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Gross nitrous oxide production drives net nitrous oxide fluxes across a salt marsh landscape

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Abstract

Sea level rise will change inundation regimes in salt marshes, altering redox dynamics that control nitrification – a potential source of the potent greenhouse gas, nitrous oxide (N₂O) – and denitrification, a major nitrogen (N) loss pathway in coastal ecosystems and both a source and sink of N₂O. Measurements of net N₂O fluxes alone yield little insight into the different effects of redox conditions on N₂O production and consumption. We used *in situ* measurements of gross N₂O fluxes across a salt marsh elevation gradient to determine how soil N₂O emissions in coastal ecosystems may respond to future sea level rise. Soil redox declined as marsh elevation decreased, with lower soil nitrate and higher ferrous iron in the low marsh compared to the mid and high marshes ($P < 0.001$ for both). In addition, soil oxygen concentrations were lower in the low and mid-marshes relative to the high marsh ($P < 0.001$). Net N₂O fluxes differed significantly among marsh zones ($P = 0.009$), averaging $9.8 \pm 5.4 \mu\text{g N m}^{-2} \text{ h}^{-1}$, $-2.2 \pm 0.9 \mu\text{g N m}^{-2} \text{ h}^{-1}$, and $0.67 \pm 0.57 \mu\text{g N m}^{-2} \text{ h}^{-1}$ in the low, mid, and high marshes, respectively. Both net N₂O release and uptake were observed in the low and high marshes, but the mid-marsh was consistently a net N₂O sink. Gross N₂O production was highest in the low marsh and lowest in the mid-marsh ($P = 0.02$), whereas gross N₂O consumption did not differ among marsh zones. Thus, variability in gross N₂O production rates drove the differences in net N₂O flux among marsh zones. Our results suggest that future studies should focus on elucidating controls on the processes producing, rather than consuming, N₂O in salt marshes to improve our predictions of changes in net N₂O fluxes caused by future sea level rise.

Introduction

Coastal wetlands play an important role in mitigating the impact of upland nitrogen (N) pollution on estuarine and marine ecosystems by intercepting up to 93% of upslope N inputs (Brin *et al.*, [2010](#)). Denitrification, an anaerobic microbial process that reduces nitrate (NO_3^-) to N gases, is a major N loss pathway in these ecosystems (Kaplan *et al.*, [1979](#); White & Howes, [1994](#)). In salt marshes, denitrification is often coupled to nitrification, an aerobic microbial process that produces NO_3^- (Tobias *et al.*, [2001](#); Dollhopf *et al.*, [2005](#); Hamersley & Howes, [2005](#); Koop-Jakobsen & Giblin, [2010](#)). Both denitrification and nitrification produce

nitrous oxide (N_2O), a potent greenhouse gas and catalyst for stratospheric ozone depletion, which can be released to the atmosphere or be further reduced to dinitrogen (N_2) via denitrification. The N_2O yield ($\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$) is generally low in salt marshes (Blackwell *et al.*, 2010; Yang *et al.*, 2015) but increases with anthropogenic N loading (Lee *et al.*, 1997). Because the processes contributing to N_2O dynamics are redox sensitive, global changes that influence soil redox conditions in coastal ecosystems, such as sea level rise, can alter N_2O dynamics to feedback on climate change.

Greater frequency and duration of tidal inundation associated with sea level rise will likely lower soil redox potential in salt marsh soils (Koch *et al.*, 1992; Warren & Niering, 1993), impacting N_2O dynamics in several ways. First, low redox favors denitrification, with higher denitrification potential in low marsh zones compared to high marsh zones (Wigand *et al.*, 2004). However, denitrification rates in coastal ecosystems are often limited by NO_3^- (Koch *et al.*, 1992; Koop-Jakobsen & Giblin, 2010) such that low soil oxygen (O_2) induced by increased tidal inundation could inhibit denitrification by suppressing the supply of the NO_3^- from nitrification. Second, low O_2 can stimulate N_2O release during nitrification (Goreau *et al.*, 1980; Zhu *et al.*, 2013), although the importance of this mechanism is questionable due to low nitrification rates under these conditions. Third, as NO_3^- becomes limiting under more reducing conditions, microbes are more likely to reduce N_2O , a less energetically favorable electron acceptor than NO_3^- (Tiedje, 1988). Indeed, N_2 production via denitrification is higher in permanently submerged tidal creek sediments than in tidally inundated marsh sediments (Kaplan *et al.*, 1979; Koop-Jakobsen & Giblin, 2010). However, differences in N_2O consumption rates among vegetated marsh zones that experience different tidal inundation regimes have not been explored. Overall, sea level rise could decrease N_2O emissions from salt marshes by inhibiting N_2O production from coupled nitrification–denitrification and stimulating N_2O reduction to N_2 via denitrification, with this effect moderated by anthropogenic N loading.

Salt marshes receiving low anthropogenic N loading can act as N_2O sinks to mitigate climate change (Schlesinger, 2013; Yuan *et al.*, 2015), and sea level rise could increase the importance of this negative feedback on climate change. Nitrous oxide uptake by soil occurs when gross N_2O consumption rates exceed gross N_2O production rates. Biological reduction of N_2O to N_2 via denitrification or nitrifier denitrification is likely responsible for the consumption of atmospheric N_2O (Chapuis-Lardy *et al.*, 2007). Thus, N_2O uptake by soil is thought to occur when N_2O consumption rates are high due to limited availability of O_2 or NO_3^- , more thermodynamically favorable electron acceptors than N_2O , or when high soil moisture impedes N_2O diffusion out of soil or allows for more N_2O dissolution (Chapuis-Lardy *et al.*, 2007). However, the mechanisms

controlling N₂O consumption rates are unclear, in part due to the difficulty in measuring N₂O reduction to N₂ (Groffman *et al.*, [2006](#)).

