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Eelgrass meadows, *Zostera marina* (L.), facilitate the ecosystem service of nitrogen removal during simulated nutrient pulses in Shinnecock Bay, New York, USA

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ABSTRACT

Seagrass meadows are important sites of nitrogen (N) transformations in estuaries, however, the role of N loading in driving relative rates of N fixation and denitrification in seagrass habitats is unclear. The current study quantified N fluxes in eelgrass meadows (*Zostera marina* (L.)) and nearby unvegetated sand in trials representing in situ and N enriched conditions. Net N_2 fluxes were low or negative under in situ conditions in both eelgrass and sand. Under N enriched conditions, denitrification was higher than N-fixation, and denitrification in eelgrass was significantly higher than sand. Denitrification of water column NO_3^- was more significant than coupled nitrification-denitrification in the eelgrass. Denitrification was likely supported by greater organic carbon and N within the eelgrass sediment compared to sand. Eelgrass meadows in Shinnecock Bay may facilitate the ecosystem service of N removal and retention during short-term nutrient pulses that can originate from groundwater discharge and stormwater runoff.

1. Introduction

Seagrass meadows provide significant ecosystem services including invertebrate and fish habitat (Blandon and zu Ermgassen, 2014; Heck et al., 2003; Watson et al., 1993), sediment stabilization (Bos et al., 2007), wave attenuation (Fonseca and Cahalan, 1992), carbon (C) sequestration (Macreadie et al., 2014), and nutrient cycling (McGlathery et al., 2007). Recent estimates of their economic value range from \$1000 ha^{-1} $year^{-1}$ for nitrogen (N) removal (Piehler and Smyth, 2011), \$178,000 ha^{-1} $year^{-1}$ for enhancing fish biomass (Blandon and zu Ermgassen, 2014), and up to \$13.7 billion $year^{-1}$ in carbon sequestration (Pendleton et al., 2012).

Despite their economic and ecological importance, seagrass coverage has declined an estimated 7% $year^{-1}$ worldwide since 1990 (Waycott et al., 2009). Currently, 15% of seagrass species are considered threatened (Short et al., 2011). The decline of seagrass habitat has been attributed to a number of anthropogenic activities, especially those that reduce water quality and clarity such as cultural eutrophication (Burkholder et al., 2007) and sediment loadings (Dennison et al., 1993). Thus, it is critical to quantify ecosystem services and ecosystem processes within existing seagrass habitat to document their ecological and societal value in support of conservation and management efforts.

Seagrasses are typically the dominant primary producer in shallow, nutrient limited coastal ecosystems (McGlathery et al., 2007), and have significant impacts on N transformations. Previous studies suggest that N fixation, or the conversion of inert dinitrogen (N_2) gas into a usable form, can provide a significant portion of total N demand in seagrass ecosystems (Cole and McGlathery, 2012). Nitrogen fixation within seagrass meadows may come from autotrophic or heterotrophic epiphytes (Cole and McGlathery, 2012; Wetzel and Penhale, 1979), although heterotrophic N fixation within the seagrass rhizosphere also occurs (Welsh et al., 2000). Heterotrophic N fixers utilize organic C such as foliar photosynthate, root exudates, or sediment organic matter. Rates of N fixation vary widely in seagrass habitats (i.e., 0–22 μmol N m^{-2} h^{-1}; see review in McGlathery, 2008) and rates in unvegetated sediment can be similar (Howarth et al., 1988) or higher (Fulweiler et al., 2007; Gardner et al., 2006). A major driver for high N fixation rates appears to be the quantity and quality of organic C available to N-fixing sulfate reducers (Cook et al., 2015; Fulweiler et al., 2013).

