Factors contributing to spatial variability of N_2O fluxes in a Virginia salt marsh

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ABSTRACT

Salt marshes are heralded for their capacity to denitrify terrestrially-derived nitrogen, but a byproduct of this is the emission of nitrous oxide (N_2O), a potent greenhouse gas. In this study I seek to characterize physical and biogeochemical factors that contribute to spatial variability of N_2O emissions within a marsh. A flow-through steady-state (FT-SS) chamber and trace gas analyzer were used to measure fluxes within a small mid-Atlantic salt marsh. Fluxes of N_2O were low (<29.9 µg N_2O -N m^{-2} h^{-1}) with the exception of one emission hotspot (158.4 µg N2O-N m^{-2} h^{-1}, average) that became the focus of the investigation. Factors thought to contribute to N_2O production including exogenous nitrate (NO_3^-) delivery and denitrifying microbial activity were evaluated within the context of the measured fluxes. There is evidence that bioturbation promotes N_2O emission by influencing both NO_3^- delivery and denitrifying activity through enhanced infiltration, water residence time, and surface area that support elevated levels of microbial N2O production. At the low and high density burrow sites (42 and 328 burrows m^{-2}, respectively), burrows expanded sediment surface area for gas and solute exchange by 7% and 59%, respectively, and overwhelmed saturated hydraulic conductivity as the primary infiltration pathway for exogenous NO_3^-. N_2O production within the marsh was typically below the detection limit (29.9 µg N_2O-N m^{-2} h^{-1}) and was limited by either NO_3^- delivery or the potential to support elevated microbial activity.

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1. Introduction

1.1. Background and motivation

Nitrous oxide (N_2O) is the fourth largest global warming contributor, accounting for approximately 6% of recent warming increases (IPCC, 2001), yet factors affecting its emissions are not well understood. The Intergovernmental Panel on Climate Change (IPCC) has cited a need for an improved budget for natural and anthropogenic N_2O to help refine global climate change models (IPCC, 2007). Although atmospheric concentrations are much lower than those of carbon dioxide (CO_2), per molecule N_2O has a global warming potential (GWP) about 300 times greater than CO_2. Therefore, accurate quantification of surface-atmosphere fluxes have significant implications for global energy budgets and climate change predictions. Unlike CO_2, which has terrestrial sinks, the only known major sink for N_2O is UV photolysis and chemical reactions with oxygen radicals in the stratosphere, that contribute to ozone depletion (Crutzen, 1970; Prather, 1998).

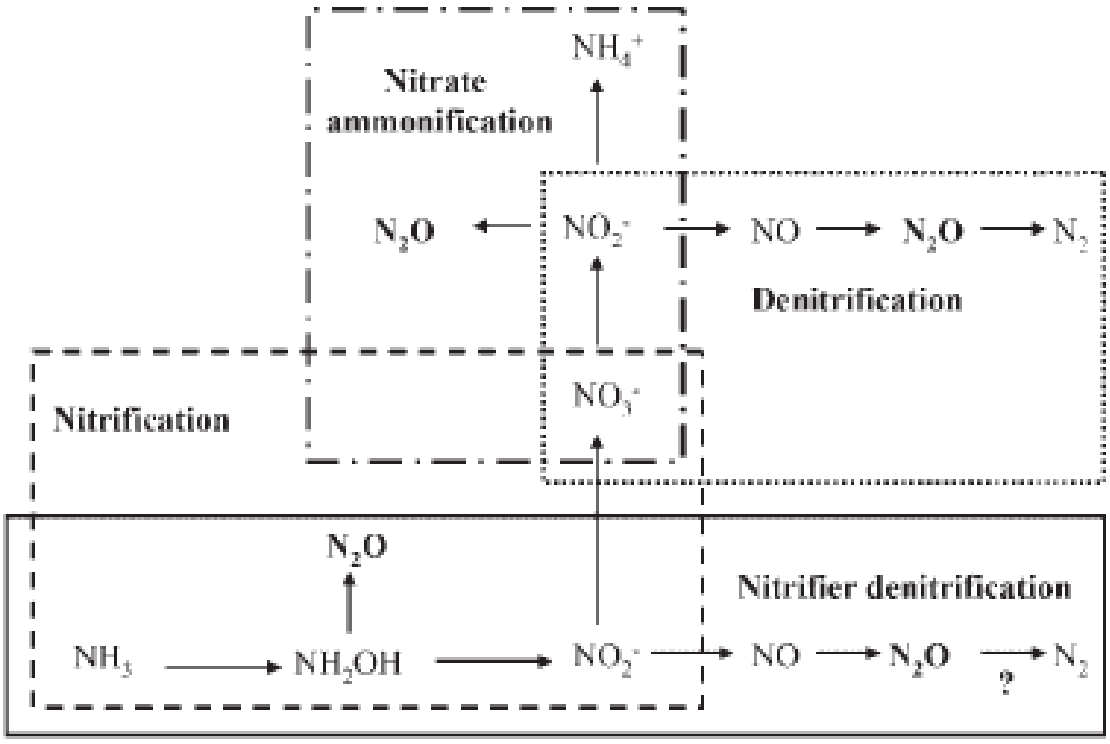
Over the past 150 years, the global mixing ratio of N_2O has increased from 270 ppbv to 314 ppbv as a result of human activities (Wolff & Spahni, 2007). Production of N_2O via chemical synthesis and nylon manufacture are relatively small sources compared to microbial sources that have been anthropogenically stimulated (Mosier *et al.*, 1998; IPCC, 2007). Industrialized agriculture and the advent of the Haber Bosch process to produce synthetic ammonium and nitrate fertilizers from biologically inert dinitrogen gas (N_2) are the main contributors to rising N_2O concentrations due to the introduction of

these fertilizers into the environment where microbial processing may occur. The application of fertilizers alone accounts for more than eighty million tons of N annually, much of which eventually ends up in waterways or is released back to the atmosphere as N_2 or other N-containing gasses including N_2O as a result of microbial processing (Galloway *et al.*, 2003).

The processes presented in Figure 1 describe the various biologic pathways of N_2O production. Bacteria are responsible for the majority of these N-cycling processes; however some archaea and fungi are also known to share some denitrifying capabilities (Shoun *et al.*, 1992). Briefly, nitrification is the aerobic oxidation of inorganic ammonium (NH_4^+) to nitrate (NO_3^-) or nitrite (NO_2^-). In a specialized subset of nitrifiers, production of NO_2^- is followed by reduction to N_2O and N_2, in a process called nitrifier denitrification (Wrage *et al*., 2001). Denitrification and nitrate ammonification (sometimes called dissimilatory nitrate reduction to ammonium or DNRA) are anaerobic processes in which NO_3^- is reduced to N_2 or NH_4^+, respectively. In N-limited environments, DNRA is believed to be important in conserving NO_3^- by recycling it directly back into the inorganic pool whereas in N-impacted environments denitrification facilitates N removal (Mohan *et al.*, 2004).

The contribution of atmospheric N_2O from various N-cycling processes is still poorly understood and efforts to budget sources are hampered by the high spatial and temporal variability of emissions (Baggs & Philippot, 2010). Therefore, it is important to quantify N_2O fluxes in environments known for rapid nitrogen cycling. Where

Figure 1. Biotic nitrogen cycling processes which produce N_2O. Adapted from Baggs (2008).



exogenous NO_3^- is a significant input, it is especially prudent to account for denitrification-derived N_2O.

Wetland areas downstream of agricultural lands are indirectly subject to fertilizers and may play a substantial role in regional and global N_2O emission budgets. According to Well and Butterbach-Bahl (2010) the uncertainty in emission estimates from wetlands and groundwater flow paths may vary by as much as two orders of magnitude, thus leaving their contribution to global N_2O budgets poorly quantified. As a result, N_2O emission estimates from these areas are currently the subject of speculation. For example, global emissions from indirect wetland sources was initially estimated at 1.6 Tg N yr^{-1} (Mosier *et al.*, 1998), but this number was significantly reduced to 0.76 Tg N $^{-1}$ based on more recent 2006 IPCC data (Del Grosso *et al.*, 2008). Recently, the number has been re-estimated specifically for rivers and estuaries as 0.3 to 2.1 Tg N yr^{-1} (Kroeze *et al.*, 2009) illustrating the high degree of uncertainty that remains in our ability to estimate the magnitude of N_2O fluxes from various wetland sources.

With a global area of 3.8×10^{11} m^2 (Woodwell, 1973), salt marshes have the potential to be significant contributors of N_2O to the global budget. According to some mass balance estimates, 16-36% of terrestrial NO_3^- loads are removed by salt marshes before reaching the marine system where excess NO_3^- causes significant eutrophication problems (Bricker *et al.*, 1999). For this reason, marshes are heralded for their capacity to intercept and remove terrestrially-derived N and so are the focus of many conservation, restoration and remediation efforts. Removal occurs partially by macrophyte sequestration and organic matter burial, but also by denitrification. While many studies

have quantified denitrification rates, few have sought to evaluate associated N_2O fluxes. It is unclear if the denitrifying capacity of salt marshes will prove to be a double edge sword by contributing to global N_2O production as the N-loading to these systems continues to increase as a result of watershed agricultural and urban growth (Boyer *et al.*, 2006; Hopkinson & Giblin, 2008). This study aims to quantify N_2O emissions from a salt marsh downstream of an agricultural watershed and to investigate factors that control spatial variability of those emissions.

Denitrification rates are directly linked to N_2O fluxes, and therefore an evaluation of the environmental controls on denitrification is a first step in targeting the likely N_2O emission 'hot spots' (McClain *et al.*, 2003). Although the potential for denitrification is typically high in marsh sediments, rates are variable in both magnitude and spatial distribution because they are dependent on the status of the microbial community and the amount of NO_3^- in the system (Cornwell *et al.*, 1999). Aside from NO_3^-, which serves as the electron acceptor in denitrification, organic carbon is known to play an important role in governing microbial activity as the primary electron donor (Dodla *et al.*, 2008; Liikanen *et al.*, 2009). Rarely is denitrification carbon limited in pristine marshes. Only in circumstances where NO_3^- is added to excess, has carbon been shown to limit denitrification rates (Dodla *et al.*, 2008). In general, elevated organic matter (OM) content tends to increase sediment metabolism, O_2 demand, and N remineralization (Cornwell *et al.*, 1999). As a result, high OM content creates anaerobic conditions that increase utilization of water column NO_3^- by sediment denitrifiers (Caffrey, 1993).

Therefore spatial variability of sediment OM may influence distribution of N_2O emissions within a marsh.

In pristine marshes where very low NO_3^- concentrations are almost exclusively the product of ammonium oxidation (nitrification), denitrification rates and resulting N_2O fluxes tend to be very low (Smith *et al.*, 1983; Anderson *et al.*, 1997). Comparison of fluxes from pristine marshes with those suffering N-loading shows that N_2O emissions are substantially higher in the latter (Liikanen *et al.*, 2009). When inorganic nitrogen, especially NO_3^- is applied to marsh soils in laboratory experiments, the response tends to be rapid (<1 hr) reflecting changes in microbial activity (Sherr & Payne, 1978; King & Nedwell, 1987; Aelion, 2010; Song *et al.*, 2010). This flexibility in microbial response may result in highly variable efficiency in the process of denitrification. As a result, the denitrification ratio N_2O: N_2O+N_2 in core incubation studies have been shown to vary from 1:250 (Seitzinger *et al.*, 1980) to 1:7 (Firestone *et al.* 1979), the former from conservative cycling with low NO_3^- and the latter in a NO_3^- -rich solution.

In situ measurements of denitrification rates and N_2O production in salt marshes remain scarce and there is a notable lack of available information relating N_2O emissions to physical marsh properties. In this study I measure N_2O production at various locations within a salt marsh and examine physical environmental characteristics that may contribute to the spatial variability of the fluxes. Accurate estimates of N_2O fluxes are notoriously difficult to quantify due to a high degree of spatial and temporal variability (Cornwell *et al.*, 1999; Jacinthe & Lal, 2006; Cheng *et al.*, 2007; van den Heuvel *et al.*,

2009; Liinkanen *et al.*, 2009). Therefore studying physical factors that may influence the variability in these systems will ensure a more accurate N_2O budget by providing spatially explicit information, that may be used to extrapolate small-scale information to larger scales (Rodrigues *et al.*, 2007; Dinsmore *et al.*, 2009).

