



Elevated temperature and nutrients lead to increased N₂O emissions from salt marsh soils from cold and warm climates

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Abstract Salt marshes can attenuate nutrient pollution and store large amounts of ‘blue carbon’ in their soils, however, the value of sequestered carbon may be partially offset by nitrous oxide (N₂O) emissions. Global climate and land use changes result in higher temperatures and inputs of reactive nitrogen (Nr) into coastal zones. Here, we investigated the combined effects of elevated temperature (ambient + 5°C) and Nr (double ambient concentrations) on nitrogen processing in marsh soils from two climatic regions (Quebec, Canada and Louisiana, U.S.) with two vegetation types, *Sporobolus alterniflorus* (= *Spartina alterniflora*) and *Sporobolus pumilus* (= *Spartina patens*), using 24-h laboratory incubation experiments.

Potential N₂O fluxes increased from minor sinks to major sources following elevated treatments across all four marsh sites. One day of potential N₂O emissions under elevated treatments (representing either long-term sea surface warming or short-term ocean heatwaves effects on coastal marsh soil temperatures alongside pulses of N loading) offset 15–60% of the potential annual ambient N₂O sink, depending on marsh site and vegetation type. Rates of potential denitrification were generally higher in high latitude than in low latitude marsh soils under ambient treatments, with low ratios of N₂O:N₂ indicating complete denitrification in high latitude marsh soils. Under elevated temperature and Nr treatments, potential denitrification was lower in high latitude soil but higher in low latitude soil as compared to ambient conditions, with incomplete denitrification observed except in

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Louisiana *S. pumilus*. Overall, our findings suggest that a combined increase in temperature and Nr has the potential to reduce salt marsh greenhouse gas (GHG) sinks under future global change scenarios.

Keywords Salt marsh · Nitrogen · Nitrous oxide · Denitrification · Greenhouse gas · Biogeochemistry · Future global change · Temperature · Reactive nitrogen loading · Quebec · Louisiana · *Sporobolus alterniflorus* · *Sporobolus pumilus* · Climate · *Spartina patens* · *Spartina alterniflora*

Introduction

Salt marshes provide wide ranging ecosystem services including carbon sequestration and nutrient filtration, with the potential to reduce some of the impacts of climate change and reactive nitrogen (Nr) pollution (Mitsch and Gosselink 2001; Chmura et al. 2003; McLeod et al. 2011). However, the value of the atmospheric carbon sequestered in marsh soils may be partially offset by nitrous oxide (N₂O) emissions, a potent greenhouse gas with a sustained-flux global warming potential 270 times higher than that of CO₂ over 100 years (Neubauer and Magonigal 2019). Salt marshes filter N through plant uptake and storage in biomass and soil organic matter and through a variety of microbial processes (Tobias and Neubauer 2019). Key microbial processes filtering N include immobilisation, denitrification, nitrification and anaerobic ammonium oxidation (anammox), with denitrification and nitrification producing N₂O.

Immobilisation is the accumulation of N into bacterial biomass, denitrification is the reduction of nitrate to N₂, nitrification is the oxidation of ammonium to nitrate and anammox is the anaerobic oxidation of ammonium to N₂ (Tobias and Neubauer 2019). Denitrification, nitrification and coupled nitrification–denitrification are the dominant pathways of salt marsh N₂O production (Foster and Fulweiler 2016; Maavara et al. 2019). Denitrification may both produce and consume N₂O during the conversion of NO₃⁻ to N₂, with the completeness of denitrification determining the ratio between N₂O and N₂ production (Kendall 1998).

N₂O fluxes from salt marshes are highly variable and are dependent on local environmental conditions. Salt marshes are typically small sources or sinks of

N₂O (Moseman-Valtierra et al. 2011; Murray et al. 2015); however, with increased Nr (reactive N) loading, these ecosystems are expected to become larger sources of N₂O (Murray et al. 2015; Chmura et al. 2016; Roughan et al. 2018).