We conducted a field study along a salt marsh elevation gradient, which served as a proxy for sea level rise, to determine if differences in redox conditions across a range of tidal inundation regimes drive variations in N₂O dynamics. Soil N₂O dynamics are difficult to study because N₂O can be produced and consumed simultaneously. Thus, most studies measure only net N₂O fluxes between the soil and atmosphere. While this is important for determining global warming potentials for a given ecosystem, it is less useful for determining mechanisms driving the patterns in net N₂O fluxes observed. We used the ¹⁵N₂O pool dilution technique to simultaneously measure gross N₂O production and consumption *in situ* (Yang *et al.*, [2011](#)). We hypothesized that gross N₂O production would decrease and gross N₂O consumption would increase from high to low marsh due to more frequent and longer tidal inundation regimes that create more reducing conditions and higher soil moisture in the low marsh. We therefore expected that the low marsh could act as a net N₂O sink whereas the mid and high marshes may not.

Soil redox potential is established by processes that consume electron acceptors (Yao & Conrad, [2000](#)), and those processes occur sequentially according to their thermodynamic yield (Patrick & Jugsujinda, [1992](#); Megonigal *et al.*, [2004](#)). However, spatial heterogeneity in *in situ* conditions may not lead to a clear thermodynamic hierarchy, and thus can allow co-occurrence of reactions along the theoretical redox ladder (Bethke *et al.*, [2011](#)). We therefore also measured a suite of redox-related soil properties (e.g., O₂, NO₃⁻, Fe(II), and CH₄ concentrations) and other soil characteristics (e.g., pH and denitrifying enzyme activity, DEA) to explore controls on gross N₂O production and consumption across the marsh elevation gradient and how those processes balance to determine the net N₂O flux.

Materials and methods

Study Site

The study site was located in the coastal wetlands of Tomales Bay (38.08°N, 122.83°W), which is part of the Point Reyes National Seashore in northern California, USA. The climate is characterized as Mediterranean, with cool wet winters and warm dry summers. Mean annual rainfall reported for a nearby grassland is 950 mm, and seasonal temperatures range from 6 °C in January to 20 °C in July (Ryals *et al.* [2015](#)). This study was conducted in three adjacent marsh zones that differ in elevation and thus also in inundation regimes. The tides are mixed semidiurnal with a maximum tidal range of about 2.5 m. The high marsh becomes saturated

during high spring tides, the mid-marsh flooded regularly at high tide, and the low marsh is flooded except at low tide. All marsh zones have loam soils (Table 1). The high marsh includes the native species *Salicornia virginica*, *Distichlis spicata*, *Grindelia stricta*, *Jaumea carnosa*, *Limonium californicum*, and *Triglochin concinna* (Traut, 2005). The mid-marsh is dominated by *Salicornia virginica* and *Distichlis spicata*, and the low marsh is dominated by *Spartina alterniflora*. It was estimated that cattle grazing within the Tomales Bay watershed increased N inputs by approximately 5 kg-N ha⁻¹ yr⁻¹ (Freifelder *et al.*, 1998) and were likely to be an important source of NO_3^- to the marsh.

Table 1. Mean ($\pm$ SE) soil characteristics (0–10 cm depth) by marsh zone across all sampling dates. Letters indicate statistically significant differences among marsh zones

	Low (n = 12)	Mid (n = 16)	High (n = 16)	df	F-statistic
Moisture (g H ₂ O g ⁻¹)	0.56 $\pm$ 0.01 a	0.53 $\pm$ 0.02 b	0.49 $\pm$ 0.01 c	2, 9	3.7*
Water-filled pore space (%)	NA	36 $\pm$ 3	41 $\pm$ 2	1, 6	3.0
Temperature (°C)	15.5 $\pm$ 0.7 a	12.3 $\pm$ 0.9 b	12.2 $\pm$ 0.9 b	2, 9	7.8**
Denitrifying enzyme activity (ng N g ⁻¹ h ⁻¹)	550 $\pm$ 121	442 $\pm$ 121	356 $\pm$ 60	2, 9	1.4
pH	6.9 $\pm$ 0.1 a	6.5 $\pm$ 0.04 b	6.6 $\pm$ 0.1 b	2, 9	16***
O ₂ concentration (%)	2.9 $\pm$ 0.5 a	3.6 $\pm$ 1.1 a	9.8 $\pm$ 0.8 b	2, 8	38***
Fe(II) concentration (mg Fe(II) g ⁻¹)	13.3 $\pm$ 2.2 a	0.70 $\pm$ 0.11 b	0.19 $\pm$ 0.03 c	2, 9	42***
NH_4^+ concentration (μ g N g ⁻¹)	6.3 $\pm$ 1.6	3.7 $\pm$ 0.6	3.1 $\pm$ 0.4	2, 9	2.2

