Accumulation of soil carbon drives denitrification potential and lab-incubated gas production along a chronosequence of salt marsh development

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ABSTRACT

We measured sediment organic carbon and nitrogen accumulation and rates of denitrification enzyme activity and greenhouse gas (CO_2, CH_4, N_2O) production from slurries of sediments of a mudflat that formed in 2002, a young (8-year-old) natural *Spartina alterniflora* salt marsh that developed on part of the mudflat, and four mature (>200 years old) salt marshes in southeastern Georgia to examine microbial processes related to carbon (C) and nitrogen (N) cycling during succession from mudflat to mature marsh. Soil organic C and N and C: N ratio (0–30 cm) increased across the chronosequence from mudflat (791 ± 35 g C/m^2, 125 ± 17 g N/m^2) to young marsh (2520 ± 131 g C/m^2, 190 ± 10 g N/m^2) to mature marshes (5827 ± 250 g C/m^2, 372 ± 20 g N/m^2). After 8 years of colonization by *S. alterniflora*, sediment organic carbon increased 3.2 times, and nitrogen increased 1.5 times relative to the mudflat. The high rate of organic C and N accumulation based on time series measurements (188 g C/m^2/yr, 7.8 g N/m^2/yr) and feldspar marker layers (359 g C/m^2/yr, 26.2 g N/m^2/yr) was attributed to high accretion (3 cm/yr) in this low elevation (0.18 m NAVD88) emerging marsh. Carbon dioxide production increased with increasing sediment organic C from mudflat to mature marshes. Un-amended denitrification enzyme activity, measured in slurry incubations, ranged from an average of 0.020 ± 0.005 µg g^{-1} hr^{-1} in the mature marshes to 0.094 ± 0.03 µg g^{-1} hr^{-1} in the young marsh. We also measured denitrification potential in slurry incubations amended with C (glucose), N (nitrate), and C + N to assess the potential for substrate limitations. Denitrification potential in the mudflat did not show strong nutrient limitation. In the young marsh, denitrification potential was C-limited, and in the mature marsh, it was co-limited by C and N. In July samples, CO_2 production showed a statistically significant increase with age from the mudflat to the mature marshes. However, in both months, CO_2 production efficiency (expressed on a per g C basis) was significantly higher in the mudflat sediment slurries than in the young marsh and mature marsh samples. *Spartina* colonization of mudflats and the subsequent accumulation of organic matter are key to enriching sediment organic C and N pools that control microbial heterotrophy, particularly denitrification and CO_2 production, which play important roles in marsh C and N cycling.

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1. Introduction

Salt marshes are the predominant intertidal habitats along temperate zone coastlines. These wetlands play an important role in sustaining biodiversity, providing habitat for finfish, shellfish, and water birds, sequestering carbon, and purifying water (Zedler and Kercher, 2005). Young ecosystems generally are characterized by relatively simple community structure and open nutrient cycles, whereas mature ecosystems are characterized by much greater complexity and more efficient internal nutrient cycles (Odum, 1969; Childers et al., 1993). Although there is a wealth of

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information regarding the development of community structure and nutrient cycling of created and restored salt marshes, there is limited data on ecosystem development of natural salt marshes. However, there have been some observations of newly formed natural marshes, including newly formed *Phragmites australis* salt marshes in the Yellow River Delta (Gao et al., 2012; Bai et al., 2012; and Ye et al., 2014), an emerging Mississippi River delta marsh (Henry and Twilley, 2013), young barrier island *Spartina alterniflora* marshes in Virginia (Tyler and Zieman, 1999; Tyler et al., 2003), and a previous study of young *S. alterniflora* marshes in coastal Georgia (Krull and Craft, 2009). Developmental trajectories in a created young marsh may not necessarily reflect those in a naturally developed young marsh due to inherent differences in hydrology, parent material, nutrient and sediment supply, and other key drivers of ecosystem development.

During their initial stages of development, tidal marshes and other young ecosystems are characterized by small pools of organic matter and nitrogen (Odum, 1969; Krull and Craft, 2009; Henry and Twilley, 2013) that may limit the development of microbial processes that drive carbon (C) and nitrogen (N) cycling. These processes are important for supporting heterotrophic food webs and contributing to water quality improvement via denitrification (Day et al., 1989; Groffman et al., 2006). However, young ecosystems, including tidal marshes, exhibit higher rates of net primary production than mature ecosystems (Craft et al., 1999). Even though they contain less sediment organic C and N than mature marshes, young natural marshes accumulate C and N at rates 5—7 times greater than accumulation rates in mature marshes (Krull and Craft, 2009; Henry and Twilley, 2013).