Global change (i.e., climate and land use change) is causing rapid change in many environmental conditions, such as temperature, nutrient loading and sea level rise, that may cause shifts in vegetation and soil properties. Increasing temperatures will lead to warmer sea surface temperatures and warmer soils, potentially impacting microbial activity and greenhouse gas emissions from coastal marshes (Kirwan and Blum 2011). Further, salt marshes have experienced an increase in Nr loading due to excess N from fertiliser use entering waterways (Liu and Greaver 2009; Seitzinger et al. 2010). Rates of N₂O fluxes and nitrogen transforming reactions, including the completeness of denitrification, are dependent on vegetation and soil properties, such as soil moisture, temperature, plant traits, substrate availability and salinity (e.g., Weier et al. 1993; Murray et al. 2015; Tobias and Neubauer 2019). These vegetation and soil properties vary with different climates (latitudes) and land-use (e.g. McCall and Pennings 2012; Bai et al. 2013); therefore, the response of N₂O fluxes and nutrient filtration to global change will likely differ geographically.

We investigated the effect of temperature and nitrogen loading on greenhouse gas (GHG) fluxes, rates of denitrification (N₂O versus N₂) and rates of nitrification-derived N₂O in salt marshes at a low latitude, subtropical humid climate (Louisiana) and a high latitude, temperate continental climate (Quebec). Recent evidence suggests that the Gulf of St. Lawrence and the Gulf of Mexico are warming faster than the global ocean average (Wang et al., 2023, Galbraith et al., 2023), therefore, it is important to understand temperature responses of salt marsh soils in these regions. Two dominant salt marsh plant communities were investigated from each latitude; one dominated by *Sporobolus alterniflorus* (Loisel; formerly *Spartina alterniflora*) and *Sporobolus pumilus* (Aiton) Muhl. (formerly known as *Spartina patens*; Peterson et al. 2014). Different climatic regions and vegetation zones may have different rates of biogeochemical reactions due to different soil properties, background nutrient concentrations and ambient temperatures. We used tracers of ¹⁵N–NO₃⁻ and

$^{15}\text{N-NH}_4^+$ to investigate rates of N_2O and N_2 production from denitrification and nitrification. We predict that increasing temperature and Nr loading would increase rates of GHG fluxes and denitrification, but that under these conditions, a greater proportion of denitrification would be incomplete favoring N_2O over N_2 production. This would be expected because of the typically higher reaction rates under increasing temperatures and increases in Nr leading to higher ratios of $\text{N}_2\text{O}:\text{N}_2$ from denitrification (Kirwan and Blum 2011; Song et al. 2014; Murray et al. 2015). We hypothesise that higher ambient temperatures in low latitude soils will result in smaller responses to elevated temperatures than high latitude soils as reaction rates have a more sensitive temperature response at lower temperatures (Kaplan et al. 1979; Ferrón et al. 2007; Hirota et al. 2007; Kirwan and Blum 2011; Moseman-Valtierra et al. 2011). We also analysed soil properties to investigate the drivers of differences between climatic region and plant species, as well as responses to global change conditions.

Materials and methods

Study sites

Intact soil cores were collected from *S. alterniflorus*- and *S. pumilus*-dominated marshes in Quebec, Canada and Louisiana, U.S. in summer 2021. In Quebec, both *S. alterniflorus* and *S. pumilus* were sampled within the same salt marsh in different elevation zones, whereas in Louisiana two separate marshes (one *S. alterniflorus* and one *S. pumilus*) were sampled. In Quebec, the salt marsh is located on the St. Lawrence estuary near la Pocatière (47°22'24.7" N 70°03'26.3" W), and in Louisiana the two marshes are located in the Barataria Basin (Coastwide Reference Monitoring System (lacoast.gov/CRMS) sites 220, 29°33'47.3"N 90°04'22.8"W, and 224, 29°29'52.2"N 89°55'00.2"W, Fig. 1a). Soil porewater salinities measured in this study averaged 21.2 and 9.8 PSU for the *S. alterniflorus* and *S. pumilus* in Quebec and 1.7 and 0.8 PSU for the *S. alterniflorus* and *S. pumilus* in Louisiana. The salinities measured in the Louisiana marshes were unusually low, likely due to high precipitation in this region in 2021, the salinity usually averages 8.8 PSU in the *S. alterniflorus* and 4.1 PSU in the *S. pumilus* marsh (lacoast.gov, 2022). The average