	Low (<i>n</i> = 12)	Mid (<i>n</i> = 16)	High (<i>n</i> = 16)	df	<i>F</i> -statistic
NO_3^- concentration ($\mu\text{g N g}^{-1}$)	0.40 ± 0.15 a	2.1 ± 0.4 b	1.1 ± 0.2 b	2, 9	29***
C concentration (%)	3.86 ± 0.46	3.17 ± 0.52	4.49 ± 0.36	2, 9	1.6
N concentration (%)	0.34 ± 0.04	0.25 ± 0.04	0.34 ± 0.03	2, 9	1.7
Sand content (%)	30 ± 2	32 ± 5	45 ± 7	2, 9	2.5
Silt content (%)	46 ± 3	49 ± 2	40 ± 5	2, 9	1.4
Clay content (%)	24 ± 2 a	19 ± 3 b	15 ± 2 b	2, 9	3.7*
Bulk density (g cm^{-3})	NA	0.27 ± 0.05	0.36 ± 0.04	1, 6	2.0

- Statistically significant at $*P \leq 0.1$, $**P \leq 0.05$, and $***P \leq 0.001$; NA = not available.

Field measurements

We conducted four field sampling campaigns to characterize N_2O dynamics over a range of environmental conditions: once during the cool winter period (December 2011) and monthly during the transition to warmer temperatures and increased plant activity (April–June 2012). We established four permanent replicate plots spaced 10 m apart along a 30-m-long transect (perpendicular to the slope of the elevation gradient) in each of the marsh zones (i.e., 12 plots total). During each of the four campaigns, we performed measurements at low tide on three consecutive days with measurements occurring within one marsh zone each day. After each trace gas flux measurement, we measured air and soil temperature next to the surface flux chamber, sampled soil from the chamber footprint for laboratory assays conducted later that day (described

below), and collected a soil gas sample from the soil equilibration chamber installed in that plot (described below).

We used the $^{15}\text{N}_2\text{O}$ pool dilution technique to simultaneously measure gross N_2O production and consumption in the field (Yang *et al.*, [2011](#)). The methodology we used is based on that described in detail by Yang *et al.* ([2011](#)) with minor modifications described here to optimize it for this study. We injected 10 mL of isotopically enriched spiking gas into the headspace of a 28 L surface flux chamber inserted 6 cm into the soil surface. The spiking gas consisted of 100 ppm N_2O at 98 atom % ^{15}N enrichment and 28 ppm SF_6 to achieve a ^{15}N - N_2O enrichment of approximately 10 atom %, thereby increasing the chamber headspace gas composition by approximately 40 ppb N_2O and 10 ppb SF_6 . The spiking gas was made volumetrically in gas bags using certified 99.8% concentration of 98 atom % ^{15}N - N_2O (Isotech, Richmond, CA, USA), 99.8% SF_6 (Scotty Specialty Gases, Richmond, CA, USA), and ultra-high-purity N_2 (Praxair, Richmond, CA, USA). We sampled the chamber headspace at 5, 30, 60, 90, 120, 150 and 180 min after spiking gas injection. We analyzed samples for N_2O , SF_6 , CO_2 , and CH_4 concentrations on a gas chromatograph (GC, Shimadzu GC-14A, Columbia, MD, USA) equipped with an electron capture detector, thermal conductivity detector, and flame ionization detector. We analyzed samples for ^{15}N - N_2O on an IsoPrime 100 continuous flow isotope ratio mass spectrometer interfaced with a trace gas preconcentration unit (Isoprime Ltd, Cheadle Hulme, UK) and Gilson GX271 autosampler (Middleton, WI, USA). The precision on five replicate analyses of 90 mL atmospheric air was 0.0029 atom % ^{15}N - N_2O .

Gross N_2O production and consumption rates were estimated using the pool dilution model as described by Yang *et al.* ([2011](#)) and Von Fischer & Hedin ([2002](#)). The iterative model solves for gross production rates based on the isotopic dilution of the isotopically enriched chamber headspace pool of N_2O by natural abundance N_2O emitted by the soil. Gross consumption rates were estimated from the empirical loss of the $^{15}\text{N}_2\text{O}$ tracer, using the loss of the SF_6 tracer to account for physical losses such as diffusion. We assumed that the isotopic composition of the N_2O produced was 0.3431 atom % ^{15}N and the fractionation factor associated with N_2O reduction to N_2 was 0.9924 (Yang *et al.*, [2011](#)). Sensitivity analyses performed by Yang *et al.* ([2011](#)) showed that the pool dilution model output is not sensitive to these assumed values at the high isotopic enrichment used. Net fluxes of N_2O , CO_2 , and CH_4 were determined from the linear change in gas concentration over time. Observed net N_2O fluxes were strongly correlated with net N_2O fluxes calculated from the difference between gross N_2O production and consumption rates ($R^2 = 0.995$, $n = 44$, slope = 0.966 ± 0.01); we report the observed net N_2O fluxes determined from the change in chamber headspace N_2O concentration.

At each field plot we installed soil equilibration chambers (Silver *et al.*, [1999](#); Liptzin *et al.*, [2011](#); Yang *et al.*, [2011](#)) for measuring soil O₂ and trace gas concentrations. Chambers were buried at 10 cm depth in all marsh zones and also at 20 and 30 cm depths in the high marsh. Deeper chambers were deployed in the high marsh to explore if subsoil conditions there were similar to lower elevation surface soils in this ecosystem. Each chamber consisted of a 5 cm diameter PVC pipe sealed to a PVC end-cap (total length 15 cm) and left open on the other end. The cap was fitted with ¼” brass tubing with a Swagelok septum port for manually sampling the chamber headspace gas. Each time we sampled the chambers we took two samples. We immediately analyzed one sample for O₂ using an O₂ meter (Model 52; Yellow Springs Instruments, Yellow Springs, OH, USA) and Clark-type electrode fitted with an airtight cell (Silver *et al.*, [1999](#)). A second gas sample was stored in a pre-evacuated glass vial and analyzed in the laboratory for N₂O, CO₂, and CH₄ concentrations on a GC.