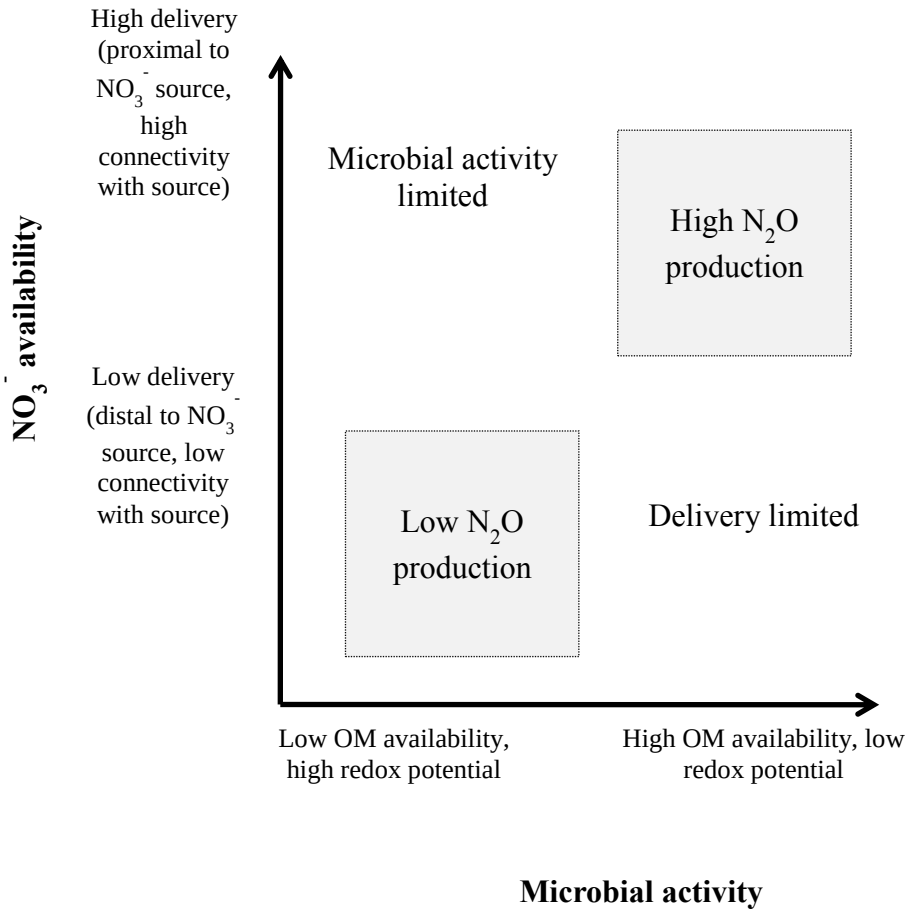
1.2. Conceptual model

To better understand factors contributing to spatial variability of N_2O production, I test a conceptual model for marsh systems (Fig 2.) in which N_2O production is largely governed by 1) NO_3^- availability and 2) microbial activity. Nitrate availability is primarily affected by proximity to the stream water NO_3^- source and its delivery to the marsh sediment, which is determined by factors such as hydraulic conductivity, infiltration and macropore density. Microbial activity is promoted by organic matter availability and redox potential which can be regarded as a combination of O_2 availability and an indication of carbon oxidation products. While denitrification rate does not directly translate to rate of N_2O production, as reduction efficiencies of NO_3^- to N_2 (as represented by the N_2O:N_2 ratio) can vary widely, the same environmental factors that affect denitrification processes ultimately influence the reduction efficiency. Although not directly incorporated into the model, reduction efficiency will be discussed later in this thesis.

1.2.1. Nitrate availability within a salt marsh

Within a salt marsh, NO_3^- availability is spatially variable depending primarily on proximity to exogenous sources. Sources of nitrate include nitrification, atmospheric

Figure 2. Conceptual model of nitrous oxide production where the interaction of independent variables, NO_3^- availability in the environment and microbial activity, influence resulting flux magnitudes. The potential for microbial activity, which is not measured directly, is inferred by high organic matter abundance and a highly reducing sediment environment. Likewise, NO_3^- availability is described by the delivery potential. The interaction of the two independent variables determines the resulting flux.



deposition and import from the terrestrial environment via tidal creeks or rivers. Groundwater, which can have high NO_3^-, is typically not a source to the marsh sediments due to low head gradient and low conductivity (Howes & Goehringer, 1994; Hicks *et al.*, 2000; Bowen & Valiela, 2001; Howarth & Marino, 2006; Wilson & Gardner, 2006; McKellar *et al.*, 2007). Nitrification is a relatively small source of NO_3^- to marshes, accounting for less than 15% of N inputs in a pristine marsh (Anderson *et al.*, 1997). Direct wet and dry atmospheric deposition of nitrogen to estuaries and marshes is also minor, contributing approximately one eighth the amount of N as from nitrification (Paerl, 1995; Anderson *et al.*, 1997). Moreover, atmospheric deposition also tends to be uniform across a landscape and so would have little direct affect on spatially variable N-cycling processes (Bowen & Valiela, 2001; Russell *et al.*, 2003). In contrast, exposure to imported NO_3^- from rivers and creeks may be highly spatially variable within the marsh. Distribution of creek water occurs when flooding seawater mixes with creek water and breaches the creek banks during high tide (McKay & DiIorio, 2010). The extent of overland and subsurface NO_3^- delivery is therefore related to proximity to the tidal creek and infiltration through the marsh soils.

Delivery of creek water NO_3^- to the denitrifying community within the surrounding marsh sediment may be a function of soil hydraulic conductivity and features like macropores from bioturbating fauna. Salt marshes are generally characterized by very low conductivity sediments with minimal porewater movement (Wilson & Gardner, 2007; Hopkinson & Giblin, 2008). However, horizontal porewater movement and corresponding NO_3^- transport is often enhanced within two meters of the creek bank due

to the presence of coarser sediments and a head gradient between the creek and adjacent bank (Howes & Goehringer, 1994). Macropores such as burrows from bioturbating fauna are often present in marshes and play an important role in subsurface water movement, often overwhelming the degree of exchange via the surrounding matrix (Beven & Germann, 1982; Nuttle, 1988; Harvey *et al*., 1995). In coarse creek bank sediments, macropores can enhance horizontal porewater movement across the bank face, thereby enhancing the mixing of high NO_3^- water from the tidal stream with the marsh subsurface (Katz, 1980). During a flood tide, burrows farther from the bank enhance infiltration by allowing overlying surface water to reach sediments at depth directly. Moreover, on ebbing tides, water is retained inside the burrows thereby enhancing water residence time (Bertness, 1985; Koretsky *et al.*, 2002). Finally, macropores are also critical in the expansion of the surface area over which NO_3^- exchange may occur with those sediments (Montague, 1982; Mayer *et al.*, 1995).

1.2.2. Microbial activity within a salt marsh

Previous studies have characterized the environmental factors known to influence denitrification activity: temperature, O_2 partial pressure, pH and organic carbon availability. As with all biologic reactions, enzyme activity is temperature sensitive and denitrifying activity is relatively higher during warm periods (Holtan-Hartwig *et al.*, 2002; Abdalla *et al.*, 2009). In the absence of O_2, which is thermodynamically favorable to NO_3^- as a terminal electron acceptor, microbes with denitrifying capabilities switch respiration pathways when nitrate reductase and nitrous oxide reductase are no longer

inhibited by O_2 (Otte *et al.*, 1996). High soil moisture content, when water filled pore space (WFPS) is >70%, is known to promote denitrification by lowering pore space O_2 concentration (Dettmann, 2001; Seitzinger, 1988; Risgaard-Petersen *et al*., 1994). Organic matter serves as the electron donor in heterotrophic denitrification so an abundance of OM often means more denitrifiers and higher denitrification rates (Dodla *et al.*, 2008; Kemp *et al.*, 1990, Galvotti, 2004) although denitrifier abundance and activity are not necessarily correlated (Miller *et al.*, 2009; Philippot *et al.*, 2009; Song *et al.*, 2010; Cuhel *et al.*, 2010). High organic content also fuels decomposition (remineralization) of the organic matter which, in a salt marsh, is generally accomplished by sulfate or Fe(III) reduction (Howarth & Hobbie, 1982). Redox potential has been demonstrated to be negatively correlated with potential for denitrification as available electron acceptors are depleted from the surrounding matrix (Cuhel *et al.*, 2010).

1.2.3. Effect of excess NO_3^- on microbial efficiency

NO_3^- (+5 oxidation state) is energetically preferred to N_2O (+1 oxidation state) as an electron acceptor, so excess NO_3^- can hinder complete reduction of NO_3^- to N_2 (Baggs *et al*., 2003). This suggests that systems with high N loadings are likely to emit gaseous forms of N with a greater N_2O:N_2 ratio (Blackmer & Bremner, 1978; Cho & Mills, 1979; Magalhaes *et al.*, 2005; Weymann *et al.*, 2008). In a freshwater marsh N-amendment study by Zhang *et al.* (2007), exogenous N additions were found to have a significant positive impact on N_2O production, increasing fluxes non-linearly by 32%, 113% and 581% at 6, 12 and 24 g NH_4NO_3-N m^{-2} additions, respectively. This illustrates that while

NO_3^- availability exerts a control on rates of denitrification, it may have a disproportionately strong influence on N_2O production.

1.3. Studies of spatial variability of N_2O fluxes

Our current understanding of spatial variability of N_2O fluxes largely derives from studies conducted in agricultural settings. Findings suggest that in situ fluxes may vary by orders of magnitude on small spatial scales (Velthof *et al.*, 1996; van den Heuvel, 2009). In such environments, the best predictors of N_2O emissions are soil moisture in determining degree of soil anoxia (Velthof *et al.*, 1996; Smith *et al.*, 1998; Dobbie & Smith, 2001; Allen *et al.*, 2010; Konda *et al.*, 2010) and NO_3^- availability (Pihlatie *et al.*, 2005; Matthews, 2010; Allen *et al.*, 2010). However in marshes, where soils are saturated or near saturated, other factors like OM abundance or properties governing gas diffusion become important in addition to NO_3^- concentration (Wang *et al.*, 2007; van den Heuvel *et al.*, 2009; Chen *et al.*, 2010; Wang *et al.*, 2009). Although there is a large body of literature on denitrification processes in marshes, there are very few studies on nitrous oxide emissions, specifically. Several studies in Northern European marshes found that N_2O emissions were low overall (<10 $\mu g\ m^{-2}\ h^{-1}$) and correlated best with sediment NO_3^- and organic acids abundance, while studies in China have focused on the spatial distribution of fluxes in similar environments such as tidal mudflats and mangroves but not on the marshes themselves (Lee *et al.*, 1997; Cheng *et al.*, 2007; Liikanen *et al.*, 2009). In the United States, there is a wealth of studies on salt marsh

denitrification, yet references to N_2O production tend to be ancillary in nature (Cornwell *et al.*, 1999; Rivera-Monroy *et al.*, 2010).

