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Changes in abundance and community structure of nitrate-reducing bacteria along a salinity gradient in tidal wetlands

Rima B. Franklin*, Ember M. Morrissey[1], Joseph C. Morina

Virginia Commonwealth University, Department of Biology, 1000 West Cary Street, Richmond, VA 23284, USA

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ABSTRACT

Primarily because of their high levels of microbiological activity, wetland soils play a key role in ameliorating nitrogen pollution and reducing nutrient loadings to coastal zones. Saltwater intrusion associated with climate change is expected to dramatically affect the biogeochemistry of these soils and induce changes in the composition of the soil microbial community that may alter their ability to process nitrogen. In this study, the abundance and community structure of microorganisms associated with two key nitrate removal pathways – denitrification and dissimilatory nitrate reduction to ammonium (DNRA) – were assessed for eight tidal wetlands along a naturally-occurring salinity gradient (0–2 ppt) in the Chesapeake Bay watershed (USA). Molecular analyses targeted functional genes specific for each pathway (denitrification: *nirS* and *nosZ*; DNRA: *nrfA*). Shifts in abundance and community structure of both groups of nitrate reducers were strongly coupled to changes in salinity, and correlation analyses suggested that the effect of salinity on these organisms may have been mediated, at least in part, by changes in soil organic matter availability. Considering the growing body of evidence that microbial community composition may help regulate ecosystem process rates, an increased understanding of how salinity affects nitrogen cycling microbial communities may help us better predict how wetland soil function will be affected by global change and issues such as sea level rise.

1. Introduction

Wetlands are sensitive to environmental stressors and the effects of global climate change are already evident in these ecosystems. Intrusion of saltwater into historically freshwater systems is of particular concern and will occur globally through a variety of mechanisms including changes in precipitation and rising sea levels (Herbert et al., 2015). Because salinity directly impacts a wide range of abiotic and biotic processes, it is considered to be a major driver of ecosystem structure and function (e.g., Brucet et al., 2012; Lozupone and Knight, 2007; Mendelssohn et al., 1999). Though salinity has been shown to impact microbial communities and associated biogeochemistry (e.g., Baldwin et al., 2006; Chambers et al., 2011; Weston et al., 2011), most prior studies consider broad salinity gradients (i.e., freshwater to marine). There is less work examining modest salinity gradients (e.g., freshwater versus oligohaline conditions such as Edmonds et al., (2009), Morrissey et al. (2014), Morrissey and Franklin (2015), Neubauer et al. (2013), and Poffenbarger et al. (2011)), even though that transition is what many freshwater systems are likely to experience as a consequence of sea level rise in the coming decades (Woodroffe and Murray-Wallace, 2012).

One of the most valuable ecosystem services provided by wetlands is the ability to remove excess nutrients, especially nitrogen (N), from surface waters (Fisher and Acreman, 2004). However, most prior research that considers saltwater intrusion focuses on how increased salinity will affect methanogenesis and sulfate reduction (e.g., Bartlett et al., 1987; Weston et al., 2006, 2011), and the effects of salinity on N cycling are less well understood (Giblin et al., 2010; Marks et al., 2016; Marton et al., 2012; Osborne et al., 2015). The study presented here considers how the soil microbial communities associated with two important N cycling functional processes, denitrification and dissimilatory nitrate reduction to ammonium (DNRA), may be regulated by

* Corresponding author.
E-mail addresses: rbfranklin@vcu.edu (R.B. Franklin), ember.morrissey@mail.wvu.edu (E.M. Morrissey), morinajc@vcu.edu (J.C. Morina).
[1] Present address: West Virginia University, Division of Plant and Soil Sciences, Morgantown, WV 26505, USA.

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salinity. In general, these groups are heterotrophic bacteria that use nitrate (NO_3^-) as the terminal electron acceptor for anaerobic respiration, yielding N-gasses (mostly N_2, with some N_2O) and ammonium (NH_4^+) respectively. Much of the N removal from freshwater wetlands is attributed to denitrification (Craft et al., 2009; Giblin et al., 2010; Herbert et al., 2015; Rysgaard et al., 1999), but appreciable rates of DNRA have also been documented (e.g., see Giblin et al., 2013). However, in many marine systems, DNRA has been found to be as important as denitrification (Bernard et al., 2015; Ma and Aelion, 2005; Tobias et al., 2001; Yang et al., 2015).

In this study, we examined how the abundance and community structure of these two groups of nitrate reducers changed in soils collected from tidal wetlands along a naturally-occurring salinity gradient in the Chesapeake Bay watershed (Virginia, USA). In an effort to limit co-variants that often confound data interpretation following gradient sampling, we chose sites that were close enough that weather, land-use, tidal influence, and underlying lithology were likely similar, and plot selection targeted nearly identical plant communities. Our analyses focused on process-specific functional genes that code for key enzymes associated with each nitrate reduction pathway. By and large, denitrifiers can be divided into two groups based on whether they possess the *nirK* gene (which codes for a copper binding dissimilatory nitrite reductase) or the *nirS* gene (which codes for the cytochrome cd1 variant) (Zumft, 1997; Graf et al., 2014). We focused on *nirS* gene as it seems predominant in this study system (10^6–10^8 copies per g of soil organic matter, compared to 10^1–10^3 copies for *nirK*; Morina et al., Unpublished data) and used it as a proxy for denitrifier abundance. For analysis of denitrifier community composition, we focused on *nosZ*, which codes for the nitrous oxide reductase that catalyzes the terminal step in complete denitrification ($N_2O \rightarrow N_2$). Not all denitrifiers possess *nosZ*, and incomplete denitrification stops with the production of N_2O. Recent work has shown that *nosZ* co-occurs with *nirS* more frequently than *nirK,* and comparative phylogenetic analysis indicates a greater degree of shared evolutionary history between *nosZ* and *nirS* than *nosZ* and *nirK* (Graf et al., 2014). To study DNRA, we used the *nrfA* gene, which codes for the nitrite reductase that generates ammonium.