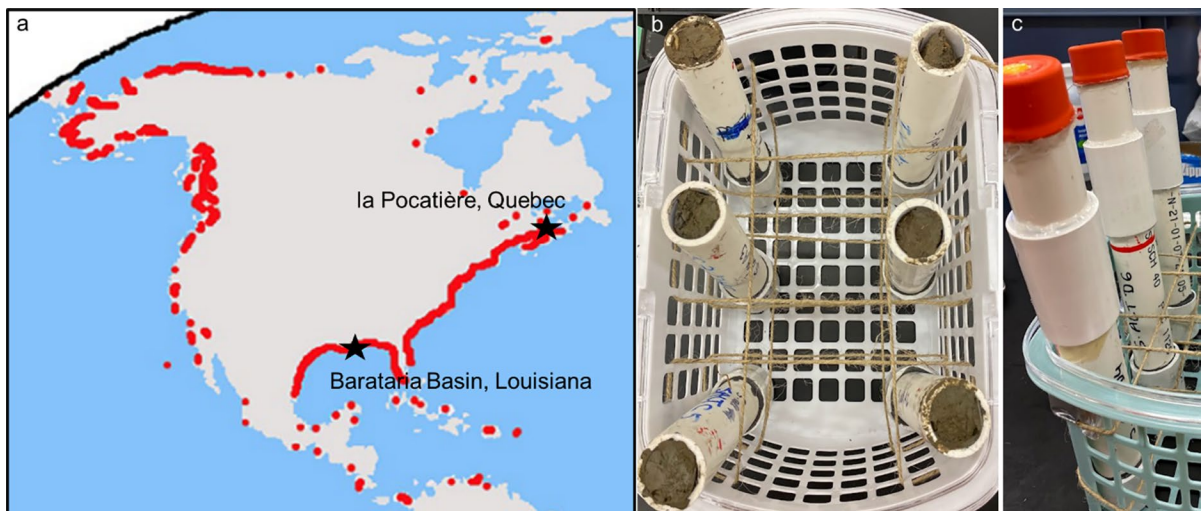


Fig. 1 a The locations of the studied salt marshes (black stars) on a map of the distribution of salt marshes in North America (red circles) illustrating the climatic gradient of major salt marsh systems that were studied, adapted from Giuliani

and Bellucci, 2019, b. Intact cores shown inside the incubation pipes and c. Incubation pipes with added pipe to create a headspace. Photo credit: Sophie Comer-Warner. (Color figure online)

tidal range at la Pocatière, Quebec is ~4.1 m (Canadian Hydrographic Service 2021) and at the coast of the Barataria Basin, Louisiana is 0.3 m (Conner and Day 1987). The climate is temperate continental at la Pocatière and subtropical humid in the Barataria Basin (Belda et al. 2014) with average annual temperature of 4.5 °C at la Pocatière and 20.6 °C in the Barataria Basin and average annual precipitation of 933 mm at la Pocatière (Environment and Climate Change Canada, 2020) and 1600 mm in the Barataria Basin (Conner and Day 1987).

Soil collection

Five sampling locations (1-m²) were chosen along a 20-m transect at each marsh vegetation site, which served as replicates for the incubation experiments. This resulted in 20 sampling locations across the four marsh sites. Soil cores were collected from the Louisiana marshes on 20–21st July 2021 and from the Quebec marsh on 9–10th August 2021. Six intact soil cores were collected from 0–15 cm depth at each sampling location using a 2.5 cm diameter soil auger or piston corer, placed onto plastic soil sampling liners and inserted into PVC tubes (2.5 cm diameter, 15 cm long), which were capped for shipping (Fig. 1b). An additional bulk sediment sample was collected from each sampling location for chemical analysis. Samples were transported on ice to the laboratory and then stored at 4 °C. Soil temperature (YSI 30, Baton Rouge, USA or DeltaTrak 11050, Pleasanton, USA) and porewater salinity (YSI 30, Baton Rouge, USA or portable ATC refractometer) were measured *in-situ* at the time of sampling with the YSI 30 (at the Louisiana sites) or 2 days after sampling in the laboratory (for the Quebec sites) for the refractometer measurements.

