Vol. 602: 87–102, 2018
https://doi.org/10.3354/meps12678

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 23

Restoration enhances denitrification and DNRA in subsurface sediments of *Zostera marina* seagrass meadows

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ABSTRACT: Seagrasses exude oxygen and labile carbon into the sediment, which can stimulate microbial activity. However, it is not clear how seagrasses impact competing nitrate reduction processes, including nitrogen removal through denitrification and nitrogen retention through dissimilatory nitrate reduction to ammonium (DNRA). Using an *in situ* push–pull incubation method, we measured denitrification and DNRA rates in the root zone of a restored *Zostera marina* meadow, in adjacent unvegetated sediments, and in experimentally cleared plots within the meadow. Denitrification and DNRA rates in the meadow sediments were highly variable and contained 'hotspots' where maximum rates exceeded median rates by more than an order of magnitude. Hotspots were not observed in bare sediments, leading to average rates 4× greater in vegetated sediments than in bare sediments. In the meadow sediments, denitrification dominated over DNRA except in fall, during seagrass senescence, and after the experimental removal of seagrass. Extrapolated rates of annual nitrate removal via denitrification were greater in the vegetated sediments compared to bare sediments (0.62 compared to 0.16 g N m^{-2} yr^{-1}) and accounted for 44% of annual N loading to the system. Similarly, annual DNRA rates were greater in the vegetated compared to bare sediments (0.45 and 0.12 g N m^{-2} yr^{-1}, respectively). The restoration of the seagrass meadow thus increased both nitrogen removal and recycling, but removal via denitrification was the dominant process. The dominance of denitrification demonstrates how seagrass restoration can enhance the filter function of shallow coastal systems.

KEY WORDS: *Zostera marina* · Denitrification · DNRA · Push–pull · Restoration

INTRODUCTION

Anthropogenic eutrophication in coastal ecosystems is a major environmental challenge (National Research Council 2000, Howarth & Marino 2006). As increasing amounts of reactive nitrogen enter the biosphere, much of that nitrogen will ultimately travel to coastal ecosystems, leading to nutrient overenrichment and associated negative effects, including algae blooms, anoxia, and fish kills (Galloway et al. 2004, Seitzinger et al. 2006, Howarth 2008). The impact of increased nitrogen loading on coastal and estuarine systems will depend in part on the capacity of these areas to filter incoming nitrogen (Cloern 2001). Coastal seagrass meadows have the potential to serve as an effective nutrient filter. Temporary accumulation of nitrogen in seagrass biomass and more permanent storage in meadow sediment are 2 important pathways through which seagrasses enhance nitrogen removal from the water column (McGlathery et al. 2007). In addition, seagrass can stimulate biogeochemical cycling in meadow sediments, potentially leading to the removal of nitrogen.

Denitrification, the microbially mediated transformation of nitrate into inert dinitrogen gas, requires a supply of nitrate, reduced carbon substrate, and

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anoxic conditions. In sediments below the sediment–water interface, the nitrate to support denitrification is typically produced via nitrification, an aerobic process that converts ammonium into nitrate. Coupled nitrification–denitrification is common in low-nutrient ecosystems; in seagrass meadows, this coupled process is generally linked to plant metabolism. Seagrass roots exude both oxygen and labile organic carbon into the subsurface sediments, creating oxidized microzones and steep redox gradients that support coupled nitrification–denitrification (Frederiksen & Glud 2006, Jovanovic et al. 2015). Oxygenation via roots may also reduce sulfide concentrations in sediments (Pagès et al. 2012), in turn reducing sulfide-inhibition of denitrification (Brunet & Garcia-Gil 1996). Seagrass meadows may also influence denitrification rates by increasing sedimentation of organic matter and thus enhancing the supply of reduced carbon in meadow sediments.

Despite the altered biogeochemical conditions in seagrass sediment, it is not clear whether seagrass meadows stimulate denitrification relative to unvegetated sediments. Several studies have measured low rates of denitrification in seagrass meadows (Rysgaard et al. 1996, Risgaard-Petersen & Ottosen 2000, Welsh et al. 2000, Russell et al. 2016), in some cases lower than in adjacent unvegetated areas (Risgaard-Petersen et al. 1998, Ottosen et al. 1999). These low rates are often attributed to competition for nitrate from benthic microalgae. However, other studies have found that denitrification rates in seagrass meadows greatly exceed rates in adjacent unvegetated tidal flats (Eyre et al. 2011, Piehler & Smyth 2011, Smyth et al. 2013). These higher rates in seagrass meadows were observed in systems with low nutrient loading, where competition for nitrate would be high. Thus, there is uncertainty in the literature over the net effect of seagrass on denitrification rates. Methodological differences may explain some of these patterns; low rates of denitrification have been measured mainly using the isotope pairing technique (e.g. Risgaard-Petersen et al. 1998, Welsh et al. 2000, Russell et al. 2016), in which an isotope tracer diffuses into surface sediments, whereas higher rates have been measured using the N_2:Ar technique (e.g. Eyre et al. 2011, Smyth et al. 2013), which integrates over a deeper sediment depth. However, methodology does not entirely explain these patterns; a recent study using the N_2:Ar method also found low rates of denitrification in seagrass sediments, comparable to the rates measured with isotope pairing (Zarnoch et al. 2017). Moreover, it is important to note that the N_2:Ar measurements of enhanced rates in seagrass meadows have relied primarily on incubations conducted under dark conditions, which would alleviate competition for nitrate from autotrophs, and could overestimate daily and annual rates. Further study of denitrification rates in seagrass meadows is therefore needed to clarify whether seagrass stimulates denitrification.

Denitrification also competes with dissimilatory nitrate reduction to ammonium (DNRA). Like denitrification, DNRA requires nitrate, reduced carbon (or sulfide), and anoxic conditions. Partitioning between DNRA and denitrification depends on factors including the relative availability of nitrate and organic carbon, the presence of sulfides, and the quality of the carbon substrate (Burgin & Hamilton 2007, Hardison et al. 2015). In contrast to denitrification, DNRA retains nitrogen in the sediment as biologically available ammonium; thus, the balance between these competing processes may alter net nitrogen removal. Relatively few studies of DNRA have been conducted in seagrass meadows to date; in some studies, DNRA was low relative to denitrification (Boon et al. 1986, Smyth et al. 2013), while in others DNRA was equal to or greater than denitrification (Rysgaard et al. 1996, An & Gardner 2002, Gardner et al. 2006). This variation suggests that further study is needed to better understand partitioning between these 2 nitrate reduction processes (Giblin et al. 2013).

Uncertainty surrounding the magnitude and partitioning of denitrification and DNRA rates in seagrass meadows may be related in part to limitations of traditional sampling methods. Conventional methods rely on laboratory incubations of cores or sediment slurries that typically do not capture rates under *in situ* conditions of light and flow that are linked to plant activity (Koch et al. 2006, Rheuban et al. 2014b) or fully capture subsurface rates or plant effects. Collection of cores may also damage below-ground biomass, leading to release of dissolved organic carbon and ammonium that can stimulate microbial processes (Hansen & Lomstein 1999, Gribsholt & Kristensen 2002). In contrast, a new push–pull method can be used in the field, where miniature piezometers inject isotopically labeled $^{15}NO_3^-$ into seagrass sediments while maintaining the complex sediment matrix and without disturbing the hydrodynamic flow, light availability, or other drivers of seagrass activity (Koop-Jakobsen & Giblin 2009). In a comparison with traditional core incubations, this push–pull method measured higher rates of both denitrification and DNRA, as well as greater variability in those rates, that were attributed to sediment heterogeneity, natural variation in field conditions, and the irregular

effects of plant exudation (Aoki & McGlathery 2017). The push–pull method has limitations as well; notably, implementation of the method is constrained by practical considerations, and because the method targets subsurface processes, it is not sufficient in systems where microbial activity at the sediment surface dominates total denitrification and DNRA rates. However, by targeting subsurface processes and therefore capturing the plant effects on redox gradients and labile carbon supply, the push–pull method is particularly appropriate for measurements of denitrification and DNRA in the complex sediment matrix of the seagrass root zone.