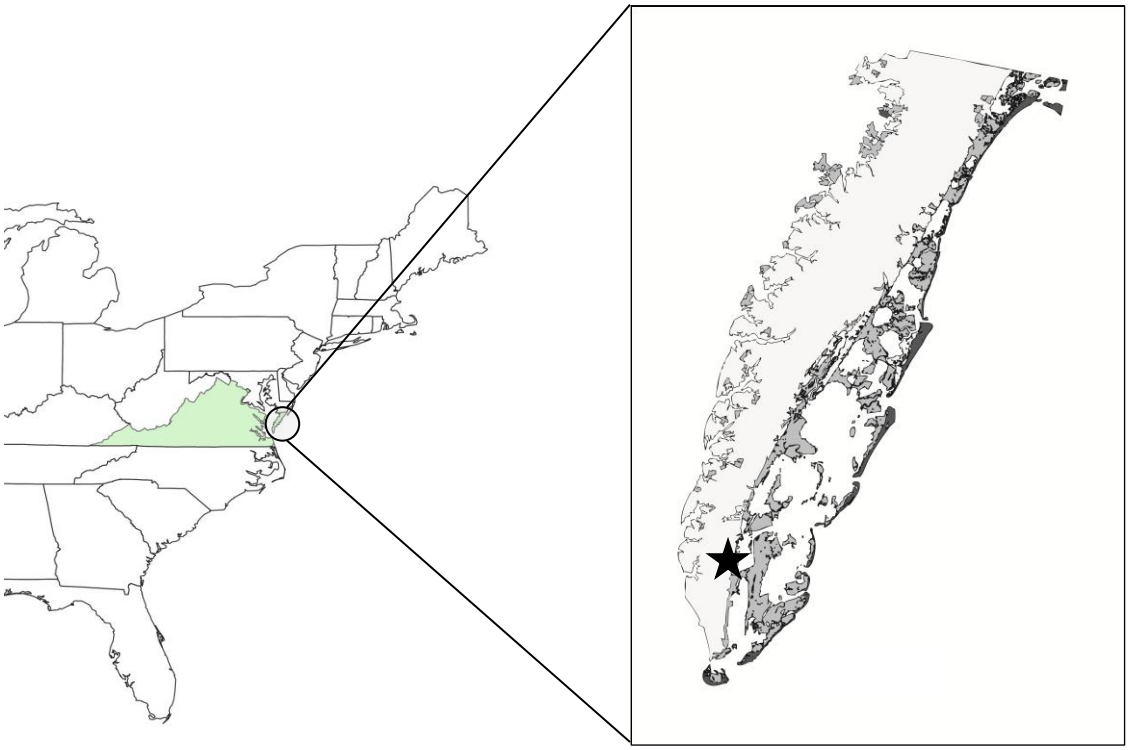
1.4. Objectives

In this study I investigated temporal and spatial N_2O emission variability in a small mid-Atlantic salt marsh. To achieve this, I employed real-time N_2O detection using a flow-through steady-state (FT-SS) chamber with a tunable diode laser (TDL) trace gas analyzer. Together, the movable chamber and TDL allowed for monitoring the temporal evolution of fluxes in situ (Denmead, 2008). Variability in NO_3^- delivery was characterized by analyzing soil properties including grain size analysis, sediment hydraulic conductivity and macropore coverage. Relative denitrifying potential was identified using soil OM content and redox status. Objectives of this study were 1) to determine the spatial variability of N_2O emissions in a marsh and 2) to characterize the physical and biogeochemical factors that contribute to the observed spatial variability.

2. Site description

The study site is Cobb Mill Creek (CMC) marsh located on the eastern shore of the Delmarva Peninsula, Virginia (+37.288° N, -75.929° W). CMC marsh is on the interior of a large marsh lagoon ecosystem that provides the site with protection from major storm wave energy (Fig. 3). The property is within the confines of the University of Virginia's Anheuser-Busch Coastal Research Center (ABCRC), which is affiliated

Figure 3. The Virginia portion of the Delmarva Peninsula, with marshes shaded in grey. The location of Cobb Mill Creek, on the interior of a large lagoon system, is marked with a star.



with the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) program. CMC marsh has a surface area of approximately 1,600 m^2 and is bordered on three sides by wooded slopes and by Crumb Hill Road on the seaward border. Tidal and creek water pass under the road through a culvert, which offers a high degree of hydrologic control for studying stream-related processes. Bisecting the marsh is Cobb Mill Creek, which drains a low relief 4.8 km^2 agricultural watershed into Oyster Harbor. Due to land management practices in the watershed, groundwater (10-15 mg NO_3^--N L^{-1}) and creek water (2 mg NO_3^--N L^{-1}) are elevated in NO_3^- relative to sea water in Oyster Harbor (0.5 mg NO_3^--N L^{-1}) (Galavotti, 2004; K. McGlathery, *pers comm*.). CMC marsh is influenced by semidiurnal tidal cycles with a mean tidal range of 1.27 m and a spring tidal range of 1.54 m. Typical of most Mid-Atlantic salt marshes, vegetation is dominated by *Spartina alterniflora* tall and short forms along the creek bank and lower marsh platform, respectively. Groundwater supply to the marsh is likely negligible due to low conductivity peaty soils and low relief topography (0-2%) (Galavotti, 2004).

3. Field and laboratory methods

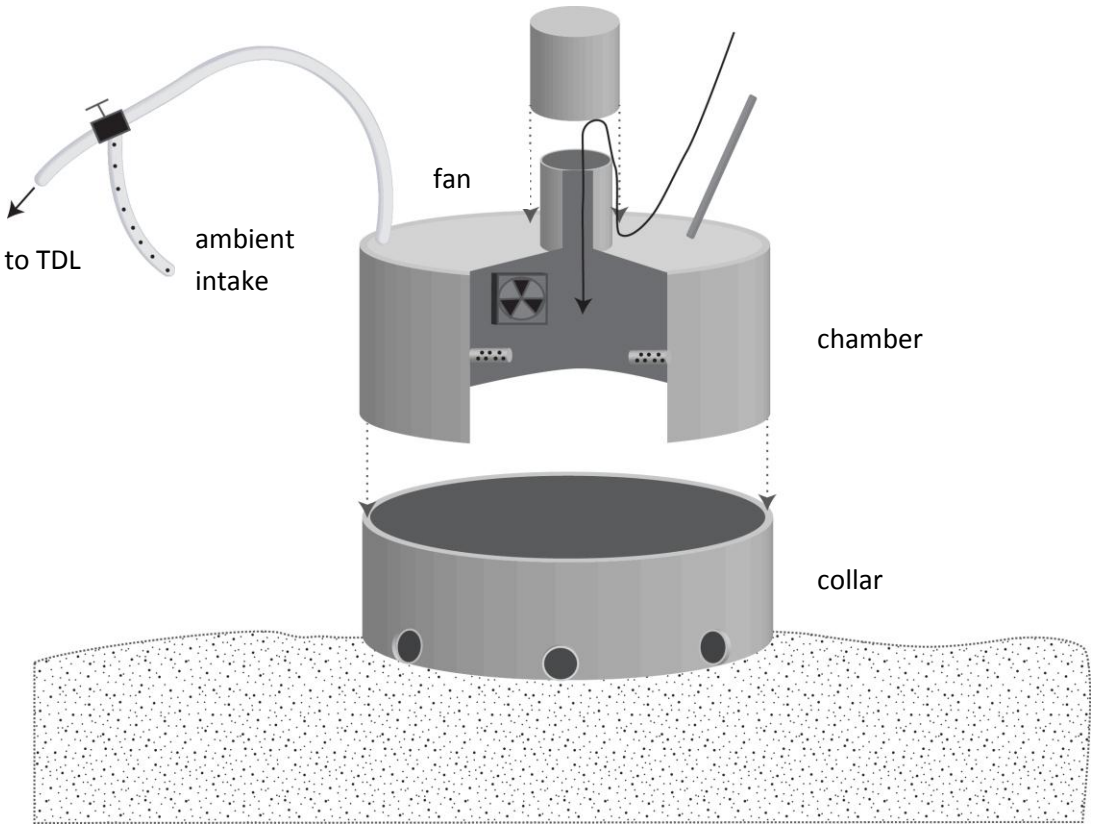
3.1. N_2O sampling equipment and technique

For *in situ* measurement of N_2O emissions, I employed a dynamic flow-through steady-state (FT-SS) chamber (Reichman & Rolston, 2002; Denmead, 2008) connected to a closed path tunable diode laser (TGA 100A Trace Gas Analyzer, Campbell Scientific, USA). The tunable diode laser (TDL) was deployed for the duration of the study period

on a wooden platform approximately 1.5 m above mean high water and within 50 m of the sample sites. The TDL and associated micrometeorological instruments were programmed to measure continuously and log data at a frequency of 10 Hz. A single movable FT-SS chamber apparatus (Fig. 4) was used to collect all flux measurements and was designed to be large enough to contain above and below-ground vegetation for several *Spartina* spp. plants with minimal disturbance. The chamber was fashioned from a 15-cm high section of 20.3-cm i.d. PVC pipe and capped with a PVC pipe end with a 7.6-cm diameter hooded chimney vent. For each sampling location a permanent 20-cm i.d. PVC collar made of the same material was driven 10 cm into the sediment to ensure location reproducibility and to create an air-tight seal with the chamber during flux measurements. Each collar was installed a minimum of two months prior to sampling. To allow the sediment within the chamber to experience realistic tidal regimes, six 1.9-cm drainage holes were evenly spaced around the collar at sediment level to allow tidal water to freely fill and drain the collar area. These holes were plugged during periods of flux measurements.

Connection of the FT-SS chamber to the TDL occurred via 50 m of 0.6-cm i.d. flexible polyethylene Bev-a-Line® hose tubing that allowed mobility within the study area. A 3-way valve spliced into the tubing near the chamber was used to manually direct air flow through the chamber or through an exterior ambient air sampling port. When the valve was turned ‘on’, air entered the chamber through the hooded chimney vent (Bain *et al.*, 2005). To ensure that air inside the chamber was fully mixed, a small

Figure 4. Flow-through steady state (FT-SS) chamber design. Air flow through chamber follows solid black arrow under the chimney hood and into the chamber. A small fan suspended from the ceiling mixes the air before it is pulled out to the TDL via perforated interior tubing. Collar drain holes are plugged prior to sampling.



(3.8-cm square) computer fan attached to a 9V battery was suspended at mid height from the chamber ceiling to circulate air within the headspace (Pumpanen *et al.*, 2001). Air exiting the chamber to the TDL was pulled through perforated tubing encircling the inner chamber wall. A vacuum pump (Busch, USA) next to the TDL and micrometeorological tower continuously drew air through a dryer on the end of the TDL instrument. Air flow was measured with a rotameter along the line that draws air to the TDL. Chamber temperature was measured with a thermometer installed in the cap and soil temperature was measured with a thermometer probe inserted into the sediment adjacent to collars.

Flux calculations for individual chamber measurements were determined by:

$$F_g = v(\rho_{g,i} - \rho_{g,a})/A, \quad (1)$$

where F_g is the flux density of N_2O (kg m^{-2} s^{-1}), v is the flow rate (m^{-3} s^{-1}), which was set to 5 standard liters per minute (SLPM), $\rho_{g,i}$ and $\rho_{g,a}$ denote the atmospheric concentration of N_2O (kg m^{-3}) coming from within the chamber or ambient air through the sampling port, respectively and A is the chamber area (Denmead, 2008). Because the sampled air was dried prior to measurement, it was not necessary to perform density corrections in the conversion of parts per million (ppm) to mass. Conversion of N_2O mixing ratio to concentration was performed assuming a constant TDL enclosure temperature of 45 °C (Campbell Scientific, *pers comm.*).

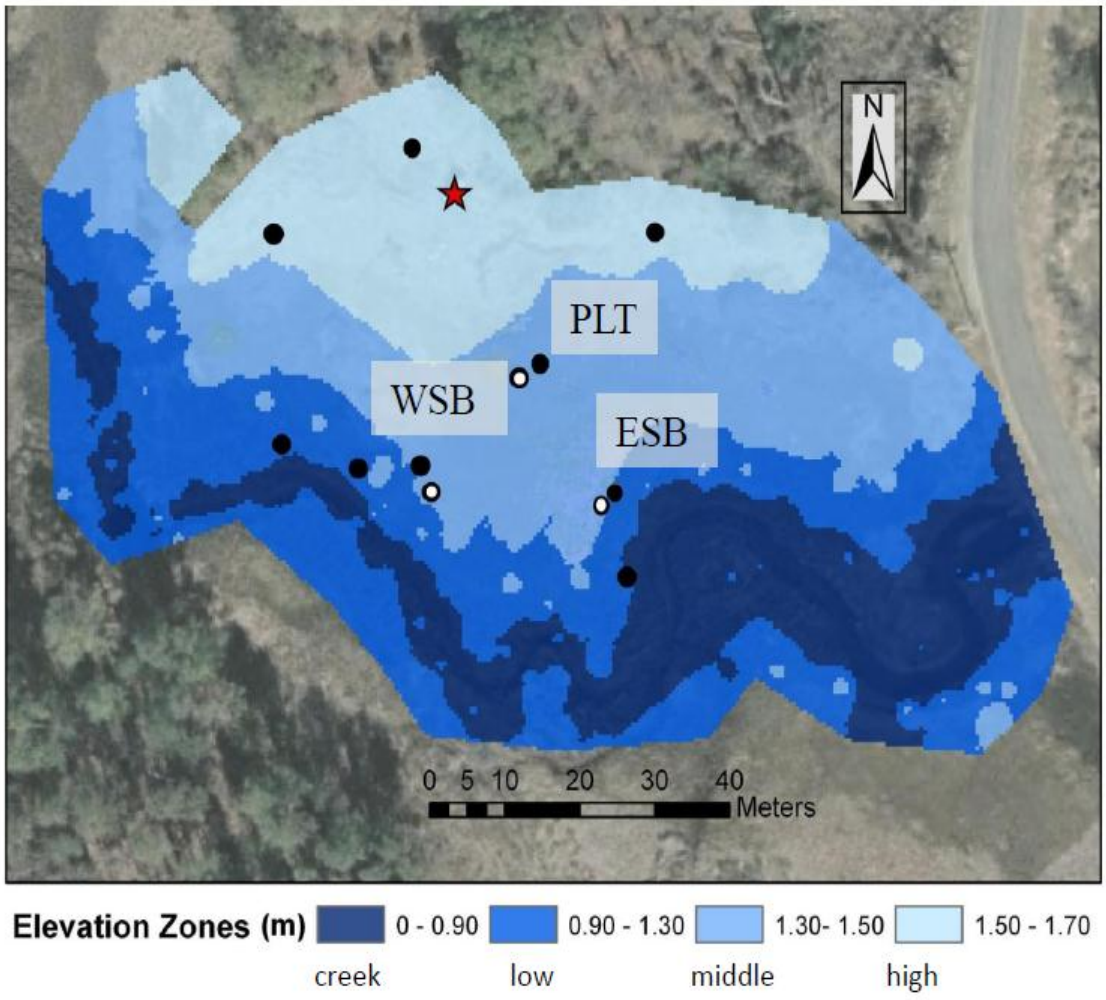
3.2. Identifying 'hot spots' of N_2O emissions

To determine the spatial variability of N_2O fluxes and the distribution of emission "hotspots" and temporally sporadic "hot moments", CMC marsh was divided into three elevation zones with three measurement sites within each zone. The elevation zones were establish as 0.9-1.3, 1.3-1.5 and 1.5-1.7 meters above the lowest point of the creek bottom and were termed the low, middle and high marsh zones (Fig. 5). Three sites within each zone were randomly selected and flux collars were installed to contain approximately equal densities of plants. To test the effect of vegetation, sites within the middle zone were paired with adjacent unvegetated marsh patches (Fig. 5, open circles) such that there were twelve flux collars at nine sites.