2. Materials and methods

2.1. Sampling

Eight tidal wetlands, varying in salinity from fresh to oligohaline (Table 1), were sampled during a two-week period in June 2010. At each site, a $10 \times 10\ m^2$ plot was established that contained at least 75% cover of *Peltandra virginica* (an obligate wetland plant common on the eastern coast of North America). Within each plot, five sampling stations were randomly selected with the caveat that the minimum separation distance between stations was 3 m. Above- and below-ground plant biomass were determined as described in Morrissey et al. (2014). In addition, samples of surface soil (0–10 cm depth) were collected and transported back to the lab on ice in airtight plastic bags.

2.2. Soil and porewater properties

Soil pH and redox potential were immediately measured (Hanna Combo pH and ORP probe, Smithfield, RI, USA) and a subsample of soil (~5 g) was archived at −20 °C for the microbial community analyses described below. Soil texture was assessed using the hydrometer method (Gee and Or, 2002). Soil organic matter (OM) concentration (% as loss on ignition, 425 °C for 12 h), and C and N content (Perkin Elmer Series II CHNS/O Analyzer 2400; Waltham, MA, USA) were determined. Porewater was collected by centrifugation (~5 ml of soil at $3000 \times g$ for 15 min), filtered (0.45 μm pore size), and then analyzed via ion chromatography (Dionex ICS-1000, Sunnyvale CA, USA) to determine Cl^-, SO_4^{2-}, and NO_3^- concentrations. Standard methods were used to analyze porewater NH_4^+ concentrations (Skalar segmented flow analyzer, Skalar Analytical, The Netherlands; Rice et al., 2012). Salinity (ppt) was calculated as described by Bianchi (2006) and log transformed prior to statistical analyses.

2.3. Microbial community assays

Whole-community DNA was extracted from frozen soils using the MoBio PowerSoil Kit (MoBio, Carlsbad, CA, USA) and quantified

Table 1
Site locations and environmental data for each wetland (mean ± SE, N = 5 per site).

Site (abbreviation)	GPS (N)	GPS (W)	Porewater Chemistry			
			Salinity (ppt)	SO_4^{2-} (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (ug L^{-1})†
James River NWF (JR)	37°16′27”	77°09′18”	0.03 ± 0.01	14.1 ± 7.4	0.1 ± 0.0	11.5
Walkerton Landing (WL)	37°43′60”	77°00′96”	0.04 ± 0.01	21.2 ± 4.1	0.6 ± 0.4	58.3
Yarmouth Creek (YC)	37°19′64”	76°52′26”	0.15 ± 0.03	20.0 ± 6.5	0.5 ± 0.3	7.9
Morris Creek (MC)	37°16′78”	76°53′38”	0.16 ± 0.01	68.2 ± 16.4	0.2 ± 0.2	2.1
Blackstump Creek (BC)	37°18′76”	76°51′89”	0.17 ± 0.02	86.1 ± 17.7	0.1 ± 0.0	0.2
Gleason Marsh (GM)	37°38′14”	76°51′39”	0.54 ± 0.04	46.5 ± 6.0	0.2 ± 0.1	209.4
Sweet Hall Marsh (SH)	37°33′02”	76°53′31”	0.88 ± 0.08	43.3 ± 7.8	0.5 ± 0.3	59.8
College Creek (CC)	37°15′08”	76°42′60”	1.86 ± 0.11	134.8 ± 47.5	0.4 ± 0.2	35.6

Site (abbreviation)	Plant Biomass		Soil Properties				
	Aboveground (kg m^{-2})	Belowground (mg cm^{-3})	pH	Redox (mV)	OM (%)	C:N	Texture† (% sand, silt, clay)
James River NWF (JR)	0.23 ± 0.04	4.8 ± 1.1	6.1 ± 0.0	−11 ± 5	37.4 ± 2.9	12.7 ± 0.6	36, 38, 26
Walkerton Landing (WL)	0.19 ± 0.04	4.4 ± 1.8	5.6 ± 0.2	309 ± 26	35.8 ± 1.7	12.6 ± 0.5	56, 30, 14
Yarmouth Creek (YC)	0.16 ± 0.02	23.8 ± 3.4	6.4 ± 0.1	−96 ± 9	10.4 ± 2.0	11.4 ± 0.2	35, 42, 23
Morris Creek (MC)	0.12 ± 0.03	6.34 ± 0.3	6.2 ± 0.2	−65 ± 28	18.2 ± 0.6	11.5 ± 0.3	35, 35, 30
Blackstump Creek (BC)	0.11 ± 0.03	30.0 ± 3.4	6.2 ± 0.1	23 ± 19	34.2 ± 5.0	12.9 ± 0.6	33, 39, 28
Gleason Marsh (GM)	0.53 ± 0.12	1.0 ± 0.2	5.6 ± 0.1	255 ± 58	15.3 ± 0.4	10.8 ± 0.4	32, 32, 36
Sweet Hall Marsh (SH)	0.14 ± 0.05	6.1 ± 2.2	6.2 ± 0.2	−64 ± 31	14.2 ± 0.6	11.3 ± 0.3	34, 36, 30
College Creek (CC)	0.20 ± 0.04	9.1 ± 2.7	6.4 ± 0.2	−73 ± 22	16.4 ± 0.7	10.8 ± 0.7	52, 26, 22

†Due to the limited amount of sample, analysis was performed using pooled material comprised of equal fractions from each replicate.