Accurate measurements of the seagrass effect on nitrate reduction processes is critical to understanding how seagrass restoration affects the coastal nutrient filter. As a large-scale and well-established restoration project, our study site in the Virginia coastal bays is an ideal system to test for these impacts. Seagrass seeding in the Virginia coastal bays has transformed over 25 km^2 of unvegetated benthos into seagrass meadow since 2001. Work at this site has shown for the first time that seagrass restoration reinstates the capacity to sequester carbon in both biomass and sediments (McGlathery et al. 2012, Greiner et al. 2013, Oreska et al. 2017), but the impacts of seagrass restoration on nutrient filtration are not yet known. By measuring nitrate reduction rates in the restored meadow and in adjacent bare sediment, we can determine for the first time whether the restoration has enhanced denitrification and therefore enhanced the nutrient filter function of the seagrass meadow.

In this study, we used the push–pull method to compare nitrate reduction rates at vegetated sites within the restored meadow to rates in unvegetated sediment outside the meadow. In addition to the external bare-site comparison, we wanted to isolate the effect of seagrass presence on sediment conditions and consequently on denitrification and DNRA rates. The external bare sites experience different environmental conditions compared to the meadow sites (i.e. deeper water column, higher flow velocities, larger sediment grains) which may impact rates. We therefore conducted a removal experiment within the meadow in which we compared rates measured in the meadow sediments to rates measured in plots within the seagrass meadow that experienced identical environmental conditions where we experimentally cleared above- and below-ground seagrass biomass. Finally, we conducted seasonal measurements within the seagrass meadow in order to understand patterns in nitrate reduction rates over time.

MATERIALS AND METHODS

Site description

South Bay is a shallow lagoon located on the Atlantic coast of the eastern shore of Virginia. The mean water depth is 1.4 m and the mean tidal range is 1.2 m (Fagherazzi & Wiberg 2009). Seagrasses were historically present in South Bay, and other Virginia coastal bays, until the mid-1930s, when a combination of the pandemic wasting disease *Labyrinthula* sp. and a severe hurricane caused a local extinction (Orth & McGlathery 2012). A landscape-scale restoration experiment was begun in 2001; over 7.5×10^6 *Zostera marina* seeds were broadcast in replicate 0.2 and 0.4 ha plots beginning in 2001. In South Bay, the original plots coalesced into a contiguous meadow that has continued to spread, covering approximately 680 ha in 2015 (Orth et al. 2012, Oreska et al. 2017). Sediments in South Bay are predominantly fine sands (McGlathery et al. 2012). Long-term monitoring has shown a shift in sediment characteristics, with smaller grain sizes and increased organic matter content in the restored meadow (McGlathery et al. 2012), and recent work has shown that the restored meadow has achieved carbon storage capacities on par with natural meadows (Greiner et al. 2013). Nutrient loading to South Bay is low compared to coastal lagoons throughout the world, at approximately 1.4 g N m^{-2} yr^{-1} (McGlathery et al. 2007, Anderson et al. 2010) and water quality is high, with dissolved inorganic nitrogen concentrations frequently undetectable in surface water.

At this site, dissimilatory nitrate reduction occurs predominantly in subsurface sediments. Denitrification and DNRA measured in surface sediments using a traditional isotope pairing core incubation were exceedingly low (approximately 0.1 µmol m^{-2} h^{-1} in both seagrass and bare sediments) and were 34 to 135× less than rates measured using the push–pull method (Aoki & McGlathery 2017). In this system, it is therefore appropriate to rely on the push–pull method to measure denitrification and DNRA. In other systems with greater contributions from surface rates, fully capturing the dissimilatory nitrate reduction rates would require combining the push–pull method with another method targeting surface rates.

Subsurface rates of denitrification and DNRA were expected to be low in the bare sediments. However, previous work has shown that (1) bare sediments in these lagoons are sufficiently permeable to allow advective transport to dominate over porewater diffusion (Huettel & Gust 1992, Rheuban et al. 2014a) and (2)

that wave energy is significantly greater in the bare sediments outside the seagrass meadow (Hansen & Reidenbach 2012). Tidally driven advection of oxygen into the upper centimeter of the bare sediments could therefore support nitrification below the surface, supplying nitrate to denitrification and DNRA that could be captured with the push–pull method. Oxygenation of macrofauna burrows could also support subsurface rates (Pelegri et al. 1994, Wenzhöfer & Glud 2004, Meysman et al. 2010). Overall, any subsurface rates were expected to be lower in the bare sediments compared to vegetated sediments.

Experimental design

The sampling design for the 3 components of this study is summarized in Table 1. For all 3 components, denitrification and DNRA rates were measured using the push–pull method, described below. All push–pull measurements were conducted during the day (i.e. with ambient sunlight available); light and flow conditions varied naturally over the course of each 6 h push–pull deployment and between deployments conducted on different days. Additional samples were collected to measure sediment characteristics, porewater chemistry, and seagrass metrics; details are included below. All sampling within the meadow was conducted within the areas of the initial seeding (3 replicate 0.4 ha plots) in order to ensure that all seagrass plots were the same age (13 yr since restoration in 2014 and 14 yr in 2015).

Table 1. Sampling design of the study

Study component	Sites	Sampling dates	Total push–pull measurements
External bare site comparison	1 meadow site	June–August 2014	10
	1 external bare site	June–August 2014	8
Removal experiment	3 meadow sites	June–July 2015	10, 12[a]
	3 cleared sub-plots	June–July 2015	10
Seasonal monitoring	1 meadow site	June–August 2014	10
	1 meadow site	October 2014	7
	3 meadow sites	April 2015	9
	3 meadow sites	June 2015	10

[a]10 replicate measurements before sub-plots were cleared, 12 replicate measurements during Weeks 2 to 4 of the experiment

External bare site comparison

Denitrification and DNRA were measured *in situ* using the push–pull technique throughout summer 2014 in order to gain data representative of the seagrass growing season. Rates were measured at one seagrass site in the interior of the meadow, and at one unvegetated, bare site located adjacent to the meadow edge. Between 2 and 4 push–pull measurements of nitrate reduction were made at both the seagrass and bare sites in June, July, and August for a total of 8 to 10 measurements at each site across the seagrass growing season (Table 1). Some environmental parameters influencing microbial activity remained constant over the summer (e.g. sediment temperature; Table 2), but other parameters, especially seagrass biomass, varied (Table 3). Temporal variability in these environmental parameters likely contributed to the overall variability in the compiled summer nitrate re-

Table 2. Sediment and porewater characteristics at the seagrass and external bare sites from June 2014 to June 2015. Values are mean (SD); nd: no data; –: months that the bare sites were not sampled. Porewater nitrate concentrations were below the detection limit (0.87 µM) across all sites and months

	Sediment Temperature (°C)		Salinity (ppt)		Porewater [NH_4^+] (µM)		Porewater [HS^-] (µM)	
	Seagrass	Bare	Seagrass	Bare	Seagrass	Bare	Seagrass	Bare
June 2014	29.0 (1.4)	29.0	32.5 (0.7)	nd	nd	nd	20.4 (13.2)	4.6 (5.4)
July 2014	28.0 (1.4)	28.0	33.5 (0.7)	33.0	nd	nd	2.5 (1.7)	1.5 (1.6)
Aug 2014	28.0	29.0	34.0	32.0	17.3 (12.0)	56.5 (15.1)	5.6 (6.8)	1.8 (1.2)
Oct 2014	21.3 (0.6)	–	34.3 (0.6)	–	56.6 (47.7)	–	4.9 (6.1)	–
April 2015	15.5 (1.5)	–	35.3 (0.6)	–	4.3 (3.8)	–	4.9 (2.2)	–
June 2015	26.5 (3.0)	–	32.3 (2.1)	–	10.1 (5.2)	–	8.5 (7.2)	–

Table 3. Seagrass shoot densities and biomass measured at meadow sites from June 2014 to June 2015. Values are mean (SD), 'nd' indicates no data, '–' indicates months that the bare sites were not sampled

	Shoot density (shoots m^{-2})	Aboveground biomass (g DW m^{-2})	Belowground biomass (g DW m^{-2})	Chlorophyll *a* (mg m^{-2})	
				Seagrass	Bare
June 2014	424 (76)	136.9 (53.5)	73.9 (13.3)	31.9 (10.4)	24.3 (4.6)
July 2014	638 (89)	167.4 (101.5)	208.2 (56.7)	19.1 (4.2)	nd
August 2014	431 (101)	201.4 (64.3)	95.3 (28.8)	91.9 (70.0)	18.2 (4.9)
October 2014	205 (58)	65.1 (8.9)	51.5 (15.8)	25.0 (21.4)	–
April 2015	320 (54)	34.7 (20.0)	44.4 (25.8)	5.6 (4.0)	–
June 2015	346 (43)	50.5 (33.1)	55.1 (22.5)	11.6 (6.4)	–

duction rates. However, variability was also driven by root exudations and non-uniform accumulation of particulate organic matter, leading to heterogeneous sediment conditions on short temporal and small spatial scales. Replicate push–pull measurements conducted simultaneously within ~3 m^2 could vary by an order of magnitude during all summer months.