For each flux measurement, the chamber was placed atop a sample collar with the 3-way valve turned to ambient for a full minute to establish local ambient air concentration. To begin a flux measurement, the valve was switched to pull air through the chamber for two minutes before the valve was returned to ambient for an additional minute. Collars were sampled in random order such that each sampling lap consisted of one measurement per collar.

Due to the imprecision of manually recorded switch times combined with high-frequency noise and low-frequency drift of the TDL signal, it was necessary to establish a detection limit for N_2O fluxes derived from visual interpretation of the N_2O mixing ratio time series. A concentration difference equal to or greater than two standard deviations of the ambient signal was chosen to define the detection limit because concentration differences could be easily identified if this condition was met. The mean standard

Figure 5. CMC marsh divided into low, middle and high marsh zones. The TDL is marked by a star and nine measurement sites are marked with black circles. Middle zone unvegetated sites are noted with empty circles.



deviation was determined from 10 randomly chosen 30 second samples of ambient time series (300 values each). A regression of the 10 standard deviations against their respective mixing ratios confirmed (r^2=0.0215) there was no trend in the magnitude of the deviations with baseline concentrations. The detection limit was applied to multiple sampling dates and the concentration difference was converted to a flux using equation (1) and determined to be 29.9 μg N_2O-N m^{-2} h^{-1}. Fluxes less than this amount were considered non-detect (ND). Although this detection limit is high compared to commonly cited values for static chambers (e.g. 8.3 μg N_2O-N m^{-2} h^{-1}, Liikanen *et al.*, 2009), it provides a conservative lower limit for these relatively short-duration flux measurements.

For the twelve sample collars, seventy two measurement attempts were carried out from August through October, 2009. Despite considerable effort to capture fluxes over a variety of tidal regimes that were hypothesized to contribute to hot spot or hot moment activity, it was revealed that only one site, which I refer to as Eastern Stream Bank (ESB), in the middle zone had detectable fluxes (i.e. >29.9 μg N_2O-N m^{-2} h^{-1}). Fluxes were detected at both the unvegetated and vegetated collar, captured at a single measurement time in early August, 2009. For this reason, the focus of the study shifted to investigate site-specific factors that could lead to local hot spots of N_2O emissions, such as those detected at ESB.

3.3. Characterizing biophysical controls on spatial variability of N_2O emissions

Following on the earlier efforts to quantify the spatial variability of N_2O fluxes within CMC marsh, I focused on identifying the factors that could promote high N_2O efflux in certain locations such as ESB. Preexisting vegetated collars at sites Platform (PLT) and West Stream Bank (WSB) from the middle zone (Fig. 5) were selected to compare with the ESB site. WSB was selected because of its similarity to ESB in both elevation and distance from the creek bank (1.5 m), yet initial data indicated at least occasional differences in emissions between the sites. The PLT site was chosen because it is farther from the creek bank (~20 m), and likely has distinct sediment properties. PLT is also of interest because it represents a large portion of the marsh surface.

Site-specific sediment characteristics thought to control NO_3^- delivery and denitrifying activity were measured at each of the three study sites. The potential for delivery of exogenous nitrate was assessed by grain size analysis, bulk density, porosity, infiltration rate and fiddler crab (*Uca pugnax*) burrow density. The potential for supporting high rates of denitrifying activity was evaluated with platinum electrode potential as a metric of sediment redox state and sediment organic matter content. To accentuate the impact of site-specific characteristics on N_2O emission potential, denitrification was stimulated with the addition of NO_3^- -amended water. Porewater NO_3^- was not analyzed because it was assumed to be near zero (Anderson *et al.*, 1997; Howes & Goehringer, 1994).

3.3.1. Characterization of factors contributing to NO_3^- availability

Grain size analysis

Sediment grain size analysis was performed on one core (7.62-cm i.d.) from each site. Cores were hammered into the sediment to a minimum depth of 20 cm, then extracted and sectioned into 5 cm segments. A 20-g representative subsample from each section was then processed with sodium hypochlorite bleach to remove organic material according to the method by Brower and Zar (1984). For each core segment, particle size analysis was performed in triplicate on a laser diffraction particle size analyzer (Beckman Coulter, USA) and results were sorted according to standard USDA size classifications (Wentworth, 1922).

Hydraulic conductivity

The saturated hydraulic conductivity of marsh sediments was measured using a falling head method. At each site, four core tubes made from aluminum drain pipe (40-cm length, 7.62-cm i.d.) were installed to a depth of 10 cm with special care taken to avoid inclusion of burrows and marsh plants. Following high tide, cores were filled to 30 cm using creek water and the time and water height recorded. Water height was monitored continuously for 18 hours, hydraulic conductivity for each site was then calculated using Hvorslev's formula (Hvorslev, 1951):

$$K = \frac{\pi D}{11(t_1 - t_0)} ln \frac{s_0}{s_1}, \quad (2)$$

where hydraulic conductivity, K (cm s^{-1}), is a function of the tube diameter, D (cm) and s_0 (cm) is the initial water height at time t_0 (s) and s_1 is the water depth at time t_1 which can be obtained from the linear portion of the semi-log drawdown plot.

Crab burrows density, diameter and coverage

Burrows belonging to the common mud fiddler crab, *Uca pugnax,* were investigated for their role in increasing hydraulic conductivity and expanding sediment surface area. Burrow density was determined by counting the burrows inside a 0.5 x 0.5 m PVC quadrat randomly dropped at four locations per site. In one quarter of each quadrat (0.0625 m^2), burrow diameters were measured to the nearest tenth of a millimeter. Burrow cross-section area was summed per quarter quadrat and then calculated as a percent of quadrat area. Burrow vertical surface area was defined as the percent increase in total surface area relative to bare ground. The vertical surface area was calculated using an average burrow diameter and assuming a uniform burrow depth of 10 cm (Bertness, 1985; Koretsky *et al.*, 2002) and the density of burrows.

Bulk density and porosity

To determine sediment bulk density, porosity, organic, and mineral content, four 3.8-cm i.d. cores were taken to a minimum depth of 15 cm at each site. Cores were extracted using a drill corer to minimize compaction and water loss and were carefully extracted into Whirlpack ® bags in the field and kept on ice. In the lab, cores were sectioned into 5 cm segments and then weighed and dried at 60 °C. Bulk density was calculated as the sediment dry weight divided by segment volume. Porosity (unitless) was determined from the difference in wet and dry mass divided by volume, assuming saturated conditions in the cores. All reported masses are expressed on a dry mass basis.

To match rooting zone depth, data have been averaged from 0-10 cm depth (Gross *et al.*, 1991).

3.3.2. Characterization of microbial activity

Redox potential

A depth profile of reduction potential was made to gain insight into the relative reducing environment between each site. One set of platinum electrode potential (PtEP) measurements was taken in November. Corers were constructed from 50 cm lengths of PVC pipe (5.08-cm i.d.) which were driven 40 cm into the sediment, then filled with site creek water, extracted and plugged at both ends. For storage and transportation, the bottom plug was sealed and cores were refrigerated at 4°C for two weeks until analysis. It was assumed that the low temperature would slow activity and relative redox relationships would be maintained. Reduction potential was measured electrometrically using an Orion SA 210 voltmeter with an 18 gauge platinum wire. ZoBell's solution (7.45 g KCl, 1.4066 g of $K_4Fe(CN)_6$, and 1.0964 g of $K_3Fe(CN)_6$ L^{-1}) was used to calibrate the Accumet Silver Chloride reference electrode. Measurements were performed with the reference electrode submerged in creek water in the core headspace and the Pt wire buried horizontally in the sediment. Starting 0.5 cm below sediment surface level, a 0.2-cm diameter hole was drilled in the PVC core and the Pt wire immediately inserted. Each measurement was recorded after the readings had stabilized; a uniform period of 10 seconds was chosen to standardize this process. As this invasive measurement process introduces some error, the recorded potentials are intended not as

absolute values but instead to differentiate redox conditions between the sites. After each measurement, the hole was immediately covered with electrical tape and the process repeated at 1-cm intervals for the complete depth of the core.

Organic carbon content

Dried sections from the bulk density and porosity cores (3.8-cm i.d.x 5 cm depth) were sub sampled, re-weighed and placed overnight in a 500 C° muffle furnace then re-weighed after cooling. All organic content was assumed to be lost-on-ignition and was calculated as the difference in dry sediment and ashed sediment weight and presented as a percent of sediment dry weight. Percent mineral content was assumed to be the remaining material.

N_2O flux in response to NO_3^- addition

N_2O fluxes were measured under natural (unamended) conditions and conditions of elevated NO_3^-. Collars were installed in quadruplicate at vegetated ESB, WSB and PLT (Fig. 5). At each site, two of the collars were dedicated to experimental NO_3^- treatments to determine the potential for N_2O fluxes under conditions of increased fertilizer use. The treatment levels were selected based on naturally occurring NO_3^- concentrations in the nearby environment and were intended to simulate a realistic range of elevated watershed NO_3^- conditions. Water from Cobb Mill Creek (2.57±0.89 mg NO_3^- N L^{-1}) was used to simulate delivery of creek water undiluted by sea water and groundwater (12.13±0.64 mg NO_3^--N L^{-1}) was collected from below the hyporheic zone

under CMC stream sediments to provide a naturally-occurring high NO_3^- concentration. To control for the effects of moisture, one collar at each site was dedicated for the application of an equal volume of deionized water to measure changes in the N_2O flux due solely to soil moisture changes caused by the addition of treatment water. A fourth collar measured natural fluxes (no additions) at each site.

Nitrate concentration for the treatments was tested to ensure accuracy and reproducibility across four sampling events. For each event, creek water and groundwater were collected during slack tide and tested with a CHEMetrics nitrate test kit (Calverton, VA). If NO_3^- concentrations were found to be low, the samples were amended with KNO_3 to reach desired concentration. At the beginning of each sampling campaign 125 mL from the stream water and groundwater samples was filtered in the field using a 45 µm membrane filter on a 60 cc syringe and kept frozen until analysis. Chemical analysis of water samples was completed on a Lachat Quik Chem 8500 Flow Injection Analysis System (Hach, USA).

Flux measurements were carried out from July 24 -26 2010, with a total of 14 measurements at each of four collars per site. Following the initial flux measurement at each collar, collars were amended with 200 mL of their respective treatments shortly after the receding tide exposed the marsh surface. Fluxes were measured at approximately 1 hour intervals for the duration of the first tidal exposure and intermittently thereafter for a total of 48 hours. Collars were tested in randomized order at each site and sites were sampled in randomized order for each round of measurements. To mimic natural tidal

conditions, collar plugs were removed prior to tidal inundation and measurements were suspended until the water receded. Concurrent with all flux measurements, soil temperature and chamber headspace temperature were recorded. During this time, much of the marsh platform including site PLT was under several cm of stagnant water, but measurements proceeded regardless.

