nitrate than in those amended with urea, even when pH was high enough for the volatilization to occur. Studies examining alternative pathways of NH_4^+ transformation are needed to understand the potential fate of urea loading to wetlands. Anaerobic NH_4^+ oxidation (anammox) is one alternative pathway, which unlike nitrification-denitrification, is completed by specialized prokaryotes that do not require oxygen (Jetten et al. 1998; Strous et al. 1999). Thus, the anammox pathway may become more important for N removal in coastal wetlands as urea fertilizer use increases (Dalsgaard et al. 2005; Nicholls and Trimmer 2009).

Based on economic and agricultural projections, the overall use and production of urea will increase in the future (Glibert et al. 2006) and will likely contribute to the delivery of excess urea to coastal wetlands. Coastal wetlands have the potential to mitigate urea loading via denitrification, yet their capacity for mitigation may be hindered with coastal development, climate change, and other environmental changes that alter hydro-edaphic conditions or contribute to wetland loss (Kirwan and Megonigal 2013). Our study demonstrates that excess urea pollution entering coastal wetlands may not be removed as efficiently as inorganic forms of N. In addition, it is important to note that impacts from agricultural runoff may not be the only pathway for increasing urea concentrations, as atmospheric deposition of urea has been found to be significant over continental regions such as the Mediterranean Basin (Bo et al. 2009, Violaki and Mihalopoulos 2011). Based on our results, the ability of coupled nitrification-denitrification to remove excess urea before it reaches adjacent estuaries and bays may be limited under saturated soil conditions, resulting in the accumulation of urea and NH_4^+ that may negatively affect the biotic structure and function of wetlands. Our study provides a direct comparison between urea and nitrate and begins to explain observations of other studies in which elevated concentrations of urea were not removed before entering coastal ecosystems (e.g., Glibert et al. 2005 and Switzer 2008). We hypothesize that future inputs of excess urea will continue to cause nutrient enrichment in coastal waters because conditions in coastal wetlands may reduce rates of coupled nitrification-denitrification. Therefore, to fully understand the impact of urea on ecosystems, additional studies are needed to explore this overlooked form of N pollution.

Acknowledgments We would like to express our sincere appreciation to Dr. Behzad Mortazavi for his support and allowing us to complete our DNP analysis in his laboratory, and Joshua Jones for his guidance at Big Branch Marsh NWR. For their help in the field and with additional microcosm and sample preparation, we thank Jamie Galloway, Adam Constantin, and Mindy Russo. Also, we thank the anonymous reviewers for their comments and edits during the review process of this manuscript. Funding for this work was provided by the University of Alabama Howard Hughes Medical Institute undergraduate research program.

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