Porewater samples were collected during the push–pull measurements for dissolved inorganic nitrogen (DIN) and sulfide analysis. DIN samples were filtered (0.45 µm) and frozen until analysis. NH_4^+ and NO_3^- concentrations were measured on a Lachat Quik Chem 8500 using standard colorimetric techniques (Zhang et al. 1997). Detection limits were 1.12 µM for NH_4^+ and 0.87 µM for NO_3^-. Sulfide samples were trapped with zinc acetate in the field and stored at 4°C until spectrophotometric analysis following Cline (1969).

At both the seagrass and bare sites, sediment samples were collected to determine porosity, organic matter, carbon, and nitrogen content of the sediment. A cut-off plastic syringe (2.5 cm inner diameter, i.d.) was used to collect 5 sediment samples to a depth of 5 cm at each site. Sediment samples were dried at 60°C to a constant weight; dry and wet weights were used to calculate sediment porosity. Organic matter was calculated based on loss on ignition after 6 h in a 500°C muffle furnace. Carbon and nitrogen content of sediments were measured on a Carlo Erba Elemental Analyzer with a 1020°C combustion tube, 650°C reduction tube, and helium as a carrier gas. Sediment samples were also collected to measure the concentration of chlorophyll *a* (chl *a*) as a proxy for benthic microalgae abundance. A small cut-off syringe (1 cm i.d.) was used to collect 5 replicate surface sediment samples (2 cm depth) at each site. Samples were kept in the dark on ice and frozen on return to the laboratory. For analysis, thawed samples were extracted overnight in a 45:45 methanol: acetone solution and analyzed spectrophotmetrically after Lorenzen (1967).

At the seagrass site, shoot densities were measured by counting individual shoots in 10 haphazardly distributed 0.25 m^2 quadrats. Seagrass biomass was measured in triplicate cores (15.24 cm i.d., 15 cm depth); cores were sieved through 1 mm mesh and seagrass biomass was sorted into above- and below-ground fractions. Biomass samples were dried to constant weight at 60°C.

Removal experiment

In summer 2015, a removal experiment was conducted in the meadow interior in order to compare denitrification and DNRA in sediments exposed to identical environmental conditions except for the presence of seagrass. Experimental sub-plots (4 m^2) were established at 3 of the original meadow plots. Plastic lawn edging was used to delineate the sub-plots and was inserted into the sediment to a depth of 8 cm. Denitrification and DNRA rates were measured in these sub-plots and in surrounding seagrass sediments, before the removal of seagrass shoots (Fig. 1). There was no statistical difference between rates in the sub-plots and surrounding sediments (Mann-Whitney *U*-test, $p > 0.05$ for denitrification and DNRA). Sediment samples were also collected to compare bulk sediment properties in the sub-plots and the surrounding sediments (see Table 5). The experiment was then begun by removing seagrass shoots within the sub-plots by hand; rhizomes in the surface sediments were also removed. Approximately 97% of living rhizome mass occurred in the upper 2 cm of sediment (based on below-ground biomass in sediment cores segmented by 2 cm increments; data not shown); by removing these surface rhizomes and attached roots, we eliminated the majority of conduits for products of plant metabolism to deeper sediments. The cleared sub-plots were then left to equilibrate and re-establish sediment redox gradients for 2 wk after clearing. The plastic lawn edging was left in place in order to prevent re-colonization of the cleared sub-plots by the surrounding seagrass

Two wk after the removal, denitrification and DNRA rates were again measured in the cleared sub-plots

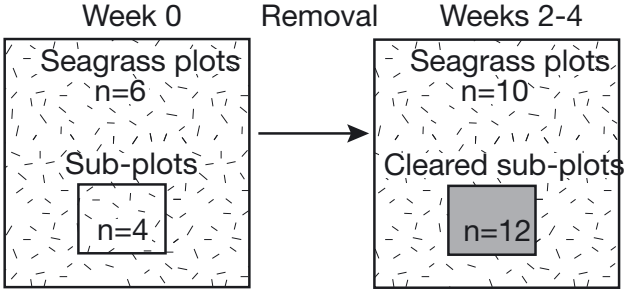

Fig. 1. Experimental design of the seagrass *Zostera marina* removal experiment, showing the number of total push–pull measurements conducted in the seagrass plots (lines) and manipulated sub-plots (gray) before and after the removal of seagrass shoots

and in the surrounding seagrass sediments; these measurements were repeated 4 wk after removal. The cleared sub-plots remained bare during the 4 wk of the experiment. Samples for porewater DIN and sulfide and for sediment properties were collected and analyzed as above. There was no statistical difference in rates between Weeks 2 and 4 (Mann Whitney *U*-test, $p > 0.05$ for both seagrass and cleared plots), so the rates were pooled. Analyses were then conducted to compare the rates in 3 data sets: (1) seagrass pre-removal (rates measured in sediments with seagrass present before the removal occurred); (2) seagrass at Weeks 2 to 4 (rates measured in sediments with seagrass present during Weeks 2 and 4); and (3) cleared (rates measured in the experimentally cleared plots during Weeks 2 and 4).

Seasonal monitoring

Additional measurements of denitrification and DNRA were made in seagrass sediments during October 2014 and April 2015. These measurements were combined with the summer 2014 and summer 2015 measurements at seagrass sites to complete a seasonal data set for seagrass sediments only. Rates were measured at one meadow plot in October 2014 (n = 7 total) and at 3 plots in April 2015 (n = 9 total). Porewater samples for DIN and sulfide and seagrass density and biomass samples were measured as above.

Push–pull incubation technique

In the experiments described above, a new push–pull incubation technique was used to measure denitrification and DNRA in the seagrass and unvegetated sediment. Building on work by Koop-Jakobsen & Giblin (2009) and Addy et al. (2002), the push–pull technique is a non-destructive approach to measuring nitrate reduction in subsurface sediments under field conditions. Details of the technique are described in Aoki & McGlathery (2017), and are summarized briefly below.

To measure dissimilatory nitrate reduction using the push–pull technique, a miniature piezometer (1.8 mm i.d.) was inserted into the sediment to a depth of 5 cm. Viton tubing connected the piezometer to a graduated cylinder that served as a reservoir. A peristaltic pump was used to slowly (~4 ml min^{-1}) pump ~200 ml of porewater out of the sediment into the graduated cylinder; a 20 ml layer of castor oil in the cylinder was used to prevent exchange between the porewater and the atmosphere. Duplicate 12 ml samples of porewater were collected in Exetainers and fixed with 50 µl of $ZnCl_2$ (100% m/v) and stored in a water bath. An additional 10 ml sample was filtered (0.45 µm) and stored on ice for DIN analysis, and two 1 ml samples were fixed with 0.01 M zinc acetate for sulfide analysis.

The porewater was then amended with a spike of artificial seawater containing $^{15}NO_3^-$ (99% ^{15}N; Cambridge Laboratories) and saturated with argon gas (Ar). After spiking, the concentration of nitrate in the porewater was approximately 100 µM. Duplicate samples were again collected, fixed, and stored in a water bath. The spiked porewater was then pumped ('pushed') into the sediment and allowed to incubate *in situ*. Additional samples were retrieved ('pulled') at half-hour intervals over the next 2 h to produce a time series. After the final porewater sample was collected, a small sediment core (2.54 cm i.d., 10 cm depth) was collected from the injection point and frozen for ammonium extraction and DNRA analysis.