Continuous flux measurements

To capture flux time series at high temporal resolution, a modified flux campaign was carried out such that fluxes at each site were measured continuously over time following groundwater amendment. These measurements were carried out at WSB, ESB and PLT over three consecutive days from August 19-22, 2010. Continuous measurement were achieved using a datalogger (CR 23X micrologger, Campbell Scientific, USA) programmed to operate a 3-way solenoid valve in place of the manual 3-way valve. At each site, the collar previously dedicated to the moisture control treatments, 200 mL of 13.8 mg NO_3^--N L^{-1} groundwater was administered and the chamber was set to measure continuously at 15-minute cycles (10 minutes of chamber air followed by 5 minutes of ambient air) for a minimum of 24 hours. As before, collar plugs were removed immediately prior to high tide and measurements were suspended until the tidal water receded from site. In place of manual temperature readings, thermocouples were installed in the chamber headspace and buried 5 cm into the soil within the collar.

Although calculations for the flux time series were performed in the same manner as the manually-operated fluxes using equation (1), the detection limit of 29.91 μg N_2O-N m^{-2} h^{-1} was not applied due to greater precision with the automated valve switches. Time was recorded relative to the addition of the groundwater treatment and the cumulative flux was calculated as the summation of all detectable fluxes for the duration of the study period. N_2O production efficiency was calculated as the cumulative flux of N_2O-N divided by the mass of added NO_3^--N. Similar to the July campaign, PLT was flooded with stagnant water for the duration of the trial. Measurements proceeded unhindered until a flooding tide 10.5 hrs following treatment forced interruption. Therefore, in order to make uniform comparisons, the cumulative flux and reduction efficiency for all sites were calculated based on the same 10.5 hour period following treatment.

3.4. Statistical analysis

All physical sediment characteristics were evaluated using Statistical Analysis System (SAS 9.2) and either general linear model procedure for analysis of variance (ANOVA) or the t-test procedure for student t-tests of two sample comparisons. A post comparison Ryan's Q test was used to determine which sites significantly differed under the standard ANOVA. When variance failed homogeneity assumption (as with bulk density, porosity, and organic matter), a Welch's ANOVA was used to compare differences between sites. When only two sites could be compared for a particular characteristic (e.g. burrow coverage) or following a Welch's ANOVA, a student's t-test

was used with either the Satterthwaite test for unequal variance or the Pooled method for equal variance. All tests were conducted at α=0.05 level of significance.

4. Results

4.1. Variability of naturally occurring N_2O emissions in CMC marsh

Initial efforts to capture the spatial heterogeneity of N_2O fluxes within the low, middle and high marsh zones revealed that most locations had emission below the 29.9 µg N_2O-N m^{-2} h^{-1} detection limit. Between August 4th and 20th, 72 measurements were attempted among the 12 collars during both spring and neap tidal cycles. Of those attempts, only the paired site ESB in the middle marsh zone had any detectable fluxes. Even there, detectable fluxes were measured only once at the vegetated (127.4 µg N_2O-N m^{-2} h^{-1}, Fig. 6) and unvegetated (189.4 µg N_2O-N m^{-2} h^{-1}) sites and were not captured again in successive attempts. The detection of an apparent transient 'hot spot' of N_2O emissions in the CMC marsh motivated further investigation of the factors that could contribute to its unusually high N_2O production.

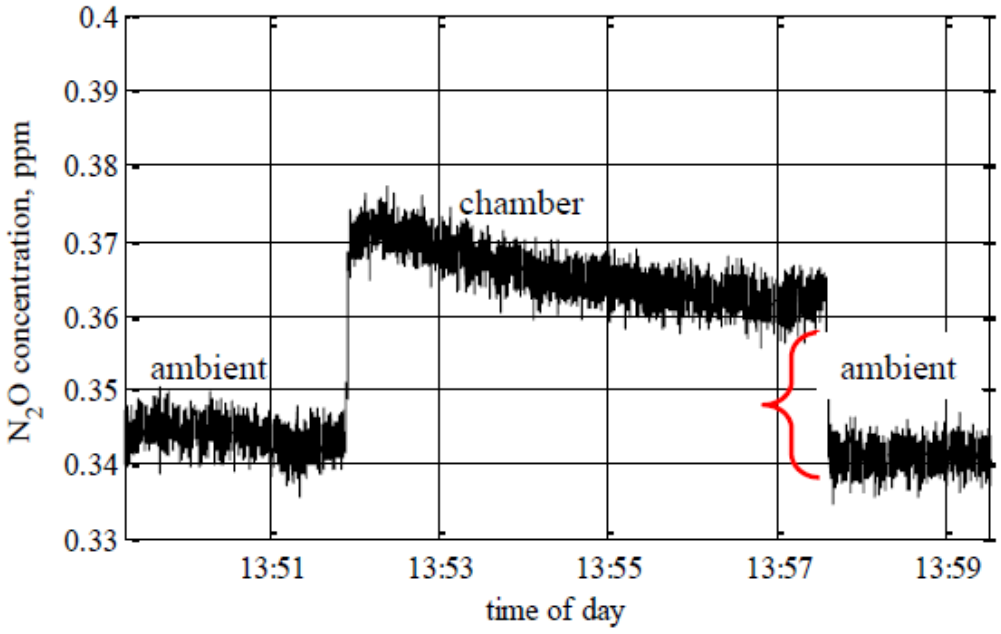
4.2 Determination of biophysical controls on spatial variability of N_2O emissions

4.2. 1. Factors contributing to NO_3^- availability

Grain size analysis

Particle size fractions are summarized in Table 1, in which the size fractions from each depth were averaged over the 20 cm depth of the core. Sites ESB and PLT were

Figure 6. Emission flux captured at ESB on August 6, 2009. The difference in concentration denoted by the bracket at the end of the measurement converts to a flux of 127 µg N_2O-N m^{-2} h^{-1}. Note that the air in the chamber initially had elevated N_2O concentrations due to accumulation in the headspace prior to flushing. Within approximately one minute, the concentration had reached steady state.



similarly dominated by fine clays and silts at 73.5% and 71%, whereas these size fractions accounted for only 30.2 % of the core at the more sandy WSB site. Per size fraction, ESB was the most homogenous over the depth of the core with an average standard deviation of only 1.39. WSB and PLT had slightly higher averaged standard deviations (3.22 and 4.05, respectively) mostly due to a compositional shift in the 15-20 cm depth segment (see Appendix, Fig. A1).

Table 1. Grain size fractions for cores averaged over 20 cm depth.

		Total core percentages (%)		
classification	**size range**	WSB	ESB	PLT
silt and clay	0.06-62.5 µm	30.2	73.5	71.0
fine sand	62.5-250 µm	24.7	19.1	17.7
med sand	0.25-0.5 mm	33.8	4.9	7.0
coarse sand	0.5-2 mm	11.3	2.4	4.2

Hydraulic conductivity

Hydraulic conductivity was found to be on the lower end of the range typical of salt marsh sediments ($10^{-3} – 10^{-5}$ cm s^{-1}, Knott *et al.*, 1987). Using a Model I ANOVA, the average hydraulic conductivity was remarkably similar between the sites ($p=0.8626$) with average conductivities of $3.77x10^{-5}$, $3.49x10^{-5}$ and $4.68x10^{-5}$ cm s^{-1} for WSB, ESB and PLT, respectively. ESB and PLT showed a high degree of uniformity with standard deviations less than 50% of the mean. In contrast, WSB showed a high degree of within-

site variability with a standard deviation 108% of the mean. These results are summarized in Table 2 and Figure 7a.

Crab burrow density, diameter and coverage

Crab burrows varied in density and coverage between all sites (Figure 7b). No burrows were present at PLT while sites WSB and ESB had significantly different densities of 42±11 and 328±61 burrows m^{-2} based on a Student's t-test with Satterthwaite method for unequal variance ($p=0.0168$). An ANOVA was used to confirm lack of variation in burrow diameters within sites ($p=0.5143$) and between sites ($p=0.2428$). Therefore, an average diameter of 0.57 cm was used to compute % ground coverage and burrow surface area for exchange based upon a generic 10 cm *U. pugnax* burrow depth (Bertness, 1985). Burrow openings covered an average of 0.11% and 0.58% of the ground surface at WSB and ESB. The addition of the cylindrical surface area of the burrows expanded the total surface area for exchange by 7.58 % at WSB and 59.2 % at ESB. Results of crab burrow analyses are summarized in Table 3.

Bulk density and porosity

Due to a high degree of variability in sediment bulk density and porosity at WSB, it was necessary to perform a Welch's ANOVA for unequal sample variance. Bulk density was significantly different ($p=0.0107$) between sites with averages 0.40±0.21, 0.23±0.03 and 0.15±0.01 g cm^{-3} for WSB, ESB and PLT, respectively. Porosity averaged 0.65±.15, 0.65±.08 and 0.79±.036 but the only significant relationship was between sites

Figure 7. Box plots for physical characteristics on which statistics were performed. For all comparisons, data was averaged over 0-10 cm depth (n=4). Differing letters indicate significant relationships.

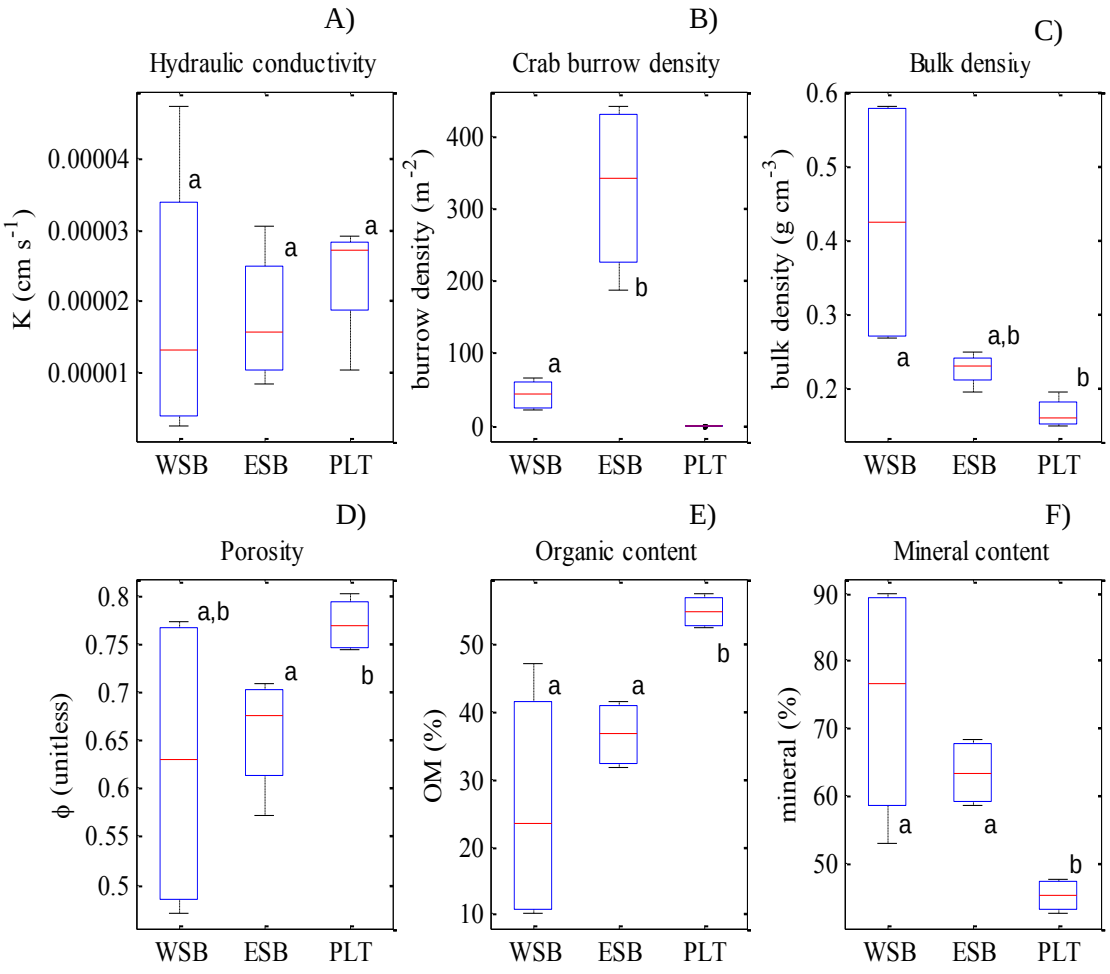


Table 2. Average soil properties ± SE, average saturated conductivity, K_{sat} ± st dev

	bulk density (g cm^{-3}) n=3	**porosity (%)** n=4	**K_{sat} (cm s^{-1})** n=4	**organic (%)** n=4	**mineral (%)** n=4
WSB	1.75 ± 0.05	65.4 ±7	$3.77x10^{-5}$ ± $4.1x10^{-5}$	26.14 ± 9.17	73.7±9.2
ESB	1.27 ± 0.09	65.0±4	$3.49x10^{-5}$ ± $1.7x10^{-5}$	36.7 ± 2.49	63.3±2.5
PLT	0.79 ± 0.04	78.9±1	$4.68x10^{-5}$ ± $1.8x10^{-5}$	54.8 ± 1.16	45.2±1.2

Table 3. Crab burrows analysis

	density m^{-2} ± SE	**Diameter** cm ± SE	**area of burrow openings** %	**exchange area*** cm^2 cm^{-2}
WSB	42 ± 10.6	0.61 ± 0.1	0.11	7.58
ESB	328 ±61.0	0.53 ± 0.2	0.58	59.2
PLT	ND	ND	ND	ND

*assume a standard depth of 10 cm, n=4

PLT and ESB (p=0.0180). Results are summarized in Table 2 and Figure 7c and 7d (t-test results are in Appendix, Table 1).