Laboratory assays

Immediately after each pool dilution measurement was completed, an intact soil core (0–10 cm) was collected from the chamber footprint and transported to the laboratory at ambient temperature. In the high marsh, we also collected soil cores from 10–20 cm and 20–30 cm depth. The soils were assayed the same day. Soil pH was measured in DI (1:1 ratio). We extracted 15 g oven-dry equivalent (ODE) soil in 75 mL of 2 M KCl for colorimetric determination of soil NH_4^+ and NO_3^- concentrations on a flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). Phosphate filtration of the soil extracts was used to remove Fe, which can interfere with the colorimetric NO_3^- assay (Yang *et al.*, [2012](#)). Approximately 1 g ODE soil was extracted in 30 mL of 0.5 N HCl for determination of Fe(III) and Fe(II) concentrations using a modified ferrozine assay (Liptzin & Silver, [2009](#)). We assayed for denitrifying enzyme activity (DEA) by adding 75 mL of degassed solution consisting of 1 mM glucose and 1 mM KNO₃ in DI water to 15 g ODE soil in a 250-mL Mason jar; we then added acetylene to achieve 10 kPa headspace concentration and used the change in N₂O concentrations measured 4 times over 40 min to estimate DEA (Yang *et al.*, [2011](#)). Chloramphenicol was not used in the DEA assay because it can inhibit the activity of existing denitrifying enzymes and the synthesis of new enzymes was unlikely to contribute substantially to DEA in the short incubation period (<1 h) used (Pell *et al.*, [1996](#); Murray & Knowles, [1999](#)). Subsamples (10 g) were oven-dried at 105 °C to determine gravimetric soil moisture and estimate the ODE mass. Soil C and N concentrations were measured on air-dried, sieved (to 2 mm), and ground subsamples using a Vario Micro Cube elemental analyzer (Elementar, Hanau, Germany). Soil texture was also measured on air-dried and sieved soil according to Gee & Bauder ([1986](#)).

On one sampling date, a 6.35 cm diameter quantitative soil corer was used to collect soil samples for the determination of bulk density in the high and mid-marsh plots in the same depth increments as the cores for laboratory assays. We were unable to collect reliable quantitative soil cores from the low marsh due to the wetness of the soil in that zone. The soil samples were sieved, handpicked to remove roots and other organic matter, and oven-dried prior to weighing them to estimate bulk density. The average bulk density value for each depth increment in each marsh zone and an assumed particle density of 2.65 g cm^{-3} were used to estimate water-filled pore space (WFPS) from gravimetric soil moisture.

Statistical analysis

We used SYSTAT version 13 (SPSS Inc., Evanston, IL, USA) to perform statistical analyses and MICROSOFT EXCEL 2007 (Microsoft Corporation, Redmond, WA, USA) to run the iterative pool dilution model. We log-transformed variables with non-normal distributions to meet the normality assumptions of ANOVA and linear regressions. We used repeated-measures ANOVA to compare soil characteristics and N gas fluxes among marsh zones (between-subjects factor) and sampling dates (within-subjects factor). The low marsh was not sampled in December 2011 due to persistent flooded conditions; thus, data from the December 2011 sampling were excluded from comparisons among the marsh zones. We used repeated-measures ANOVA to compare high marsh soil characteristics among soil depths (between-subjects factor) and sampling dates (within-subjects factor). Mean values and standard errors are reported in the text. Statistical significance was determined at $P < 0.05$ unless otherwise noted.

Results

Net fluxes of greenhouse gases

Net N_2O fluxes ranged from -16 to $60 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ across all marsh zones and sampling dates. Fluxes differed by marsh zone ($F_{2,9} = 8.3$, $P = 0.009$) and by sampling date ($F_{2,18} = 7.9$, $P = 0.003$). Net N_2O fluxes were significantly higher in the low marsh compared to the mid and high marshes (Fig. [1a](#)). Across all sampling dates, net N_2O fluxes averaged $9.8 \pm 5.4 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$, $-2.2 \pm 0.9 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$, and $0.67 \pm 0.57 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ in the low, mid, and high marshes, respectively. Net N_2O fluxes were positively correlated to soil pH ($R^2 = 0.31$, $P < 0.001$, $n = 44$) and net CH_4 fluxes ($R^2 = 0.37$, $P < 0.001$, $n = 44$). Together, soil pH and net CH_4 fluxes explained 65% of the variability in net N_2O fluxes ($P < 0.001$, $n = 44$).

Figure 1

[Open in figure viewerPowerPoint](#)

Mean (a) net N₂O flux and (b) gross N₂O production (black bars) and gross N₂O consumption (gray bars) by marsh zone across all sampling dates. Error bars represent standard errors. Letters indicate statistically significant differences in net N₂O fluxes and gross N₂O production rates among marsh zones.