Denitrification rates, or the microbial respiration of nitrate (NO_3^-)

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to N_2, can be highly variable in seagrass habitats and, when exceeding rates of N fixation, can represent a net loss of N from the ecosystem. Environmental factors that influence denitrification rates in shallow coastal ecosystems include organic matter quality and quantity, sediment oxygen, and water column N concentrations (Cornwell et al., 1999; Eyre et al., 2013; Seitzinger et al., 2006). Denitrification is unlikely to be limited by C in seagrass habitats as the below-ground tissues release organic material and their three-dimensional structure promotes settlement and microbial decomposition of seagrass detritus, phytoplankton, and other seston (Eyre et al., 2011; Gacia et al., 2002). However, the source of N for denitrification is variable and potentially limiting. Denitrifying bacteria acquire NO_3^- from the water column (i.e., direct denitrification) or via nitrification, the aerobic conversion of ammonium (NH_4^+) to NO_3^- by chemolithotrophs (Cornwell et al., 1999). Coupled nitrification-denitrification is considered the main pathway for N removal in ecosystems with low water column NO_3^- (i.e. < 10 μM; Seitzinger et al., 2006), which is typical of most seagrass-dominated ecosystems. Rates of coupled nitrification-denitrification in seagrass meadows can be high (Caffrey and Kemp, 1992; Piehler and Smyth, 2011), however, direct denitrification is often the dominant form of N removal in seagrass meadows where nitrification is inhibited or water column NO_3^- concentrations are elevated (Bartoli et al., 2008). Inhibition of nitrification in seagrass meadows can occur during periods of sediment anoxia or with competition for sediment NH_4^+ among plants, nitrifying bacteria, and benthic microalgae (Ottosen et al., 1999; Risgaard-Petersen and Ottosen, 2000). Cultural eutrophication affects rates of denitrification and N fixation, but few studies have examined how rates of N cycling in seagrass meadows vary with changing N availability.

The objective of this study was to determine how short-term NO_3^- enrichments affect denitrification and nutrient fluxes in sediment collected within eelgrass meadows (*Zostera marina*) and compare these to fluxes from adjacent, unvegetated sand. The study was conducted in Shinnecock Bay which is part of the Long Island South Shore estuary (Long Island, New York, USA). The estuary has experienced significant losses of eelgrass over the last 85 years (Peterson et al., 2013). Similar to other developed watersheds, water quality in this estuary is affected by NO_3^--enriched submarine groundwater discharge and terrestrial runoff (Bernard et al., 2014; Capone and Bautista, 1985; Cole et al., 2006; Gobler and Sanudo-Wilhelmy, 2001). Fluxes of benthic nutrients and N_2 were measured in continuous-flow core incubations from three sites, and performed in trials with in situ water column conditions (control) and simulated N-enriched conditions through an addition of + 20 μM $^{15}NO_3$-N to site water (+^{15}N treatment). Anaerobic conditions and available organic C within the eelgrass meadows were predicted to support higher rates of denitrification than the unvegetated sand. In addition, lower rates of N fixation and higher rates of denitrification were expected during the NO_3^--enriched trials (+^{15}N treatment).

2. Methods

2.1. Study sites

Shinnecock Bay is a shallow lagoon and barrier beach estuary located on the south shore of eastern Long Island, New York (Fig. 1). The total area of the bay is 39 km^2 and the average depth is 2 m (Green and Chambers, 2007). Seagrass coverage in the bay is approximately 2 km^2 (Peterson, unpublished). The bay is connected to the Atlantic Ocean by a narrow inlet with strong tidal velocities (2.5 knots s^{-1}) and it is also connected to the Peconic Bay estuary on its northern shore by a man-made canal (USFWS, 1997). The tidal range at the inlet is 0.88 m (Buonaiuto and Bokuniewicz, 2008). Salinity in the bay is relatively high (30 PSU) due to the ocean influence and low freshwater inputs (Green and Chambers, 2007). The portion of the bay west of the inlet has reduced flushing and higher phytoplankton biomass (Carroll et al., 2008), including annual brown-tide blooms (*Aureococcus anophagefferens*; Gobler et al., 2005). The study sites included one site in the eastern portion (Cormorant Point) and two sites in the western portion of the bay (Tiana Bay and Tiana Beach; Fig. 1).