4.2.2. Microbial activity

Redox potential

All redox measurements at the sites were negative, with potentials ranging from -300 to -900 mV with distinct depth-integrated reduction potentials of -476, -580 and -815 mV for WSB, ESB and PLT, respectively. At each site, potentials were the highest near the surface sediment layer and exhibited decreasing reduction potential with depth. Starting at 5-cm depth, a five-point running average was computed for each core with outliers omitted (Fig. 8, solid symbols). Sediment porewater at PLT has the greatest reduction potential with the most negative profile. The cores from ESB and WSB were likewise negative, but to a lesser degree.

Organic carbon content

Mean organic content at each of the three sites was 26, 36 and 55% at WSB, ESB and PLT, respectively. Within-site OM variability was relatively low at ESB and PLT (stdev < 14% of mean), indicating spatial uniformity of sediments. In contrast, sediment OM at WSB was highly variable (stdev up to 80% of mean), ranging from 11-47% of dry weight for the four cores. Organic (and therefore mineral as well) content was significantly different between sites ESB and PLT (p=0.0006, n=4) but not between sites WSB and PLT or ESB due to the high variability at WSB. These results are illustrated in

Figure 8. Platinum reduction potential cores of redox potential including a 5 point moving average beginning at 5 cm depth. Shaded points have been omitted from averages.

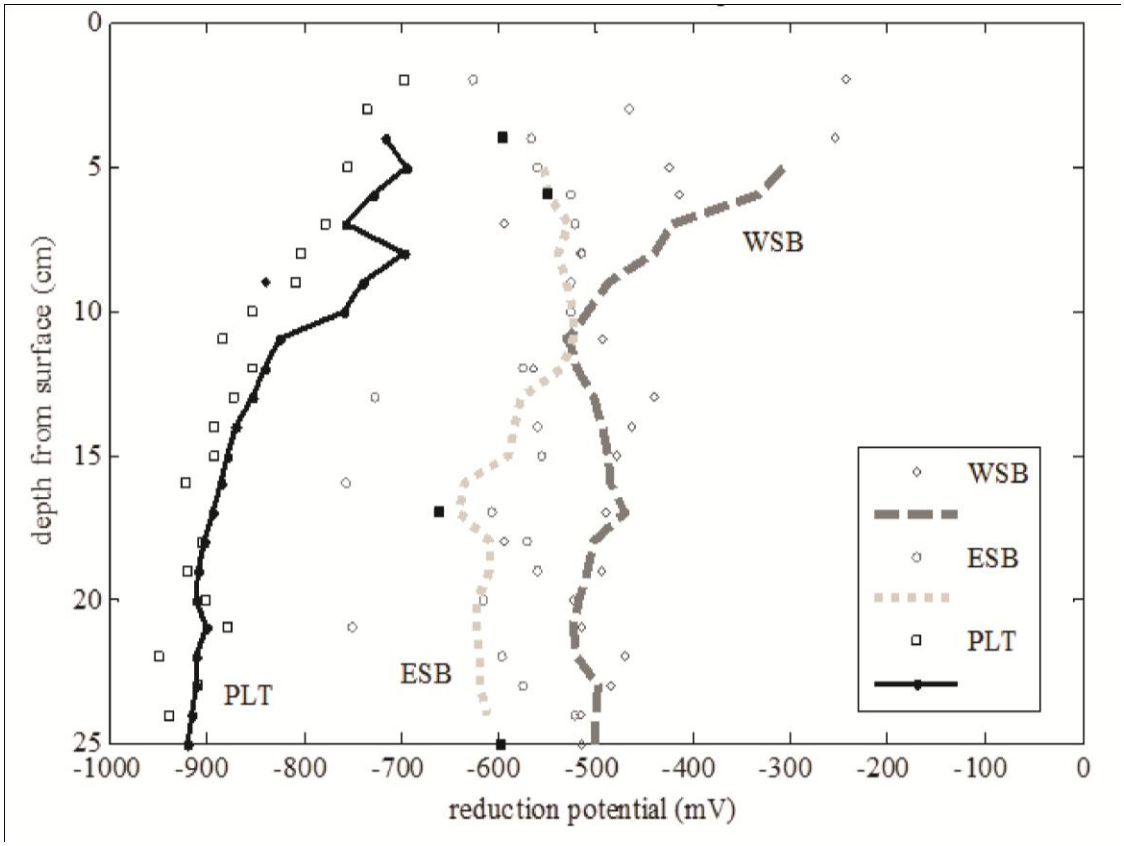


Figure 7e and 7f and summarized in Table 2.

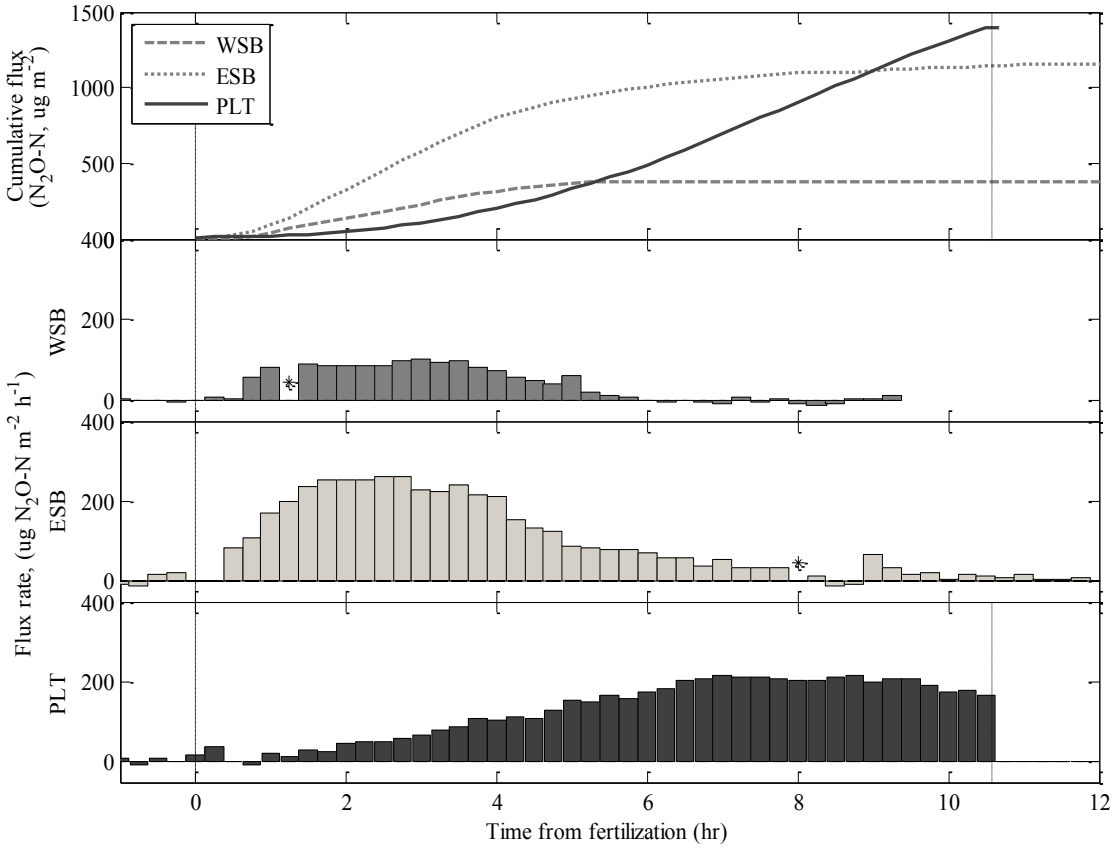
Response to NO_3^- addition

At all sites, fluxes from the 'natural' control, the moisture control and the creek water collars were below detection. Collars treated with groundwater produced fluxes at all sites with a total of 19 fluxes above detection in 41 attempts. As a consequence of these observations, only groundwater treatment was applied in the more detailed investigation of the temporal response of N_2O emissions to NO_3^- addition.

Continuous flux time series

The time series of N_2O fluxes measured in August after amendment with nitrate levels commonly found in groundwater demonstrated a clear difference in N_2O emission behavior between the sites (Fig. 9). The lowest overall N_2O flux occurred at WSB with a peak flux of 102.4 µg N_2O-N m^{-2} h^{-1} three hours following treatment. After six hours, fluxes had returned to pre-treatment levels and the 10.5 hour cumulative N_2O emission was 0.38 mg N_2O-N m^{-2}, accounting for 0.45% of the added NO_3^--N emitted as N_2O-N. Fluxes from ESB followed a similar pattern with emissions peaking at 2.5 hours with a rate of 262.3 µg N_2O-N m^{-2} h^{-1}, more than double the peak at WSB and lasted approximately six hours longer such that the cumulative flux of 1.14 mg N_2O-N m^{-2} was approximately three times greater and accounted for 1.35% of added NO_3^--N. In contrast, the platform site demonstrated more gradual response and took seven hours to reach a peak flux of 215.3 µg N_2O-N m^{-2} h^{-1}. When the measurements were terminated at 10.5 hours, the cumulative flux was 1.39 mg N_2O-N m^{-2}, accounting for 1.64% response.

Figure 9. First 12 hours of N_2O-N fluxes following treatment with 13.8 mg NO_3^--N L^{-1} groundwater (dashed line) with cumulative N_2O flux (top) and flux rates for 15 minute periods (bar graphs). Individual flux measurements with suspected error due to interference with chamber fan have been removed and denoted with '*'. Dotted line refers to termination of PLT flux due to flooding tide.



These findings are summarized in Table 4.

In general, chamber headspace temperature was considerably warmer than ambient air temperature and only slightly warmer than soil temperature inside the chamber. Across the sampling dates, soil temperature ranged from 31.1 to 42.3 C°. Average temperatures reported in Table 4 were computed for the first 10.5 hours following groundwater treatment are at each site.

Table 4. Automated chamber N_2O fluxes based on 10.5 hour period following treatment until termination at PLT site. Reported temperatures are averaged over the first 10.5 hour following groundwater treatment.

	avg air temp (C°)	**headspace temp** (C°)	**soil temp** (C°)	**time to peak** (hr)	**max** (μg m^{-2} h^{-1})	**cumulative** (μg m^{-2})	**NO_3^--N as N_2O-N** (%)
WSB	29.5	34.8	31.1	3	102.4	382.0	0.45
ESB	31.0	42.3	42.3	2.5	262.3	1144.9	1.35
PLT	32.8	41.5	35.7	7	215.3	1390.3	1.64

5. Discussion

Fluxes of N_2O were generally below detection limit (29.9 μg N_2O-N m^{-2} h^{-1}) across CMC marsh, with evidence of naturally occurring emission activity at only one of nine sites. Fluxes at that site, ESB, exceeded fluxes at other marsh sites by at least 400% during reconnaissance efforts to explore the spatial variability of marsh N_2O sources.

Following from these efforts, my goal was to determine why emissions were elevated at ESB compared with other sites.