Incubation experiments

The incubation experiments were designed with ambient and elevated treatment conditions. Results are therefore interpreted as potential fluxes and rates due to experiments being conducted in the laboratory and not *in-situ*. The ambient experiments were conducted at the soil temperature at time of sampling (16 °C and 28.1 °C for Quebec and Louisiana, respectively) and with no additional nutrients added. The elevated treatment experiments were conducted

at ambient+5 °C and double the ambient nutrient concentrations with both NO₃⁻ and NH₄⁺ concentrations elevated. Table 1 gives the ambient concentrations of NO₃⁻ and NH₄⁺ for each marsh site. Four sets of incubation experiments were performed, Louisiana cores under ambient treatment, Louisiana cores under elevated treatment, Quebec cores under ambient treatment and Quebec cores under elevated treatment. Three intact cores were used in the ambient and three intact cores used in the elevated experiments. Of these three cores, one received no tracer to investigate unmodified rates of GHG fluxes. A second core received K¹⁵NO₃ (98 at. % ¹⁵N, Sigma Aldrich) tracer to investigate rates and completeness of denitrification (including the dominant products of denitrification – N₂ versus N₂O). The third core received ¹⁵NH₄Cl (98 at. % ¹⁵N, Sigma Aldrich) tracer to investigate rates of N₂O fluxes from nitrification (Ambus et al. 2006; Matson et al. 2009; Sgouridis and Ullah 2015, 2017).

Core pipes (pre-weighed) containing soils were weighed to determine the mass of soil in each core, incubation experiments were conducted in an incubator so that the temperature could be controlled over a wider range (Isotemp 3720A, Fisherbrand, Ottawa, Ontario, Canada). The actual incubator temperature (HOBO Temperature Data Logger, Onset, Bourne, Massachusetts, U.S.) throughout the incubation experiments, as well as the within incubator variation, was measured with temperature variation at the time of experiments between 0.7 and 1.2 °C. Artificial seawater (Aquaforest Reef Salt) was made to the average salinity of the porewater for each marsh vegetation site at the time of sampling. For the elevated treatments artificial seawater was also made with NH₄NO₃ to double the concentration of these nutrients in the soil cores.

The day before incubation experiments began, the incubator was calibrated to the desired temperature. Core pipes were uncapped at the surface end, placed into baskets to hold them upright and a section of pipe was added to create a 60.5 cm³ -headspace. Six core pipes were placed into each of five baskets so that replicates from each marsh vegetation site were evenly distributed throughout the incubator and any effect from differences in temperature within the incubator was spread across replicates. A 2 ml-aliquot of artificial seawater (with nutrients for elevated treatment) was injected throughout each core using a

Table 1 Potential biogeochemical reaction rates and environmental variables for all four of the salt marsh sites (from Louisiana (LA) and Quebec (QC)) and both treatment levels (ambient (amb.) and elevated (elev.)) studied here.

Variable	Site			
	LA <i>S. alterniflorus</i>	LA <i>S. pumilus</i>	QC <i>S. alterniflorus</i>	QC <i>S. pumilus</i>
Amb. N ₂ O–N (ng g dry soil ⁻¹ hr ⁻¹)	– 0.02±0.01	– 0.00±0.02	– 0.01±0.01	– 0.01±0.02
Elev. N ₂ O–N (ng g dry soil ⁻¹ hr ⁻¹)	1.19±1.09	0.67±1.01	0.92±1.06	0.70±0.65
Amb. CH ₄ –C (μg g dry soil ⁻¹ hr ⁻¹)	0.03±0.02	0.03±0.03	0.01±0.02	0.00±0.00
Elev. CH ₄ –C (μg g dry soil ⁻¹ hr ⁻¹)	0.03±0.02	0.01±0.01	0.02±0.02	0.00±0.00
Amb. CO ₂ –C (μg g dry soil ⁻¹ hr ⁻¹)	1.12±0.55	0.12±0.74	1.18±0.34	1.57±0.43
Elev. CO ₂ –C (μg g dry soil ⁻¹ hr ⁻¹)	1.02±0.64	0.71±0.41	2.04±0.50	2.50±1.25
Amb. denitrification-derived N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Elev. denitrification-derived N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.29±0.42	0.09±0.09	1.30±1.34	0.37±0.25
Amb. denitrification-derived N ₂ (ng N g dry soil ⁻¹ h ⁻¹)	0.00±0.00	0.55±1.00	11.80±9.44	5.56±4.50
Elev. denitrification-derived N ₂ (ng N g dry soil ⁻¹ h ⁻¹)	0.05±0.10	1.31±1.46	0.46±0.29	0.23±0.14
Amb. Total Denitrification - N ₂ +N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.00±0.00	0.55±0.99	11.80±9.44	5.56±4.50
Elev. total denitrification - N ₂ +N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.33±0.44	1.40±1.40	1.75±1.41	0.60±0.36
Amb. nitrification-derived N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Elev. nitrification-derived N ₂ O (ng N g dry soil ⁻¹ h ⁻¹)	0.05±0.08	0.00±0.00	0.04±0.04	0.00±0.00
Soil temp. (°C)	27.0±0.3	29.2±1.9	17.2±0.8	15.2±0.1
Salinity	1.7±0.1	0.8±0.2	21.2±2.8	9.8±1.0
Gravimetric soil moisture (%)	73.9±5.9	82.1±4.2	44.7±2.3	52.4±2.4
DOC (μg C g wet sed. ⁻¹)	83.1±6.2	74.5±19.5	99.7±12.7	89.4±11.6
TDN (μg N g wet sed. ⁻¹)	4.8±1.7	2.9±0.8	3.3±1.0	3.0±0.4
NO ₃ ⁻ (μg g wet sed. ⁻¹)	0.5±0.5	5.7±4.7	3.2±0.5	2.9±0.2
NH ₄ ⁺ (μg g wet sed. ⁻¹)	7.3±6.8	2.8±1.9	2.0±0.2	2.5±0.5
SRP (μg g wet sed. ⁻¹)	12.3±1.9	4.4±1.6	33.1±5.8	26.3±9.2
TC (%)	8.5±2.6	15.3±3.8	3.4±0.5	4.3±0.5
TN (%)	0.5±0.2	1.0±0.2	0.2±0.0	0.3±0.0
δ ¹³ C (‰)	– 16.6±1.4	– 20.6±1.1	– 19.2±0.7	– 19.0±0.7
δ ¹⁵ N (‰)	1.6±0.3	1.3±0.2	45.4±16.1	51.8±4.1
C:N	17.7±1.7	15.8±2.0	17.0±1.6	16.8±1.4