Porewater samples were held in the water bath at or below the field temperature until analysis using membrane inlet mass spectrometry (MIMS) within 6 wk. MIMS was used to determine the concentrations of denitrification products ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$) and Ar in the samples (Kana et al. 1994). A copper reduction column heated to 500°C was included inline with the MIMS to remove oxygen from the gas analyte before analysis. Previous work has shown that oxygen can interfere with detection of other gas signals, leading to overestimation of denitrification using the isotope pairing technique (IPT) equations (Eyre et al. 2004, Lunstrum & Aoki 2016). Ar concentrations were used to correct for diffusion and gas loss; $^{29}N_2$ concentrations were also corrected to account for

mixing with ambient porewater and impurities in the $^{15}NO_3^-$ spike (Koop-Jakobsen and Giblin 2009, Aoki & McGlathery 2017). Linear production rates (p_{29} and p_{30}) were calculated from the corrected time series of $^{29}N_2$ and $^{30}N_2$. Isotope pairing equations (Eqs. 1 and 2) were then used to calculate D_{14}, the denitrification of ambient nitrate, and D_{15}, the denitrification of the amended $^{15}NO_3^-$ nitrate (Nielsen 1992):

$$D_{15} = p_{29} + (2 \times p_{30}) \quad (1)$$

$$D_{14} = D_{15} \times \frac{p_{29}}{(2 \times p_{30})} \quad (2)$$

These rates were converted from units of µM h^{-1} to areal rates (µmol N m^{-2} h^{-1}) using the sediment porosity and integrating over the depth of the incubation (calculated from the volume of amended porewater returned to the sediment, see Aoki & McGlathery 2017 for details).

DNRA analysis was conducted using a modified OX/MIMS method (Yin et al. 2014). The frozen sediment cores were thawed and ammonium was extracted with 90 ml of 2 M KCl. After extraction, each sample was centrifuged, and 5 replicate Exetainers were filled with the supernatant. A hypobromite solution, prepared as in Yin et al. (2014), was added to 3 of the 5 Exetainers, causing the ammonium to oxidize to N_2. All 5 vials were then analyzed using MIMS for $^{29}N_2$ and $^{30}N_2$ concentrations. Excess $^{29}N_2$ and $^{30}N_2$ in the oxidized vials compared to the unoxidized vials was assumed to result from the oxidation of $^{15}NH_4^+$, the product of DNRA in the sediment. $DNRA_{15}$, the reduction of the $^{15}NO_3^-$ spike, was calculated as the production of $^{15}NH_4^+$ over time. $DNRA_{14}$ was then calculated from Eq. (3), which assumes that the probability of reducing $^{14}NO_3^-$ or $^{15}NO_3^-$ is the same for DNRA as for denitrification (Christensen et al. 2000):

$$DNRA_{14} = DNRA_{15} \times \frac{D_{14}}{D_{15}} \quad (3)$$

Again, rates were integrated over the depth of the incubation to determine areal rates. For both denitrification and DNRA, the reduction of $^{14}NO_3^-$ (D_{14} and $DNRA_{14}$) was considered the ambient rate, or the underlying rate under natural conditions. Because nitrate concentrations in this system were very low (consistently below the detection limit of 0.87 µM in porewater), the ambient rates refer to low-nitrate conditions. In contrast, the reduction of the added $^{15}NO_3^-$ spike (D_{15} and $DNRA_{15}$) was considered the potential rate, or the rate under high-nitrate conditions.

Statistical analysis

The denitrification and DNRA rates measured in the seagrass sites were often non-normal, with maximum rates exceeding the median value by an order of magnitude, and log-transformations did not achieve normality. Conservative non-parametric methods were therefore used to compare the data sets, and boxplots were used to assess differences in the distributions. Mann Whitney *U*-tests were used for the comparison with the external bare site, and Kruskal-Wallis tests were used for the removal experiment and the seasonal data. Statistical analyses were conducted in R v.3.3.3 (R Core Team 2017).

RESULTS

External bare site comparison

Ambient denitrification and DNRA rates were on average 4 times greater at the seagrass site compared to the bare site (mean denitrification and DNRA rates were 19.7 and 12.2 µmol N m^{-2} h^{-1}, respectively, at the seagrass site compared to 4.9 and 3.1 µmol N m^{-2} h^{-1} at the bare site). The rates measured at the seagrass site were also characterized by extreme rates that exceeded median rates by an order of magnitude, whereas extreme rates were not observed at the bare site (Fig. 2). Due to the high variability in the seagrass rates, the differences between sites had low statistical significance (Mann-Whitney *U*-tests, p = 0.10 for denitrification, p = 0.09 for DNRA).

Dissimilatory nitrate reduction at both the seagrass and bare sites was limited by nitrate availability. Concentrations of nitrate in the porewater were undetectable at both sites (Table 2), suggesting that all dissimilatory nitrate reduction was coupled to nitrification. Potential rates (measured as reduction of the excess $^{15}NO_3^-$ spike) were significantly greater than ambient rates across both sites (Mann-Whitney *U*-test, $p < 0.0005$ for both denitrification and DNRA), indicating a nitrate limitation under ambient conditions (Fig. 3). There was no significant difference in potential rates between the sites (Mann-Whitney *U*-tests, p = 0.48 for denitrification and p = 0.30 for DNRA). Comparing the distributions, the potential DNRA distributions were very similar between the 2 sites, whereas potential denitrification had a higher median value and greater spread at the seagrass site. Spatially and temporally variable competition for nitrate from the seagrass likely contributed to the

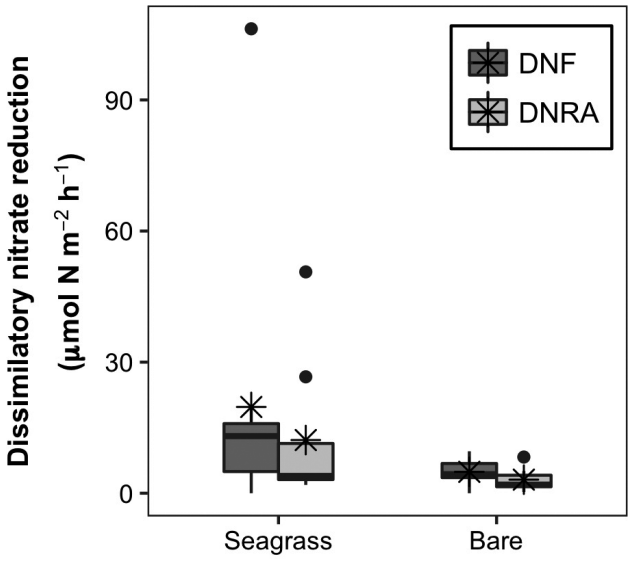

Fig. 2. Ambient denitrification (DNF) and dissimilatory reduction to ammonium (DNRA) rates measured in the *Zostera marina* seagrass meadow interior during summer 2014 had higher mean values, greater variability, and extreme maximum values compared to rates measured at the external bare site. Box-and-whisker plots show the 25th to 75th quartiles in the box, with black bars at the median and stars at the mean rates. Whiskers denote maximum and minimum rates up to 1.5× the length of the box; outlier rates are shown individually as black dots