[Caption](#)

Carbon dioxide effluxes were significantly higher in the mid-marsh than in the low marsh ($F_{2,9} = 15.2$, $P = 0.001$, Fig. 2a). Effluxes averaged 104 ± 25 mg C m⁻² h⁻¹ in the low marsh, 185 ± 24 mg C m⁻² h⁻¹ in the mid-marsh, and 155 ± 18 g C m⁻² h⁻¹ in the high marsh. Net CH₄ fluxes ranged from -18 to 57 μg C m⁻² h⁻¹ across all marsh zones and sampling dates. Fluxes were significantly higher in the low marsh compared to the mid and high marshes ($F_{2,9} = 3.3$, $P = 0.08$, Fig. 2b). Neither CO₂ effluxes nor CH₄ fluxes differed among sampling dates.

Figure 2

[Open in figure viewerPowerPoint](#)

Mean (a) CO₂ efflux and (b) net CH₄ flux by marsh zone across all sampling dates. Error bars represent standard errors. Letters indicate statistically significant differences among marsh zones.

[Caption](#)

Gross N₂O fluxes

Across all marsh zones and sampling dates, gross N₂O production rates ranged from 0 to 64.9 μg N m⁻² h⁻¹ and gross N₂O consumption rates ranged from 0 to 12.1 μg N m⁻² h⁻¹. Gross N₂O production differed by marsh zone and sampling date (zone, $F_{2,9} = 6.9$, $P = 0.02$, sampling date, $F_{2,18} = 12.1$, $P < 0.001$); rates were significantly lower in the mid-marsh compared to the low and high marshes (Fig. 1b). Gross N₂O consumption rates did not differ among marsh zones or sampling dates (Fig. 1b), averaging 2.1 ± 0.3 μg N m⁻² h⁻¹ overall.

Gross N₂O production rates were weakly positively correlated to pH ($R^2 = 0.21$, $P = 0.002$, $n = 44$). Gross N₂O consumption rates were weakly positively correlated to gross N₂O production rates ($R^2 = 0.22$, $P = 0.001$, $n = 44$). Soil NH_4^+ concentrations and CO₂ emissions together explained 27% of the variability in gross N₂O consumption rates ($P = 0.002$, $n = 44$), with both variables positively correlated to N₂O consumption.

Surface soil characteristics by marsh zone

Soil temperature differed significantly among marsh zones (Table 1), with higher temperature in the low marsh compared to the mid and high marsh zones. Soil temperature differed among

sampling dates ($F_{2,8} = 334$, $P < 0.001$), increasing from April (11.8 ± 0.2 °C) to June (16.3 ± 0.2 °C); soil temperature in the high and mid-marsh averaged 7.0 ± 0.1 °C in December. Soil moisture differed significantly among the marsh zones, ranging from an average of 0.49 ± 0.01 g H₂O g⁻¹ in the high marsh to 0.56 ± 0.01 g H₂O g⁻¹ in the low marsh (Table 1). Soil moisture also differed significantly among sampling dates ($F_{2,8} = 4.4$, $P = 0.03$) with lower soil moisture in June compared to the other sampling dates. However, WFPS did not differ between the mid and high marshes nor among sampling dates (Table 1), averaging $36 \pm 3\%$ in the mid-marsh and $41 \pm 2\%$ in the high marsh across all dates. Soil pH was significantly lower in the high and mid-marshes than the low marsh and was lower in May than in June or April (Table 1, $F_{2,18} = 25$, $P < 0.001$).

Soil O₂ concentrations ranged from 0.2 to 15.0% across all marsh zones and sampling dates. There was greater soil O₂ availability in the high marsh than in the mid and low marsh zones (Table 1). Soil NO_3^- concentrations were lowest in the low marsh whereas soil NH_4^+ concentrations did not differ among marsh zones or sampling dates (Table 1). The low marsh had significantly higher soil Fe(II) concentrations than the mid and high marsh zones (Table 1). Mean DEA tended to increase from high to low marsh zones, but differences were not statistically significant among marsh zones or sampling dates (Table 1); DEA was weakly positively correlated to soil moisture only ($R^2 = 0.22$, $P < 0.001$, $n = 44$). Total soil C and N concentrations also did not differ significantly among marsh zones or sampling dates (Table 1). Sand and silt content did not differ among marsh zones but clay content was highest in the low marsh (Table 1).

Soil N₂O concentrations ranged from 0 to 611 ppb across all marsh zones and sampling dates. In the mid-marsh, soil N₂O concentrations did not exceed 86 ppb whereas in the other marsh zones, concentrations above and below atmospheric N₂O concentrations were observed (Table 2). Soil N₂O concentrations were lower in the mid-marsh than the low and high marshes ($F_{1,3} = 66$, $P = 0.004$). Soil CH₄ concentrations were greater in the low and mid-marsh zones than in the high marsh ($F_{1,3} = 48$, $P = 0.006$, Table 2), and soil CO₂ concentrations were also higher in the mid-marsh than in the low and high marshes ($F_{1,3} = 79$, $P = 0.003$, Table 2).