Soil microbial processes are critical to the development and maintenance of C and N biogeochemical cycles, as well as important ecosystem functions associated with these cycles, such as denitrification and C sequestration. Yet, little is known about how quickly these functions change or what limits their development during succession. Few studies have investigated how denitrification potential and microbial production of CO_2, CH_4, and N_2O (greenhouse gases) change during salt marsh ecosystem development. Coastal ecosystems are often N-limited, and denitrification is one of the pathways by which reactive nitrogen is removed from the water column. Denitrification requires labile C to transform NO_3^- to N_2, and studies of created salt marshes indicate that denitrification is lower in created marshes compared to natural marshes due to low carbon availability (Broome and Craft, 2009). N_2O, a greenhouse gas with 265 times the global warming potential of CO_2 (Myhre et al., 2013), is a product of incomplete denitrification. The production of N_2O has been observed to increase with available C substrate (Bergstrom et al., 1994; Weier et al., 1993), with the C: N ratio of organic material (Huang et al., 2004), and with available nitrate (Moseman-Valtierra et al., 2011). Although the mechanism is not well understood, these studies suggest there could be substantial changes in denitrification rates and N_2O production as ecosystems age and accumulate C.

Due to concerns about global climate change, there is growing emphasis on the regulation of greenhouse gas (GHG) production. Wetlands can be sources or sinks of greenhouse gases such as CO_2, CH_4, and N_2O (Moseman-Valtierra et al., 2011). Since carbon is a key driver of CO_2 and CH_4 production from sediments, production of these greenhouse gases could be expected to change as C accumulates in marsh sediments. Studies of created marshes show that overall soil organic matter mineralization increases with age as soil organic C accumulates (Cornell et al., 2007). Similarly, additions of nitrate can increase N_2O production from a tidal salt marsh, enough to change it from a sink to a source of N_2O (Moseman-Valtierra et al., 2011). These results suggest that fluxes of GHGs from young wetlands can be expected to increase over time as organic matter

and nutrients accumulate.

To provide a better understanding of the development of microbially-mediated soil biogeochemical processes in newly developed salt marsh ecosystems, we measured denitrification potential in sediment slurries along a chronosequence from a mudflat to a young (8-year-old) salt marsh to mature (>200 years old) marshes. We used data which Krull and Craft (2009) collected at the young marsh in 2006, hereafter referred to as the 3-year-old marsh, to provide an additional time series measurement. We hypothesize that sediment organic matter limits microbial processes, thus as a mudflat develops to a marsh and accumulates sediment organic C and N, denitrification should increase. We directly assessed carbon and nitrogen limitation of denitrification potential by amending sediments with NO_3^- and labile C (glucose). We also measured greenhouse gas production (CO_2, CH_4, N_2O) in additional sediment slurries from the same sites, with a similar hypothesis that as organic matter accumulates, greenhouse gas production will increase. We also measured rates of sediment organic C and N accumulation using time series measurements of sediment C standing stocks (0—30 cm) and feldspar marker layers.

2. Methods

2.1. Site description

A mudflat formed at the mouth of the Altamaha River in 2002 and was partially colonized by *S. alterniflora* in 2003 (Krull and Craft, 2009). Krull and Craft (2009) sampled the sediment of the unvegetated mudflat and the portion of the mudflat now colonized by the young (3-year-old) marsh in 2006. Since then, the island has continued to recruit vegetation and build large expanses of mudflat and marsh. We sampled the mudflat, the young (8-year-old) marsh and four mature marshes at Sapelo Island, Georgia in 2011. The four mature marshes are part of the Georgia Coastal Ecosystems Long Term Ecological Research Project (GCE3, GCE6, GCE9 and GCE10; Fig. 1). Based on historical data, the mature marshes are greater than 200 years old. All marshes are dominated by *S. alterniflora* and experience tidal inundation of 2.3 m (see Craft, 2007 for a detailed site description). The salinity of tidal flood water at the mudflat, young marsh, and mature marsh (GCE9; Fig. 1) near the mouth of the Altamaha River ranged from 15 to 20, while the salinity of the other mature salt marshes (GCE 3, 6, and 10) further from the Altamaha River was higher, 32—37, during the study period (2011).

2.2. Sediment carbon and nitrogen accumulation

To estimate sediment C and N pools and accumulation during ecosystem development, five cores of young (8-year-old) marsh sediment (8.5 cm diameter by 30 cm deep) were randomly collected in May of 2011 and divided into 0—10 and 10—30 cm depths, dried at 70 °C, weighed, ground, and sieved through a 2 mm mesh screen (Craft et al., 2003). For consistency across years, the field sampling methods used were the same as those used by Krull and Craft (2009). Bulk density was calculated from the dry weight per unit volume for each depth increment. Organic C and N were measured using a Perkin—Elmer 2400 CHN analyzer (Perkin—Elmer, Norwalk, Connecticut). Subsamples were tested for carbonates by adding one drop of dilute (0.1 mol L^{-1}) HCl and observing whether effervescence occurred. Samples containing carbonates were treated with 0.1 mol L^{-1} HCl prior to CHN analysis. Bulk density and C and N concentrations were weighted by depth (1× for 0—10 cm, 2× for 10—30 cm) and mean values were calculated. Nutrient pools were calculated from the mean values as follows:

Nutrient Pool (g m^{-2}) = bulk density (g cm^{-3})·C, N(g/g)·