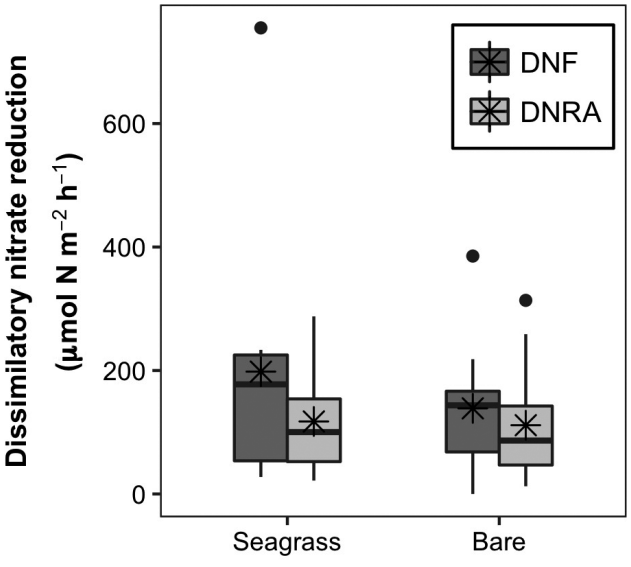

Fig. 3. Potential nitrate reduction rates (rates under high nitrate conditions) measured in the *Zostera marina* seagrass meadow and external bare site in summer 2014 were an order of magnitude greater than ambient rates (shown in Fig. 2). See Fig. 2 for explanation of box-and-whisker plot parameters

greater spread in potential denitrification rates at the seagrass site compared to the bare site. However, the minimum and maximum potential rates were higher at the seagrass site. At the bare site, multiple incubations produced undetectable potential denitrification rates (i.e. no measureable production of $^{30}N_2$ or $^{29}N_2$), and the maximum rate was about half the maximum rate at the seagrass site. These differences suggest that seagrass presence did have a stimulatory effect on denitrification, despite additional competition for nitrate.

Removal experiment

In the seagrass removal experiment, denitrification and DNRA showed contrasting patterns following removal (Fig. 4). Specifically, mean denitrification rates declined from 15.2 µmol N m^{-2} h^{-1} in seagrass plots before removal to 11.1 µmol N m^{-2} h^{-1} in the seagrass plots at Weeks 2 to 4 and 5.3 µmol N m^{-2} h^{-1} in the cleared plots at Weeks 2 to 4 (Kruskal-Wallis test, p = 0.11). In contrast, mean DNRA rates were relatively constant between the treatments, at 11.8 µmol N m^{-2} h^{-1} in the pre-removal seagrass plots, 13.7 µmol N m^{-2} h^{-1} in the seagrass plots at Weeks 2 to 4, and 15.4 µmol N m^{-2} h^{-1} in the cleared plots (Kruskal-Wallis test, p = 0.74). Consequently, while DNRA accounted for only 45% of total dissimilatory nitrate re-

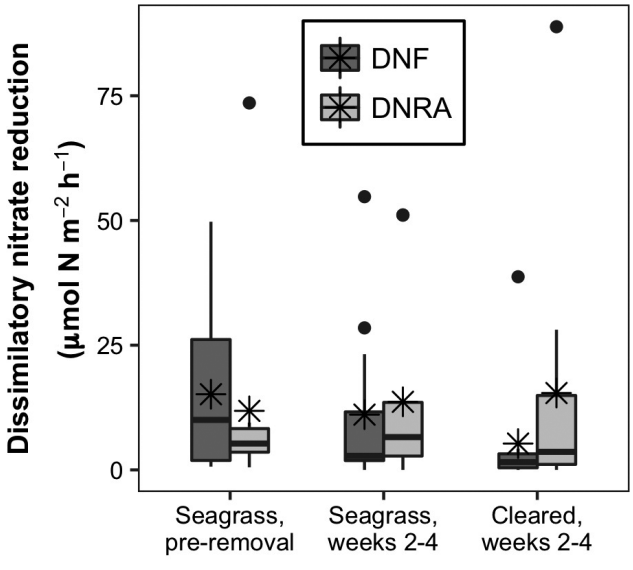

Fig. 4. Ambient rates of denitrification (DNF) declined in the seagrass *Zostera marina* and cleared plots after removal, but rates of dissimilatory nitrate reduction to ammonium (DNRA) remained constant. See Fig. 2 for explanation of box-and-whisker plot parameters

duction in the pre-removal seagrass plots, DNRA dominated in both the seagrass plots at Weeks 2 to 4 and the cleared plots, accounting for 61 and 71% of total dissimilatory nitrate reduction, respectively. These contrasting patterns suggest that the seagrass removal altered conditions in the sediment to favor DNRA over denitrification. A decrease in nitrification could have led to that change by creating high-carbon, low-nitrate conditions favorable to DNRA. The presence of extreme outliers throughout the data set again suggests that these effects on the sediment were heterogeneous over small spatial scales.

Comparing the seagrass rates before removal and at 2 to 4 wk is complicated by the fact that the meadow experienced a die-back event after the removal experiment was initiated, likely caused by high surface water temperatures. Shoot densities declined from over 350 shoots m^{-2} in the pre-removal seagrass plots to 150 shoots m^{-2} in the seagrass plots at the end of the experiment. With lower seagrass densities, the effects of seagrass activity on sediment biogeochemistry were likely reduced compared to the pre-removal seagrass plots. The comparison of measurements in the cleared plots with the seagrass plots at 2 to 4 wk is therefore a conservative estimate of the seagrass effects on nitrogen removal.

Changes in porewater chemistry were also observed following the seagrass removal in the cleared plots, where porewater ammonium concentrations increased by an order of magnitude, possibly indicating the lack of plant uptake (Table 4). A similar effect may have occurred in the seagrass plots at 2 to 4 wk, where seagrass shoot densities declined rapidly in response to the high-temperature event. Sulfide concentrations were similar in the seagrass plots throughout the experiment but were slightly elevated in the cleared plots. The seagrass removal may have increased sulfide concentrations by eliminating the transfer of oxygen from roots to the sediment; however, this effect was limited as sulfide concentrations in the cleared plots remained low compared to coastal ecosystems with highly sulfidic (100 to 1000 µM) sediments such as salt marshes.

Table 4. Porewater concentrations of ammonium and sulfide during the *Zostera marina* seagrass removal experiment. Values are mean (SD). Porewater nitrate concentrations were below the detection limit (0.87 µM) throughout the experiment

	Porewater $[NH_4^+]$ (µM)	Porewater $[HS^-]$ (µM)
Seagrass, pre-removal	10.9 (5.5)	8.5 (7.1)
Seagrass, Weeks 2–4	175.6 (119.4)	11.0 (16.8)
Cleared, Weeks 2–4	154.6 (105.5)	44.2 (74.1)

Under high-nitrate conditions, potential rates in the removal experiment were significantly greater than ambient rates (Mann-Whitney *U*-test, $p < 0.005$ for both denitrification and DNRA) and followed similar patterns as the ambient rates under low-nitrate conditions (Fig. 5). Specifically, potential denitrification dominated in the pre-removal seagrass plots and declined following removal in the seagrass plots and cleared plots, whereas potential DNRA was constant before and after removal in all plots. This pattern again suggests either greater carbon availability or greater nitrification in the pre-removal seagrass plots, although the trends in potential rates were not statistically significant (Kruskal-Wallis tests, $p = 0.24$ for denitrification and $p = 0.12$ for DNRA).

Denitrification rates were similar in the cleared sediments within the meadow and the external bare sediments outside the meadow (5.3 and 4.9 µmol m^{-2} h^{-1}, respectively). In contrast, DNRA rates were higher in the cleared sediments compared to the external bare sediments (15.4 and 3.1 µmol m^{-2} h^{-1}, respectively). Nitrate availability was low in both the cleared and bare plots (ambient nitrate concentrations were undetectable and the nitrate spike produced significantly higher potential rates). However, the cleared plots in the removal experiment had higher bulk organic matter and bulk carbon content

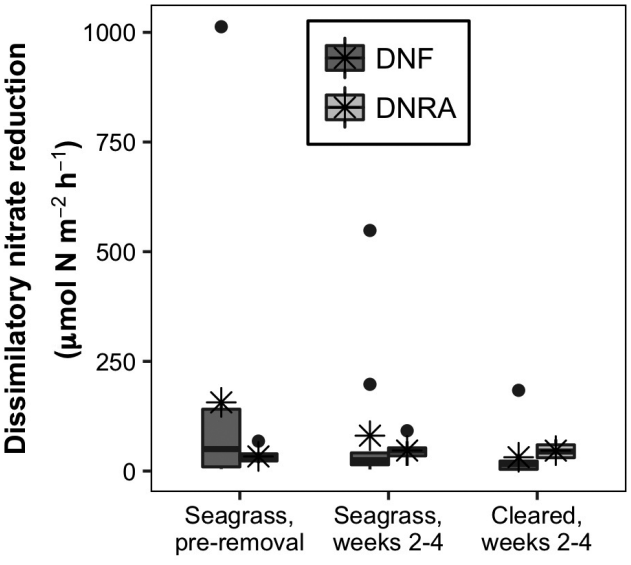

Fig. 5. Potential nitrate reduction rates (rates under high nitrate conditions) in the *Zostera marina* seagrass removal experiment followed similar trends to the ambient rates in Fig. 4. See Fig. 2 for explanation of box-and-whisker plot parameters