This study is unique in its focus on landscape-scale spatial variability within a single marsh area. In the literature, N_2O flux studies are typically broad in spatial scope, comparing different geographic regions subjected to different N-loading conditions (Delaune & Jugsujinda, 2003; Kenny *et al.*, 2004; Wang *et al.*, 2007; Liikanen *et al.*, 2009). I found that fluxes from CMC marsh are typical of those found under pristine conditions where fluxes are commonly below 10 μg N_2O-N m^{-2} h^{-1} but may range widely, for example 2.8 μg N_2O-N m^{-2} h^{-1} to 238 μg N_2O-N m^{-2} h^{-1}, within a single marsh (Wang *et al.*, 2007). Results from this study suggest that the high spatial variability seen across regional scales, with coefficients of variation ranging from 50-250% (DeLaune & Jugsujinda, 2003; Kenny *et al.*, 2004; Liikanen *et al.*, 2009; van den Huevel, 2009) can also be found on a much smaller scale within an individual marsh. Mean N_2O fluxes from sites located in N-enriched marshes, where exogenous N-loads may be as high as 10 mg L^{-1} NO_3^--N, commonly range from 200-1200 μg N_2O-N m^{-2} h^{-1} during warm weather trials (Kenny *et al.*, 2004; Liikanen *et al.*, 2009). Emissions at CMC following amendment with ~10 mg NO_3^--N L^{-1} are within this range.

Attempts to describe the spatial variability of N_2O emissions in wetland environments have been rare. In terrestrial environments, spatial emission patterns are primarily driven by soil moisture but in wetlands, moisture is seldom limiting. Fluxes followed no pattern with regard to landward or seaward position in mangroves (Chen *et*

al., 2010) or with regard to water level in littoral freshwater marshes (Chen *et al.*, 2010). Identification of local controls on denitrification rates and N_2O emissions may prove more informative than searching for large-scale gradients alone.

The experimental design of this study addresses the physical and biophysical sediment characteristics that contribute to the spatial N_2O emissions from a salt marsh, primarily as a result of denitrification. The conceptual model introduced in Figure 2 provides a convenient means to describe the relationship between N_2O fluxes captured at CMC marsh and the independent variables of microbial activity and NO_3^- availability. I also address the question of microbial efficiency and the factors that could result in a variable N_2O:N_2 ratio.

5.1. *Microbial activity and N_2O production*

The potential for supporting elevated levels of microbial activity was assessed indirectly by measuring OM content and PtEP. Organic matter content was assumed to be indicative of the potential to support elevated microbial activity by the abundance of electron donors (Knowles, 1982; Caffrey, 1993; Sirivedhin & Gray, 2006) while PtEP was interpreted as indicative of environmental conditions appropriate for denitrifying activity (Seo & DeLaune, 2010).

Organic matter was found to be significantly greater at PLT than ESB or WSB. The 54% average OM content at PLT is considered high but still within the range of values typical for peat-rich marshes (Harvey & Nuttle, 1995; Kostka *et al.* 2002; Davis *et al.*, 2004) while lower values at ESB and WSB (36.7 % and 26.1 %, respectively) are

typical of loamy and sandy loam marsh sediments (Thomas & Blum, 2010). By percent, CMC marsh sediments are carbon-rich, however most of carbon is likely bound in recalcitrant root material. As a result, the fraction of OM that is actually used by microorganisms is relatively small. Evidence presented by Boschker *et al.* (1999) suggests that the primary carbon substrate for salt marsh microorganisms is not the result of direct macrophyte mineralization but of plant exudates and particulates, specifically other bacteria and algae. So despite high total carbon content, labile carbon may still be limiting (Dodla *et al*., 2008). Denitrification rate is frequently positively correlated to OM content which suggests that as the fraction of OM increases, so does the potential to support elevated microbial activity when NO_3^- is provided (Hill & Cardaci, 2004; Dodla *et al.*, 2008). OM is significantly greater at PLT than ESB or WSB, which suggests a greater capacity for maintaining high rates of microbial activity at the site.

Platinum electrode reduction potential was used to represent soil conditions that are likely to support denitrification. Negative PtEP confers anaerobic conditions appropriate for denitrification with more negative potentials indicating a low pH and a predominance of sulfate reducing bacteria (SRB) that produce sulfide as a reduction product. In this study, PtEP results suggest that sediments from all sites are appropriate for denitrifying activity (Fig. 8). Sediment reduction potential in marshes and wetlands is notoriously complex and is primarily impacted by localized geochemistry which provides competitive advantage to functional groups of microorganisms in the system, such as denitrifiers, iron reducers and SRB (Howarth & Teal, 1979; Howes *et al*, 1984; Kostka *et al.*, 2002; Davis *et al.*, 2004). I speculate that low reduction potential at PLT is indicative

of substantial SRB activity in the absence of NO_3^-, which would be consumed immediately by denitrifiers in the near-surface suboxic layer (Sundback *et al.*, 2004). Below the sub-surface, the relationship of SRB activity to denitrification cannot be deduced from the PtEP results, alone. Further work is required to attribute differences in carbon oxidation pathways to denitrification potential and microbial activity.

At CMC marsh the N_2O emissions following NO_3^- amendment are consistent with what might be expected based on the percent OM content and sediment redox potential (Tables 2 and 4, Figs. 7, 8 and 9). Cumulative emissions were highest at PLT which agrees with the high OM of the local sediments. Emissions were less substantial at ESB where there is significantly less carbon in the sediments. Low sediment carbon and higher redox conditions may suggest that sediments at WSB are not capable of the same high rates of denitrification to produce comparable emissions of N_2O. The observed high fluxes at ESB under natural conditions may be promoted by enhanced microbial activity due to extensive bioturbation.

Bioturbation by fiddler crabs (*Uca* spp.) contributes significantly to sediment reworking and enhanced carbon and nitrogen cycling (Wolfrath, 1992; Mayer *et al.*, 1995; Kristensen & Kostka, 2005; Dollhopf *et al.*, 2005; Wang *et al.*, 2010b). When exogenous NO_3^- was added to the flux collar at ESB, denitrification increased rapidly, resulting in a relatively large emission of N_2O. WSB followed the same flux evolution as ESB but fluxes there were lower, possibly attributed to a more modest burrow density. Presumably, increased microbial activity associated with burrows would also mean a

greater microbial population and more active mineralization of OM (Dollhopf *et al.*, 2005). I believe this is reflected in the rapid rise and decline of fluxes at bioturbated ESB and WSB. Two possibilities may account for the emission decline. The first is the simple explanation that the flux peak and decline correspond to consumption of the added NO_3^- and the subsequent improvement in reduction efficiency that would produce a lower N_2O:N_2 ratio (Kaplan *et al*., 1979). Alternately, the peak of the flux could correspond to when denitrification rate becomes limited by the availability of the most labile carbon (Dodla *et al.*, 2008). I speculate that enhanced OM decomposition by fiddler crab activity and enhanced carbon oxidation by burrow-associated microorganisms increases availability of labile carbon that would support a rapid but unsustainable rise in denitrification. As a result, denitrifying activity lessens when only more recalcitrant carbon remains and denitrification proceeds at the rate of OM oxidation.

Uca activity can uncouple nitrogen dynamics in sediments from external controls by increasing rates of nitrogen fixation, nitrification and denitrification, and thereby force faster transformation of available and unavailable carbon and nitrogen (Botto *et al.*, 2005). Data from this study corroborates these findings and suggests that bioturbation can be an important term in describing N_2O sources from coastal wetland environments with and without exogenous nitrogen. The data suggest that the substantial unamended fluxes from the ESB vegetated and unvegated collars are due in part to enhanced microbial activity and N_2O production from both nitrification and denitrification associated with the burrows and sediment bioturbation.

5.2. *NO_3^- delivery*

The potential for exogenous nitrate to be important in N_2O production was evaluated by the likelihood of delivery to sediment microorganisms. Nitrate delivery was assumed to be related to the proximity of sites to the NO_3^- source and sediment physical and biophysical properties that were hypothesized to be important in controlling infiltration of the NO_3^--containing water to sediment denitrifiers.

Macropores such as crab burrows serve as conduits for delivery of water to the sediments. Burrow densities reported in Table 3 are typical of *Uca pugnax* and other *U.* spp which have been found to inhabit densities ranging from 0-480 burrows m^{-2} (Aspey, 1978; Katz, 1980; Bertness, 1985). Between creek sites, burrow density is nearly an order of magnitude greater at the muddy ESB than at sandy WSB site (Table 2). As a result, the surface area at ESB is enhanced nearly 60% over bare sediment which allows greater solute and O_2 exchange into the denitrifying suboxic sediment layer (Teal & Kanwisher, 1961). By trapping NO_3^- -laden water at depth, burrows also help to increase residence time which has been found to be an important factor in promoting denitrification (Cornwell *et al.*, 1999). Where burrows are few or absent, such as at PLT, I believe that NO_3^- delivery occurs via diffusion or perhaps by infiltration into the sediment when not waterlogged.

The timing to emission peak may provide some indication for the primary vertical delivery pathway of NO_3^- -enriched water to the subsurface microbes. Multiple studies have noted that, where present, macropores overwhelm sediment conductivity as the

predominant infiltration pathway (Harvey & Nuttle, 1995; Hughes *et al.*, 1998; Koretsky *et al.*, 2002; Bertics *et al.*, 2010). I found this to be true as well. Fluxes at ESB and WSB responded immediately to NO_3^- additions, resulting in substantial fluxes (>50 μg N_2O-N m^{-2} h^{-1}) within 30 minutes of treatment whereas the flux response at PLT was relatively delayed.

At ESB and WSB there is a possibility that some solute delivery may occur via horizontal porewater movement within 1-2 meters of the stream. The fact that I was unable to account for horizontal intrusion of NO_3^--containing water because I did not fertilize the stream itself is justified given that near-stream horizontal porewater movement is believed to be more important at reducing salt and sulfide stress than for NO_3^- delivery (Beven & Germann, 1982; Nuttle, 1988; Gardner, 2005; Wilson & Gardner, 2006).

At PLT, delivery is limited by downward diffusion. As a result, delivery occurs at a slow and steady rate, evident in the comparatively long (7 hr.) ramp-up time before fluxes reached their peak. Fluxes never peaked dramatically because there was never a 'pulse' of NO_3^- to depth as there was at the bioturbated sites. Furthermore, any N_2O produced would have been temporarily trapped in the water thereby also dampening any pulse of N_2O produced in the sediment before it could diffuse out of the water and into the air.

5.3. Other factors affecting N_2O fluxes

Vegetation is known to be important in controlling sediment biogeochemical processes by enhancing sediment oxygenation and therefore nitrification and denitrification (Teal & Kanwisher, 1966; Howes & Teal, 1994). In response to high porewater sulfide and salinity, marsh macrophytes respond by putting more energy into below ground biomass, root exudates and sediment aeration (Sherr & Payne, 1978; Howes *et al.*, 1984). Despite efforts to locate collars in areas of approximately equal shoot densities, the low bulk density and high OM content at PLT suggest that relatively more nitrification and denitrification could be occurring at the site due to enhanced below-ground activity. However, the contribution of vegetation to N_2O production remains unclear because plant exudates may also help control reduction efficiency, thereby effectively lowering the N_2O:N_2 ratio (Henry *et al*., 2008). Finally, diffusive gas transport through plant aerenchyma can be important in explaining flux variability in vegetated and unvegetated sediments (Wang *et al.*, 2007; Cheng *et al.*, 2007; Dinsmore *et al.*, 2009). However given equal shoot density among the collars, it is unlikely that vegetative transport is a controlling factor in emission variability between sites.

Differences in the percent of added NO_3^- that was released as N_2O may be due in part to factors affecting the efficiency at which microbial denitrification proceedes. High sulfide concentration is known to stress denitrification step by interfering with nitrous oxide reductase in the same way as low temperatures, low pH and high salinity (Miller *et al.*, 1986). Although sulfide, pH and salinity were not measured directly, the reducing conditions at CMC marsh, and PLT in particular, likely compromise denitrification in favor a higher N_2O:N_2 ratio. This would help explain the higher recovery of added NO_3^-

as N_2O at PLT (1.64%) relative to ESB (1.35%) and WSB (0.45%). Fluxes were terminated at this site after 10.5 hours but the fluxes had not yet returned to baseline as they had at other sites so recovery of 1.64% is likely an underestimation of this conversion.