Table 2. Soil trace gas concentrations by marsh zone and soil depth. Letters indicate statistically significant differences among marsh zones

	<i>N</i>	N₂O (ppb)			CH₄ (ppm)			CO₂ (%)		
		Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Low, 0–10 cm	8	84	611	266 ± 68 a	9	1754	360 ± 215 a	4.1	8.2	5.4 ± 0.4 a
Mid, 0–10 cm	13	0	86	51 ± 6 b	5	764	201 ± 63 a	7.0	13	10 ± 0.7 b
High, 0–10 cm	15	149	534	281 ± 33 a	1.1	5.8	2.1 ± 0.3 b	3.7	12	7.0 ± 0.7 a
High, 10–20 cm	14	94	906	306 ± 53	0.9	7.9	2.3 ± 0.5	3.3	13	6.7 ± 0.8
High, 20–30 cm	15	143	2084	563 ± 150	0.9	2.6	1.4 ± 0.1	3.3	13	7.0 ± 0.7

Soil characteristics by soil depth in the high marsh

Gravimetric soil moisture decreased from an average of 0.49 ± 0.01 g H₂O g⁻¹ at 0–10 cm depth to an average of 0.36 ± 0.02 g H₂O g⁻¹ at 20–30 cm depth ($F_{2,9} = 13$, $P = 0.002$, Table 3). In contrast, WFPS was lowest at 20–30 cm depth but did not differ between 0–10 cm and 10–20 cm depths ($F_{2,9} = 4.3$, $p = 0.05$, Table 3). Soil O₂ concentrations differed among sample dates but not among soil depths ($F_{3,21} = 16$, $P < 0.001$, Table 3). Across all soil depths, O₂ concentrations were higher in December ($13.4 \pm 0.5\%$) than all other sampling dates ($8.6 \pm 0.5\%$). Fe(II) concentrations averaged 326 ± 102 μg g⁻¹ overall and did not differ among soil depth or sampling dates (Table 3). There were also no significant differences in soil pH or texture (Table 3).

Table 3. Mean (±SE) soil characteristics by soil depth in the high marsh. Letters indicate statistically significant differences among soil depths

	0–10 cm depth (<i>n</i> = 16)	10–20 cm depth (<i>n</i> = 16)	20–30 cm depth (<i>n</i> = 16)	df	<i>F</i> - statistic
Moisture (g H ₂ O g ⁻¹)	0.49 ± 0.01 a	0.44 ± 0.01 b	0.36 ± 0.02 c	2, 9	13**
Water-filled pore space (%)	41 ± 2 a	39 ± 2 a	31 ± 2 b	2, 9	4.3**
Denitrifying enzyme activity (ng N g ⁻¹ h ⁻¹)	356 ± 60 a	44 ± 12 b	18 ± 8 c	2, 9	37***
pH	6.6 ± 0.1	6.6 ± 0.04	6.6 ± 0.1	2, 9	0.49
O ₂ concentration (%)	9.8 ± 0.8	10.2 ± 0.8	9.6 ± 1.0	2, 7	0.25
Fe(II) concentration (mg Fe(II) g ⁻¹)	0.19 ± 0.03	0.2 ± 0.05	0.6 ± 0.3	2, 9	0.021
NH ₄ ⁺ concentration (μg N g ⁻¹)	3.1 ± 0.4 a	1.1 ± 0.1 b	0.7 ± 0.1 c	2, 9	40***
NO ₃ ⁻ concentration (μg N g ⁻¹)	1.1 ± 0.2 a	1.2 ± 0.3 a	0.6 ± 0.1 b	2, 9	4.7**

	0–10 cm depth (<i>n</i> = 16)	10–20 cm depth (<i>n</i> = 16)	20–30 cm depth (<i>n</i> = 16)	df	<i>F</i> - statistic
C concentration (%)	4.49 ± 0.36 a	2.99 ± 0.13 b	1.99 ± 0.17 c	2, 9	15***
N concentration (%)	0.34 ± 0.03 a	0.24 ± 0.01 b	0.16 ± 0.01 c	2, 9	14**
Sand content (%)	45 ± 7	31 ± 5	50 ± 9	2, 9	1.7
Silt content (%)	40 ± 5	47 ± 3	34 ± 7	2, 9	1.8
Clay content (%)	15 ± 2	22 ± 2	16 ± 3	2, 9	2.2
Bulk density (g cm ⁻³)	0.36 ± 0.04	0.41 ± 0.03	0.45 ± 0.05	2, 9	1.0

- Statistically significant at *P < 0.1, **P < 0.05, and ***P < 0.001.

Soil C and N availability decreased with depth in the high marsh. Total C and N concentrations for soils ranged from 0.85 to 6.5% and 0.08 to 0.54%, respectively, and decreased significantly with depth (soil C, $F_{2,9} = 15$, $P = 0.001$; soil N, $F_{2,9} = 14$, $P = 0.002$, Table 3). Soil C concentrations were strongly and positively correlated to soil moisture ($R^2 = 0.72$, $P < 0.001$, $n = 48$) and also exhibited a strong relationship with soil N concentrations, with a slope of 13.5 ± 0.1 ($R^2 = 0.99$, $P < 0.001$, $n = 48$). Both soil NH_4^+ and NO_3^- concentrations decreased significantly with depth (Table 3, NH_4^+ , $F_{2,9} = 40$, $P < 0.001$; NO_3^-

, $F_{2,9} = 4.7$, $P = 0.04$) and differed among sampling dates (NH_4^+ , $F_{3,27} = 5.0$, $P = 0.007$; NO_3^- , $F_{3,27} = 8.4$, $P < 0.001$). Soil NH_4^+ was lower in December than in April through June; soil NO_3^- was lower in December and April compared to May and June.