Table 5. Bulk sediment characteristics for seagrass, external bare, and cleared plots. Values are mean (SD)

Site and year	Organic matter (%)	C content (%)	N content (%)	C:N	Bulk density (g cm^{-3})	Porosity (%)
Seagrass, 2014	2.53 (0.74)	0.57 (0.13)	0.04 (0.01)	13.3 (3.4)	1.45 (0.15)	0.52 (0.10)
External bare, 2014	1.39 (0.21)	0.42 (0.16)	0.02 (0.002)	17.5 (4.3)	1.46 (0.36)	0.44 (0.10)
Seagrass, 2015	2.01 (0.45)	0.41 (0.10)	0.03 (0.01)	14.1 (2.2)	1.37 (0.17)	0.60 (0.05)
Cleared, 2015	2.00 (0.44)	0.47 (0.10)	0.03 (0.01)	13.7 (1.6)	1.29 (0.12)	0.55 (0.06)

than the bare plots (Table 5). Some amount of belowground biomass was also likely present in the cleared plots, despite efforts to remove rhizomes from the surface sediments, and any remaining roots could have leached organic carbon into the sediments. Thus, more organic carbon was likely available at the cleared plots, creating low-nitrate, high-carbon conditions that favor DNRA over denitrification (Burgin & Hamilton 2007).

Seasonal patterns in nitrate reduction

Measurements of nitrate reduction in the meadow from June 2014 to June 2015 showed that denitrification was on average greater than DNRA during spring and summer (Fig. 6). Denitrification showed a seasonal pattern, with the highest mean rates in the summer and the lowest mean rates in the spring (Kruskal-Wallis test, p = 0.13). DNRA also showed peak rates in summer, but there was no trend between seasons (Kruskal-Wallis test, p = 0.48). Low nitrate reduction rates in spring may indicate competition for nitrate from rapidly growing seagrass; although porewater nitrate levels were undetectable throughout the year, porewater ammonium concentrations were at a minimum in spring, suggesting greater plant uptake of nitrogen (Table 2). Lower mineralization rates in spring might also account for the low porewater ammonium concentrations.

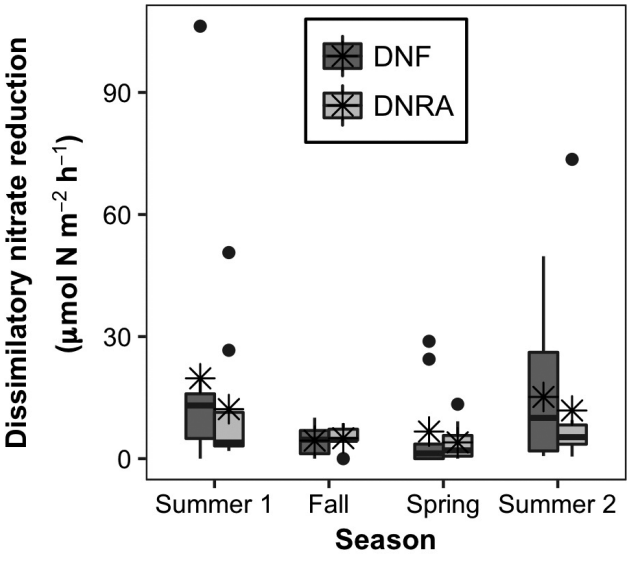

Fig. 6. Seasonal monitoring of ambient denitrification (DNF) and dissimilatory nitrate reduction to ammonium (DNRA) rates in the *Zostera marina* seagrass meadow interior showed extreme rates throughout spring and summer. See Fig. 2 for explanation of box-and-whisker plot parameters

As noted above, in summer 2014, the maximum rates of both denitrification and DNRA were roughly an order of magnitude greater than the median rates. This pattern was also evident in spring and summer 2015 for DNRA and in spring 2015 for denitrification. These maximum rates indicate that within the heterogeneous sediment matrix, conditions existed to support very high rates of dissimilatory nitrate reduction during spring and summer. In contrast, maximum rates in the fall were approximately 2× the median rates for both denitrification and DNRA, suggesting conditions were less conducive to supporting high dissimilatory nitrate reduction rates.

Under high-nitrate conditions, both potential denitrification and potential DNRA were significantly enhanced across all seasons compared to the ambient rates (Mann-Whitney *U*-test, $p < 0.005$; Fig. 7). Significant differences were observed between summer 2014 and spring 2015 for potential denitrification and between summer 2014 and summer 2015 for potential DNRA (Kruskal-Wallis test, $p < 0.05$). More interestingly, the pattern of extreme rates was evident for potential denitrification across the seasons, with maximum rates that exceeded median rates by 4 to 47 times. In contrast, potential DNRA rates were not as strongly enhanced by the nitrate spike, with maximum potential rates no more than 3× the potential median rates across all seasons. Thus, while extreme rates of both DNRA and denitrification were possible under the low-nitrate ambient conditions, the addition of the excess nitrate spike enhanced the maximum rates of denitrification compared to DNRA.

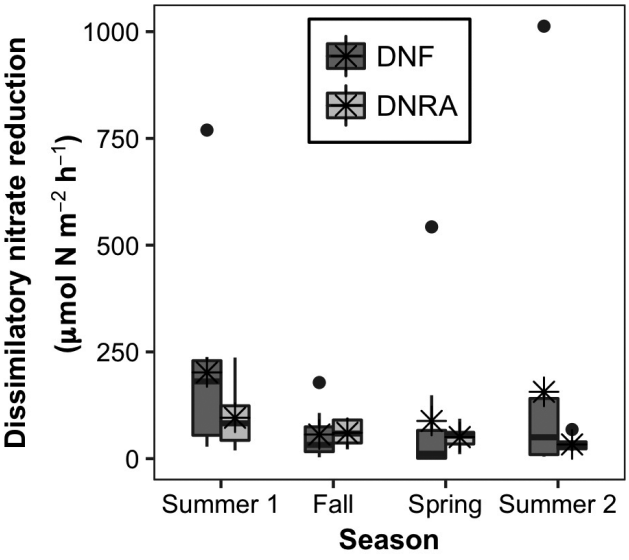

Fig. 7. Seasonal monitoring of potential rates (rates under high nitrate conditions) in the *Zostera marina* seagrass meadow interior showed extreme rates for denitrification (DNF) but not dissimilatory nitrate reduction to ammonium (DNRA). See Fig. 2 for explanation of box-and-whisker plot parameters

Extrapolations to daily and annual rates

Given the presence of extreme values and consequent non-normal distribution of the data, we used bootstrapping to verify that the arithmetic mean rates of denitrification and DNRA were representative before scaling to daily and annual rates. Combining the 2 summers, we had a total of 20 individual rate measurements in seagrass sediments during summer (Fig. 6). The arithmetic mean rates of denitrification and DNRA over those 20 measurements were 17.5 and 12.0 μmol m^{-2} h^{-1}, respectively. We subsampled with replication over 1000 bootstrap replicates to calculate bootstrapped mean rates; over 10 repeated analyses, bootstrapped mean rates varied from 17.2 to 17.6 μmol m^{-2} h^{-1} for denitrification and from 11.8 to 12.2 μmol m^{-2} h^{-1} for DNRA. As these bootstrapped means agreed very well with the arithmetic means, we were confident in scaling up the summer rates from the hourly arithmetic means. The sample sizes for the fall and spring rates were too small to apply bootstrapping (n = 7 and n = 9, respectively). However, the fall and spring rates had fewer extreme values and smaller ranges (Fig. 6), so we concluded that the arithmetic means were reasonable to scale up.