5.4. Implications

The results of this research could have implications for assessing the N_2O emission potential of wetlands in scenarios of increased N-loading. Both bioturbated creek bank and platform marsh sites showed similar emissions in response to nitrate additions. However, the difference in hydrologic regime between the locations could conceivably influence their respective annual N_2O emissions. Creek banks are frequently inundated so the possibility for porewater exchange and NO_3^- delivery may be greater than other areas of the marsh which are inundated less regularly. Meanwhile, stagnant water on the platform only exchanges during high tide events when flooding water is almost exclusively N-depleted seawater (e.g. spring tides and storm surges). Thus, there is realistically little opportunity for delivery of NO_3^- to these areas. This pattern might temporarily change following spring rains and crop fertilization (when rivers transport more nitrogen and organic matter to the marsh) and enriched water could pond on the surface and consequently cause the platform to become the predominant N_2O source within the system.

Although salt marshes do not appear to be a large source of N_2O compared to open water estuaries (Smith *et al.*, 1983; Seitzinger *et al*., 1998; Bange, 2006; Liikanen *et*

al., 2009), this research has shown that marshes do have the potential to be significant sources of N_2O when NO_3^- is supplied. North American salt marshes are facing disturbance due to urban growth and nutrients from residential and agricultural practices. Under low and moderate N-loading (<200 mg N m^{-2} y^{-1}), marshes appear to effectively process terrestrial N-loads by efficiently denitrifying the exogenous NO_3^- and should be preserved for this purpose (Kroeze & Seitzinger, 1998; Valiela & Cole, 2002). Coastal zone management that has as its goal an increase in denitrifying activity to mediate larger terrestrial N-loads, should be cautioned about the risk of greater N_2O emissions and implement management strategies accordingly.

This study highlights that there are N_2O emission hotspots in wetlands, and the commonly used measurement techniques and study designs for these systems are not adequately capturing the spatial emission patterns. More work is necessary to determine the overall importance of these emissions to the total emission budget for the marsh. It is important when planning future studies to identify the presence of significant emission hotspots, for example densely inhabited creek banks and large topographic depressions subject to continuous inundation, to provide a more accurate spatial representation of marsh emissions.

6. Summary

Fluxes of N_2O from CMC marsh were generally low and are typical of other coastal wetland environments. Sites were assessed individually according to the

conceptual model (Fig. 2) for their potential to emit N_2O based on 1) potential to support enhanced denitrifying activity and 2) the availability of exogenous NO_3^- for denitrification. The relative importance of the physical and biophysical characteristics used to assess the potential for N_2O production are summarized in Table 5 with the most influential factors identified.

Characteristics at PLT suggest a high potential for microbial activity based on high OM content and strongly reducing conditions. However, the delivery of exogenous NO_3^- at this site is limited by its distance from the stream and by an absence of macropores that could promote infiltration. This suggests that fluxes would be low under natural conditions but the potential to produce significant amounts of N_2O would increase with a substantial rise in water water-NO_3^- concentrations. At WSB, moderate OM content and redox suggest a limited potential to support denitrifying activity while low macropore coverage implies that the site may be delivery-limited, despite its close proximity to the stream. This suggests that WSB is not likely to be a large source of N_2O with increased water-NO_3^-, as supported by the measured fluxes. In contrast, at ESB the potential to support microbial activity is high due to the prevalence of burrows and moderately high OM and redox. NO_3^- availability is also high due to rapid infiltration via macropores and close proximity to the stream. Together, this translates to a high emission potential at ESB under a range of NO_3^- conditions.

Table 5. Summary of physical and biophysical characteristics important to N_2O fluxes

	Potential to support microbial activity			NO_3^- delivery		
Sites	**OM**	**redox**	**bioturbation**	K_{sat}	**burrows**	**proximity**
WSB	low	low	low	med.	low	near
ESB	med.	med.	high*	med.	high*	near
PLT	high	high	N/A	med.	N/A	far

* characteristics believed to most influential at CMC

Based on the assessment of physical and biophysical site characteristics and of the quantification of fluxes following amendment, I argue that the conceptual model adequately guides an understanding of the relationship between marsh features and N_2O production. This study demonstrates that under naturally low NO_3^- conditions, bioturbating fauna may be the most important factor controlling N_2O emissions in a salt marsh, even though their presence may be restricted to a small percentage of the total marsh area. Muddy sites near the creek typically dominated by fiddler crabs are exposed to NO_3^- -containing creek and tidal water on a diurnal basis. The increased surface area in burrows provides ample opportunity for water retention and denitrification after the tidal water has receded. At sites that have fewer burrows, receding tidal water drains off the surface and porewater exchange of nutrients is limited to the short period of inundation. However, if topography impedes complete drainage of the marsh, slow but sustained fluxes may contribute significantly to the overall N_2O budget, provided an increase in delivery of NO_3^-.

7. Application of methodology

7.1. Uniqueness of methodology

The real-time high resolution flux data obtained from this study is unique and provides novel insight towards flux behavior that is not captured by more traditional techniques. Static chambers require headspace accumulation over time so for trace gasses such as N_2O, measurements taken over several hours are regressed to obtain a single rate measurement (Denmead, 2008). As a result, flux measurements collected by static chambers represent mean rates that gloss over time-specific emissions that could imply sudden changes in microbial function. In this study, I speculated that the abrupt decline in emissions following the flux peak shown in Figure 9 was the result of substrate-limitation of denitrifying activity. Most studies on denitrification potential and actual denitrificaton rates are concerned with substrate limitation and are laboratory-based, utilizing the acetylene block method (e.g. DeSimone & Howes, 1996; Hamersley & Howes, 2003) or stable isotope tracers (e.g. Lindau & DeLaune, 1991; Matheson *et al.*, 2003). With either technique, the emphasis is generally on the starting and ending products and emission rates obtained from the same linear regressions method as static chambers. These techniques pose the obvious shortfall that any time-specific changes in N_2O production are obscured. In contrast, the FT-SS chamber technique provides real-time data in situ that reflects microbial activity in dynamic environmental conditions which enables the study of microbial functional group limitations in the environment.

7.2. Avenues for future research

Organic carbon quality and availability remains the least understood factor controlling denitrifier N_2O production (Pfenning & McMahon, 1996; Dodla *et al.*, 2008; Baggs & Philippot, 2010). Denitrification has long been shown to have a positive relationship with various kinds of organic carbon, e.g. soluble (Burford & Bremner, 1975) or easily mineralizable (Bijay-singh *et al.*, 1988), but the relationship to N_2O production is considerably more complicated. This is because organic carbon oxidation consumes oxygen, thereby altering environmental conditions which affect the denitrification N_2O:N_2 ratio. The FT-SS chamber method presented here provides opportunity to investigate this relationship in multiple ways.

Denitrification substrate preference and N_2O production

The influence of different organic carbon substrates on N_2O emission activity could be used to help resolve denitrifier substrate preference. One such example is the use of various root exudate mimics, some of which have been shown to lower the N_2O:N_2 ratio in laboratory denitrification studies (Henry *et al.*, 2008). The addition of various C compounds (e.g. acetate, sugars, sugars + amino acids) to soil samples would help identify those that are used the most readily and their effect on the N_2O:N_2 ratio based on the timing, magnitude and cumulative emission of the resulting fluxes.

Organic carbon oxidation pathways and denitrification

The same methodology could also be applied to study the effects of different organic carbon oxidation pathways on denitrification and N_2O production. This could be accomplished with the addition of a single substrate type to various sample locations dominated by different C-oxidation pathways. This would be particularly informative in a salt marsh where a spectrum of sulfate reduction to iron reduction dominance of C-oxidation pathways results in variable reduction products (sulfide vs. pyrite) that influence sediment redox and pH and therefore the N_2O:N_2 ratio. The FT-SS technique presented here provides a novel opportunity to investigate N_2O production as influenced by oxidation pathway. Automated measurements could be taken long-term (preferably in an area not subject to flooding) with daily additions. Intermittent soil samples assays could be used to follow changes in enzyme activity in response to substrate additions.

For either of the above investigations, simultaneous N_2O and CO_2 detection using an infrared gas analyzer (IRGA) would help provide some measure of total soil respiration (C-mineralization), however parsing out the CO_2 contribution from denitrification versus other C-oxidation pathways would be difficult. Alternately, more elaborate N-cycling process measurements could be performed using a ^{15}N tracer and membrane inlet mass spectrometry (MIMS) from which N_2 loss can be calculated.

APPENDIX

Figure A1. Sediment grain size distribution by depth interval (cm). Grain size class intervals may be found in Table 1.

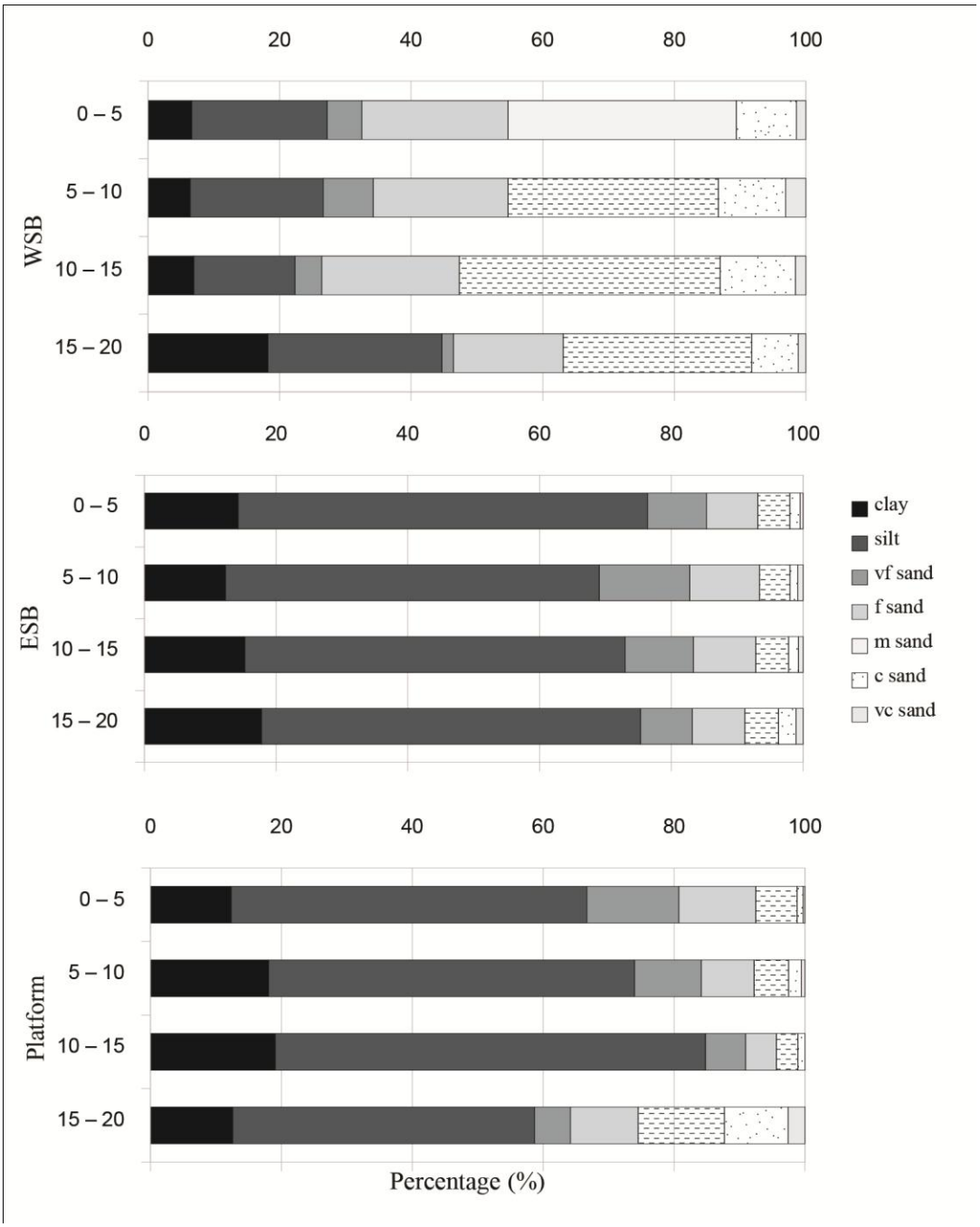


Table A1. t-test results for soil properties

Bulk density

sites	*n*	Df	t_{value}	method	Pr > t
WSB and ESB	4	6	-1.58	Satterthwaite	0.2092
ESB and PLT	4	6	5.57	pooled	**0.0014**
WSB and PLT	4	6	-2.32	Satterthwaite	0.1032

Porosity

sites	*n*	df	t_{value}	method	Pr > t
WSB and ESB	4	6	-0.06	Pooled	0.9565
ESB and PLT	4	6	-3.23	pooled	**0.018**
WSB and PLT	4	6	1.82	Satterthwaite	0.1607

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