Denitrifying enzyme activity also decreased with depth in the high marsh ($F_{2,9} = 37$, $P < 0.001$, Table 3). Denitrifying enzyme activity was positively correlated with soil C concentration ($R^2 = 0.46$, $P < 0.001$, $n = 48$), soil moisture ($R^2 = 0.50$, $P < 0.001$, $n = 48$, Fig. 3a), and soil mineral N concentrations ($R^2 = 0.47$, $P < 0.001$, $n = 48$, Fig. 3b) across all soil depths and sampling dates. Soil moisture and mineral N concentrations together best predicted DEA, explaining 62% of the variability in DEA ($P < 0.001$, $n = 48$).

Figure 3

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Denitrifying enzyme activity versus (a) gravimetric soil moisture and (b) soil mineral N concentrations across all soil depths in the high marsh. The filled circles represent 0–10 cm depth, the open circles represent 10–20 cm depth, and the triangles represent 20–30 cm depth. The linear regression lines for all soil depths together are shown.

[Caption](#)

Soil N_2O concentrations trended higher with soil depth in the high marsh (Table 2), ranging from 149–534 ppb at 0–10 cm depth to 143–2084 ppb at 20–30 cm depth. However, they were not significantly different among soil depths or sampling dates. Soil CH_4 and CO_2 concentrations averaged 1.9 ± 0.2 ppm and $6.9 \pm 0.4\%$, respectively, and also did not differ significantly among soil depths or sampling dates (Table 2).

Discussion

N₂O dynamics by marsh zone

Across all marsh zones, net N₂O fluxes ranged from -16 to $60 \mu\text{g N m}^{-2} \text{ h}^{-1}$, which is comparable to fluxes reported for other salt marshes (Hirota *et al.*, [2007](#); Blackwell *et al.*, [2010](#); Chmura *et al.*, [2011](#); Moseman-Valtierra *et al.*, [2011](#); Murray *et al.*, [2015](#)). The measurement of *in situ* gross N₂O production and consumption rates is still relatively new, so there are not many published rates to compare our results against. Gross N₂O production rates, which ranged from 0 to $64.9 \mu\text{g N m}^{-2} \text{ h}^{-1}$, were much lower in this salt marsh than in a managed temperate grassland with high soil mineral N concentrations that drove high gross N₂O production rates averaging $350 \pm 133 \mu\text{g N m}^{-2} \text{ h}^{-1}$ (Yang *et al.*, [2011](#)). Gross N₂O consumption rates were also much higher in that managed grassland, averaging about 40 times higher than in this salt marsh.

Soil redox increased with elevation in the Tomales Bay salt marsh as indicated by differences in soil concentrations of O₂, NO₃⁻, Fe(II), and CH₄ among the marsh zones, but N₂O dynamics did not follow the same monotonic trend as redox. Instead, the mid-marsh consistently exhibited negative net N₂O fluxes whereas both positive and negative fluxes were observed in the low and high marsh. Ostensibly, this suggests that the potential for N₂O consumption was greater in the mid-marsh than the other marsh zones. However, gross N₂O consumption rates did not differ significantly among marsh zones. Rather, the net N₂O uptake observed in the mid-marsh was due to gross N₂O production rates that were consistently lower than gross N₂O consumption rates and also low compared to the other marsh zones.

None of the soil variables we measured were strongly correlated with gross N₂O production rates across the marsh zones to elucidate the drivers of low N₂O production in the mid-marsh. This contrasts with the Yang *et al.* ([2011](#)) study that showed a strong correlation between DEA and gross N₂O production rates measured *in situ* across a microtopographical gradient in a managed grassland. The lack of relationships in this study is likely because the relative importance of nitrification and denitrification – two distinct processes with different underlying controls – varied among the marsh zones. Denitrifying enzyme activity trended higher from the high marsh down to the low marsh, suggesting that the relative contribution of denitrification to gross N₂O production may have increased as marsh elevation decreased. Denitrification, whether heterotrophic or autotrophic, dominates N₂O production at very low O₂, such as found in the low marsh, whereas nitrification and coupled nitrification–denitrification become important N₂O sources at higher O₂, such as found in the high marsh (Baggs, [2011](#); Zhu *et al.*, [2013](#)). The mid-

marsh may have been poised at soil O_2 and redox conditions to suppress N_2O production from both nitrification and denitrification.

The highest N_2O emissions were found in the low marsh due to high gross N_2O production rates without concomitantly high gross N_2O consumption rates. The low marsh soil exhibited the most reducing conditions and highest soil moisture so we expected that higher denitrification activity and lower gas transport rates would together increase N_2O consumption in that zone. The unexpected similarity in N_2O consumption rates across marsh zones could be due to inhibition of N_2O reduction in the low marsh by sulfide, a chemical species found under highly reducing conditions (Sørensen *et al.*, [1980](#); Gould & Mccready, [1982](#); Brunet & Garcia-Gil, [1996](#)). Although we did not measure sulfide concentrations, the strong smell of H_2S was apparent only when we handled soil from the low marsh. Sulfide can also inhibit nitrification (Joye & Hollibaugh, [1995](#)), a process that can directly lead to N_2O production and supplies NO_3^- for N_2O production via denitrification. The complex role of sulfide in regulating both N_2O production and consumption in salt marshes should be further explored, especially given rising sea levels that could increase the importance of the interaction between the S and N cycles.