Calculating daily rates required consideration of denitrification and DNRA under dark conditions, since the push–pull measurements were conducted only during the day. Under dark conditions, the seagrass effects from root exudation will be reduced but not eliminated; radial oxygen loss from root tips of *Zostera marina* declined by approximately 70% in the dark compared to saturated light conditions but did not fall to zero (Jovanovic et al. 2015). Thus, root exudation could continue to support some level of denitrification and DNRA even in the dark. Additionally, previous work has shown higher rates of coupled nitrification–denitrification in surface sediments under dark conditions; the enhanced rates were attributed to decreased competition for nitrate from the plants (Welsh et al. 2000). Therefore, it is not unreasonable to expect some amount of dissimilatory nitrate reduction under dark conditions. However, the high hotspot rates observed in the seagrass sediments would likely not occur in the dark. For comparative purposes, we therefore calculated a range of daily rates. For the minimum predicted daily rates, we assumed that no denitrification or DNRA occurred in the dark and scaled the daytime rates by 12 h. For the maximum predicted daily rates, we removed the outliers from the data sets and used the median of the remaining points as the dark rate; we scaled the daylight and dark rates by 12 h each.

Based on these assumptions, we predicted that the daily denitrification rates would fall between 53 and 109 μmol N m^{-2} d^{-1} in the fall, 80 to 81 μmol N m^{-2} d^{-1} in spring, and 209 to 351 μmol N m^{-2} d^{-1} in summer. Daily DNRA rates would range from 60 to 116 μmol N m^{-2} d^{-1} in fall, 48 to 63 μmol N m^{-2} d^{-1} in spring, and 144 to 191 μmol N m^{-2} d^{-1} in summer. We further hypothesized that rates were minimal during winter due to low sediment temperatures and decreased seagrass presence (data not shown); we therefore estimated winter rates as half of the fall rates, based on seasonal differences in other seagrass meadows (Eyre et al. 2013, Russell et al. 2016). Using the range of daily rates for each season, we estimated annual denitrification and DNRA in the meadow sediments as 34 to 54 and 26 to 39 mmol N m^{-2} yr^{-1}, respectively. We estimated annual rates in the bare sediments as a percentage of the annual rates in seagrass sediments, based on the ratio of bare to seagrass rates in summer; bare rates were 9 to 14 and 7 to 10 mmol N m^{-2} yr^{-1} for denitrification and DNRA.

DISCUSSION

Denitrification hotspots in seagrass sediment

This study provides important evidence for the presence of denitrification hotspots in subtidal sea-

grass sediments. Extreme rates were consistently measured in the vegetated sediments but not in the bare sediments, suggesting the presence of localized denitrification hotspots and/or hot moments (i.e. temporal hotspots) associated with seagrass presence. These hotspots likely indicated areas and times where the seagrass strongly altered nitrate and/or labile carbon availability. This effect was heterogeneous over small spatial scales (<1 m^2) and was variable over time, as many of the measured rates in seagrass sediments were low and similar to rates in the unvegetated sediments. Overall, there was a clear pattern of enhanced and more variable denitrification rates measured in the vegetated sediments, driven by the extreme rates occurring in hotspots and hot moments.

The presence of these hotspots and hot moments in subsurface sediments highlights the importance of accounting for subsurface denitrification and DNRA rates, and raises questions about scaling these rates both spatially and temporally. Our measurements suggest that sediment heterogeneity on small spatial scales (i.e. m^2) is comparable to heterogeneity at larger scales (i.e. between 0.4 ha plots). The mean rates presented here may therefore be broadly applicable within the seagrass meadow, even though spatial coverage was limited to 3 plots. However, in this particular meadow, sediment conditions and seagrass metrics show spatial patterns at the meadow scale (km^2) (Oreska et al. 2017), and it remains to be seen whether these differences influence the variability of denitrification and DNRA rates. Areas near the edge of the meadow, where seagrass shoot densities are lower, may have lower and/or less variable rates. In terms of temporal variability, extreme denitrification and DNRA rates were measured in spring and summer, but not in fall, indicating the importance of the seagrass growing season in supporting these hotspots. Additional measurements of subsurface denitrification and DNRA in other seagrass meadows and across seasons are needed to establish the general importance of subsurface hotspots.

The push–pull method used in this study improves on conventional core methods by conducting the incubation *in situ* and thus capturing the variability in rates driven by heterogeneous field conditions in subsurface seagrass sediments (Aoki & McGlathery 2017). Previous studies using core incubations have shown mixed impacts of seagrass on sediment denitrification, with some measuring higher rates in vegetated sediments (Eyre et al. 2011, Piehler & Smyth 2011, Smyth et al. 2013) and others showing higher rates in bare sediments (Risgaard-Petersen et al. 1998, Ottosen et al. 1999), no significant difference (Russell et al. 2016), or contrasting site-specific effects (Zarnoch et al. 2017). Differences in nutrient status do not explain the mixed findings, as studies that found no enhancement in seagrass include both low (e.g. Russell et al. 2016) and high nutrient sites (e.g. Ottosen et al. 1999). However, none of these studies showed a hotspot effect in vegetated sediments, likely due to the more constrained conditions in core incubations that do not replicate hydrodynamic flow and the interactions of light and flow that can alter seagrass activity (Koch et al. 2006, Rheuban et al. 2014b). The push–pull method also directly measures subsurface processes, in contrast to isotope pairing core incubations that rely on diffusion of the isotope tracer from surface water into the sediments. These earlier studies may therefore have underestimated coupled denitrification rates and may have minimized the difference between vegetated and bare rates. More widespread application of the push–pull incubation method would help to better understand how seagrass affects denitrification rates.

Although this study showed the presence of denitrification hotspots in the restored meadow, the areal denitrification rates were low (19.7 µmol m^{-2} h^{-1} in summer) compared to most recent measurements in subtidal seagrass meadows (28 to 824 µmol m^{-2} h^{-1}; Eyre et al. 2011, 2013, Piehler & Smyth 2011, Smyth et al. 2013). These studies used the N_2:Ar method rather than isotope pairing, and there is some concern that methodological differences between the 2 techniques lead to higher rates in N_2:Ar studies (Eyre et al. 2013). However, another recent study using N_2:Ar measured rates comparable to this study (Zarnoch et al. 2017), which suggests that methodology is not the only source of difference in measurements of seagrass denitrification rates. Furthermore, it is critical to note that the higher rates of denitrification were measured primarily under dark conditions, which alleviate competition for nitrate from autotrophs. Under light conditions, Eyre et al. (2011) reported denitrification rates of <20 µmol N m^{-2} h^{-1} in a *Zostera capricorni* meadow measured via N_2:Ar, which is comparable to the mean rate of 19.7 µmol N m^{-2} h^{-1} reported here. The agreement between these 2 studies suggests that the push–pull isotope pairing method is an effective alternative to N_2:Ar and also raises the possibility that much higher rates of denitrification might be measured via push–pull under dark conditions.

Relative importance of DNRA

In the seagrass sediments, DNRA rates were in general lower than denitrification rates, but the relative importance of DNRA fluctuated between seasons. In spring and summer, during periods of peak seagrass growth, DNRA was between 38 and 48% of total nitrate reduction, whereas in the fall, the relative importance of DNRA increased to 57%, making DNRA the dominant dissimilatory nitrate reduction process during seagrass senescence. Of the previous studies comparing DNRA and denitrification in seagrass meadows, Gardner et al. (2006) measured comparable rates, while others have found dominance of denitrification (Smyth et al. 2013) or DNRA (Boon et al. 1986, Rysgaard et al. 1996, An & Gardner 2002). The results of this study suggest that the dominance of DNRA versus denitrification can vary seasonally, following seasonal patterns in seagrass growth (Table 3).