2.2. Sediment cores and physiochemical data collection

On 13 July 2013, 12 sediment cores were collected at each site (acrylic, 30 cm or 25 cm length × 7.6 cm i.d.) using a corer designed to minimize disturbance of the sediment-water interface (Gardner et al., 2006). Six cores were collected within the interior portion of the eelgrass meadows (*Zostera marina*) and 6 were collected from non-vegetated sediments (sand) approximately 10 m removed from the eelgrass. A diver collected the cores from the eelgrass meadows to capture eelgrass within the core and minimize sediment disturbance. Sediment depth in the cores was 12–15 cm which would include eelgrass rhizosphere. The majority of below-ground biomass is found between 2 and 5 cm in Shinnecock Bay (Furman, unpublished), which is consistent with other studies of *Z. marina* (McGlathery et al., 1998). Cores were sealed with black rubber caps and placed in a dark cooler for transport to the laboratory (< 3 h). Half of the cores were used to measure sediment microprofiles, and half were used for solute and gas flux measurements. Cores used for microprofiles were 25 cm and flux cores were 30 cm in length. Three 20 l carboys were filled with site water from each sampling site for use in continuous-flow measurements. Temperature and dissolved oxygen (DO) were measured in the middle of the water column at each site with a Hach HQ30d luminescent DO probe, and salinity was measured with a refractometer. These measurements were made from 9 am through 12 pm. DO stratification is infrequent at the study sites given the shallow water (< 2 m) mixing from wind and tides. At each site, triplicate 20-ml water samples were filtered using 0.2 μm nylon syringe filters (Thermo Scientific, Rockwood, TN, USA) and frozen prior to dissolved nutrient analysis (see below). For water column chlorophyll *a*, triplicate samples of 0.5 l were filtered with a 0.45-μm nitrocellulose membrane and stored in the dark at − 20 °C until analyzed (N = 3 site^{-1}). Membranes were extracted overnight at 4 °C in 90% acetone and measured spectrophotometrically (Parsons et al., 1984).

2.3. Gas and nutrient fluxes measured via continuous-flow core incubations

Continuous-flow incubations of sediment cores were performed to measure nutrient and gas fluxes (Gardner and McCarthy, 2009; Hoellein et al., 2015). After transporting the cores to the laboratory we removed ~90% of the overlying water in each core and then carefully added new site water using a 60 ml syringe and tubing to adjust the total volume to ~230 ml. Cores were fitted with a rubber cap on the bottom and a plunger with a rubber O-ring on the top. The plunger maintained a tight seal and its surface had two holes which were plumbed with polyetheretherketone inlet and outlet tubing (PEEK; Zeus. Inc., Branchburg, NJ, USA). All cores were incubated at in situ temperature (water bath of 26 °C) and kept dark to avoid photosynthetic activity. There are limitations to studying a photic system under dark conditions, and these are carefully noted when interpreting the results.

Two sequential trials were performed by manipulating the inflow water in the continuous-flow cores. In the first trial, no nutrients were added to the site water (i.e. control). The second trial followed 24 h later and + 20 μM 15NO_3-N (referred to as the +^{15}N treatment) was added to the site water (N = 3 cores habitat^{-1} site^{-1}) to simulate N-enriched conditions. The isotopic form of NO_3^- was used so that denitrification of added $^{15}NO_3^-$ could be differentiated from natural sources as well as to estimate total denitrification and N fixation (Gardner et al., 2006; Gardner and McCarthy, 2009). For each trial, aerated site water was gently passed over the intact cores for 24 h at a rate of 1.1 ml min^{-1} (turnover time = 4.2 h). Water was collected directly from each inflow carboy and from each of the core outflows and