Dissimilatory NO_3^- reduction to NH_4^+ (DNRA), an anaerobic microbial process, could also both indirectly and directly influence patterns in gross N_2O fluxes across the salt marsh. This process can compete with denitrification for NO_3^- to inhibit N_2O production via denitrification, and a previous study in the high marsh at our study site demonstrated that DNRA rates were comparable to denitrification rates (Yang *et al.*, [2015](#)). Although there is some evidence that DNRA organisms can produce N_2O (Baggs, [2011](#); Vieillard & Fulweiler, [2014](#)), this is still under debate (Giblin *et al.*, [2013](#)). Even if DNRA can act as a source of N_2O , conditions that promote DNRA (i.e., high C to NO_3^- availability) appear to be different from those that lead to greatest N_2O production rates by DNRA organisms (i.e., low C to NO_3^- availability) (Streminska *et al.*, [2012](#)). Thus, in the mid-marsh, DNRA organisms could have outcompeted denitrifiers to reduce NO_3^- to NH_4^+ without N_2O production. In addition, DNRA organisms can harbor atypical *nosZ*, a gene encoding for N_2O reductase (Sanford *et al.*, [2012](#)), to contribute to N_2O consumption decoupled from N_2O production. This could explain how the mid-marsh exhibited similar gross N_2O consumption rates but lower gross N_2O production rates compared to the other marsh zones.

Plant community composition differed among marsh zones, potentially influencing the patterns in gross N_2O fluxes across the salt marsh. Plant community composition has been shown to alter

N₂O dynamics in coastal ecosystems (Kaplan *et al.*, 1979; Yuan *et al.*, 2015) through multiple mechanisms. First, some species, such as *S. alterniflora* which dominated the low marsh, can oxygenate the rhizosphere to affect soil redox (Koretsky *et al.*, 2008). However, multiple biogeochemically relevant measures of soil redox decreased monotonically from the high to the low marsh as expected from marsh zone differences in inundation regimes. Second, plant species with high productivity, such as *S. alterniflora*, can also suppress N₂O production through plant competition with microbes for N (Zhang *et al.*, 2013); however, the low marsh dominated by *S. alterniflora* had the highest gross N₂O production rates. Third, plant species can also directly affect rates of microbial N cycling processes, such as nitrification, by shaping the microbial community composition (Hawkes *et al.*, 2005). Our data do not address this mechanism, which warrants further investigation.

N₂O dynamics along soil depth profile in the high marsh

Trends in soil characteristics with soil depth in the high marsh suggest that denitrification was concentrated in the surface soil due to C limitation at depth. First, DEA decreased significantly with depth, with rates at 10–30 cm depth averaging 5–13% of those at 0–10 cm depth. This is consistent with a similar depth trend in DEA observed in another marsh sediment (Tobias *et al.*, 2001). Both NO_3^- concentrations and total C concentrations decreased with soil depth, but NO_3^- was only weakly correlated to DEA. In contrast, we observed a strong positive relationship between DEA and soil C across all soil depths, suggesting that the decrease in DEA with depth may have been driven by a decrease in C rather than NO_3^- availability. Second, the highest soil N₂O concentrations were observed at 20–30 cm depth. Higher WFPS in the top 20 cm of soil could have impeded gas transport from the deeper soil into the surface soil to cause the higher N₂O concentrations at depth. However, given the decrease in DEA, NO_3^- , and C with soil depth, it is likely that this trend in N₂O concentrations arose from low N₂O consumption rates rather than high N₂O production rates at depth.

The decrease in DEA with soil depth has positive implications for the use of the ¹⁵N₂O pool dilution technique in salt marsh soils where high soil moisture can impede the diffusion of the ¹⁵N₂O and SF₆ tracers into the soil to possibly cause underestimation of gross N₂O consumption rates if substantial N₂O consumption occurs at depth (Yang *et al.*, 2011). To address this potential issue, we used a three hour sampling period to give the tracers more time to diffuse into the soil. The depth trends in DEA and N₂O concentrations suggest that this measure was likely sufficient to obtain accurate estimates of gross N₂O consumption because denitrification activity was concentrated in the top 10 cm of soil. We recognize that the pool dilution technique

cannot measure complete denitrification that occurs intracellularly (Yang *et al.*, [2011](#)); therefore, we do not equate gross N₂O consumption with N₂ production by denitrification. Instead, our estimates of gross N₂O consumption rates consider only N₂O that could potentially be exchanged between the soil and the atmosphere to affect net N₂O fluxes.

Implications for understanding controls on N₂O dynamics

Our study provides a new perspective on the mechanisms that control net N₂O uptake by soil by demonstrating that variations in gross N₂O production rather than consumption drove patterns in net N₂O fluxes across the salt marsh landscape. Chapuis-Lardy *et al.* ([2007](#)) hypothesized that the potential for net N₂O uptake by soil is controlled by rates of N₂O reduction to N₂, the rate of N₂O diffusion through the soil (i.e., slower diffusion allows more N₂O to be consumed in soil before it exchanges with the atmosphere), and the dissolution of N₂O in water. This perspective focuses on how changes in the magnitude of gross N₂O consumption cause soils to switch from N₂O sources to N₂O sinks. Although potential N₂O uptake rates can vary depending on soil conditions (Frasier *et al.*, [2010](#)) and across a large range on the global scale (1–100 $\mu\text{g N m}^{-2} \text{ h}^{-1}$, Schlesinger, [2013](#)), in our study, net N₂O uptake occurred whenever gross N₂O production rates dropped below gross N₂O consumption rates, which were similar across a wide range in environmental conditions. In conclusion, our data suggest that elucidating controls on the specific processes leading to gross N₂O production in salt marshes will be critical to improving predictions of how net N₂O fluxes in these coastal ecosystems will respond to sea level rise.

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