The removal experiment results provide additional evidence that seagrass activity modulates the relative importance of DNRA in this system. Denitrification decreased and DNRA increased slightly in the cleared plots, increasing the relative importance of DNRA following seagrass loss. The pattern was more dramatic than the seasonal shifts observed above, with DNRA accounting for 71% of total nitrate reduction in the cleared plots, compared to only 45% in the pre-removal seagrass plots. DNRA importance also increased to 61% in the seagrass plots during Weeks 2 to 4, when the seagrass suffered shoot losses following a high-temperature event. Overall, these results indicate that seagrass presence supports an environment more favorable to denitrification than DNRA.

The shift toward increased dominance of DNRA following the removal of seagrass could have been caused by a decrease in nitrification. Porewater concentrations of both ammonium and sulfide were enhanced in the cleared plots compared to the pre-removal seagrass plots (Table 2). Higher sulfide concentrations suggest more reduced conditions and therefore lower nitrification rates, while the increase in ammonium concentration could indicate either decreased nitrification or decreased uptake of nitrogen by the seagrass following the removal. Under low-nitrate conditions, DNRA-capable microbes are known to outcompete denitrifiers if sufficient carbon substrate is available (Burgin & Hamilton 2007, Hardison et al. 2015). The changes in porewater chemistry therefore suggest that nitrification was more limited following seagrass removal, leading to the shift toward DNRA dominance. Likewise, these changes also suggest that the presence of seagrass enhanced denitrification by supporting nitrification.

Ambient versus potential nitrate conditions

Given an abundant supply of labile carbon, as in the seagrass meadow sediments, DNRA-capable microbes are predicted to out-compete denitrifiers if nitrate availability is low, whereas denitrifiers will dominate if nitrate availability is high (Tiedje et al. 1982, Burgin & Hamilton 2007). Differences between the ambient nitrate reduction rates (reduction of the ambient $^{14}NO_3^-$, reflecting low nitrate conditions) and the potential rates (reduction of the $^{15}NO_3^-$ spike, reflecting high nitrate conditions) in the seagrass sediments support this hypothesis. Ambient rates of both denitrification and DNRA included extreme values in hotspots that were an order of magnitude greater than median values. However, the potential rates included extreme values only for denitrification, not for DNRA. This pattern was observed across all the data sets, and it suggests that with higher nitrate availability in seagrass sediments, maximum denitrification rates will outweigh maximum DNRA rates.

Differences in the distributions of potential denitrification rates between the seagrass and bare sediments suggest the importance of labile carbon supplied by seagrass exudates. The excess nitrate available in the spike should have relieved nitrate limitations on the potential denitrification rates in both the seagrass sediments and the bare sediments. However, maximum and median potential denitrification rates were still higher in the seagrass sediments compared to both the external bare site and the cleared sediments from the removal (Figs. 3 & 5). This difference may indicate that the seagrass enhanced labile carbon availability and thus boosted the maximum potential rates. However, more data, such as porewater DOC concentrations in the seagrass and bare plots, would be needed to fully support this conclusion.

The observed pattern of enhanced denitrification under high-nitrate conditions, as well as the increased dominance of DNRA following seagrass loss, provide insight into the possible trajectories of nitrate reduction in seagrass sediments experiencing increasing nutrient loading. As long as seagrass growth is undisturbed by higher nutrient loads, a greater availability of nitrate should lead to increased deni-

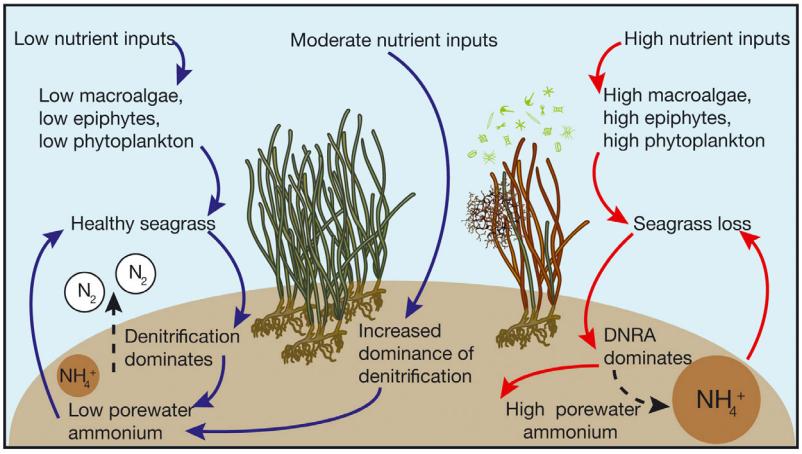

Fig. 8. Conceptual model showing the possible positive feedbacks supporting denitrification dominance under low nutrient inputs, increased denitrification dominance under moderate nutrient inputs, and DNRA dominance under high nutrient inputs

trification. Increased denitrification would in turn serve as a buffer against higher nutrient loading (up to a point) by removing reactive nitrogen from the system. In contrast, if higher nutrient loads impair seagrass growth or cause loss of seagrass, for example by increasing phytoplankton in the water column, epiphytes on seagrass leaves, or macroalgae, and reducing light availability, DNRA is likely to increase relative to denitrification, leading to greater retention of reactive nitrogen. This shift could drive a positive feedback, with increased porewater ammonium concentrations that negatively affect seagrass growth and contribute to seagrass loss (Fig. 8).

Implications for restoration

The results of this study suggest that the seagrass restoration has a pronounced effect on nitrate reduction rates because vegetated sediment can support hotspots with much higher rates of both denitrification and DNRA than unvegetated sediment. This increase in dissimilatory nitrate reduction is important in the context of very low nitrogen loading to the Virginia coastal bays. Recent work has estimated loading rates of 1.4 g N m^{-2} yr^{-1} to the bays from allochthonous sources (atmospheric and terrestrial) (Anderson et al. 2010). Spatial and temporal variability in the measured rates introduce uncertainty into extrapolated daily and annual rates, but our data clearly show that denitrification peaked in summer. Using the minimum daily rates described above (e.g. scaling hourly daytime rates by a 12 h day), denitrification in the meadow would remove 19% of allochthonous nitrogen inputs per m^2 during the fall and 76% during the summer. In comparison, nitrogen removal via denitrification in bare sediments would be only 21% of allochthonous nitrogen inputs in the summer. The effect of the restoration on nitrate removal in the lagoon is thus nontrivial and serves to enhance the nutrient filtering capacity of the lagoon. At the same time, nitrate retention through DNRA was also enhanced by the restoration. Internal recycling is known to be an important source of nitrogen to the Virginia coastal bays, providing as much as 77% of total nitrogen inputs (Anderson et al. 2010). DNRA may therefore play an important role in supporting high rates of productivity in the restored meadow by recycling nitrate into more bioavailable ammonium. Removal of nitrate via denitrification was greater than recycling via DNRA in spring and summer, whereas recycling was greater than removal during the fall. Because maximum rates of both processes occurred during summer, denitrification outweighed DNRA on an annual basis. The net effect of the restoration on nitrate reduction was therefore to enhance nitrogen removal.

The effects of the seagrass restoration on nitrogen cycling extend beyond enhanced nitrate reduction processes. Seagrass assimilation of nitrogen in biomass, as well as burial of particulate nitrogen in the meadow sediments, likely outweigh nitrate reduction fluxes by an order of magnitude (McGlathery 2008). Nevertheless, nitrate removal via denitrification helps maintain positive feedbacks that support continued seagrass growth. Given the global declines in seagrass meadow area, as well as increasing anthropogenic nitrogen loading to coastal waters, the enhanced nutrient filter observed in this restored seagrass meadow provides additional motivation to protect and restore these coastal ecosystems.

Acknowledgements. We gratefully thank M. L. Delgard, B. Shayka, A. Bartenfelder, M. Oreska, and the staff at the University of Virginia's Anheuser-Busch Coastal Research Center for assistance in the field. We also thank A. Lunstrum and M. Miller for assistance in the laboratory, and M. Pace for use of his mass spectrometer. This work was funded by the University of Virginia and by the National Science Foundation grant DEB-1237733 to the Virginia Coast Reserve Long-Term Ecological Research project.

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Editorial responsibility: Robinson Fulweiler, Boston, Massachusetts, USA

Submitted: December 20, 2017; Accepted: June 25, 2018
Proofs received from author(s): August 15, 2018