Variable abbreviations are: *DOC* dissolved organic carbon, *TDN* total dissolved nitrogen, *SRP* soluble reactive phosphate, *TC* total carbon and *TN* total nitrogen.

syringe and needle. Core pipes were then placed into the incubator overnight to pre-incubate to the desired temperature while the pipe headspace was kept open.

Incubation experiments were conducted as follows. At t=0 one basket at a time was removed from the incubator, 1 ml of tracer solution was added in order to achieve 40% ¹⁵N enrichment of the ambient nitrate or ammonium soil pool in each relevant core pipe. Two gas samples were immediately taken from within the headspace; A 7-ml gas sample was extracted to fill a 3.5-ml exetainer (Labco,

UK) for GHG analysis and a 20-ml gas sample was then extracted to fill a 12-ml exetainer for isotopic analysis. A rubber stopper was added to enclose the headspace. Once all pipes were sampled the basket was replaced into the incubator (Fig. 1c). At t=5 h one basket was removed at a time and a 7-ml gas sample taken through the rubber stopper and transferred to a 3.5-ml exetainer for GHG analysis. At t=24 h one basket was removed at a time and 7- and 20-ml gas samples were extracted for GHG and isotopic analysis, respectively.

Chemical analysis

Greenhouse gases

An Agilent 7890A Gas Chromatograph (GC) interfaced with a PAL3 autosampler (Agilent Technologies Ltd, USA) and equipped with a splitless 1-ml sample loop was used to measure concentrations of N₂O, CH₄ and CO₂ in all samples. The GC was fitted with a flame ionisation detector (FID) for CH₄ analysis and a micro electron capture detector (μ ECD) for N₂O analysis. As the FID only detects CH₄, the CO₂ was methanised to CH₄ prior to passing into the FID and analysed as CH₄. The temperature of the GC oven was 60 °C, of the FID was 250 °C and of the μ ECD was 350 °C. The FID had an N₂ make-up flow of 2 ml min⁻¹ with 48 ml min⁻¹ of hydrogen and 500 ml min⁻¹ of air. The μ ECD had a make-up argon and methane flow of 2 ml min⁻¹. The run time was 9 min with CH₄, CO₂ and N₂O eluted at 3.6, 5.0 and 7.0 min, respectively. The instrument precision, at atmospheric concentrations for the 3 gases, was determined from repeated analyses of 8 lab air samples and the relative standard deviation was <5% for all of the gases, while the minimum detectable concentration difference (MDCD) was 9 ppb N₂O, 72 ppb CH₄ and 31 ppm CO₂, respectively. The MDCD was calculated from repeated manual analyses of reference standards as in Sgouridis and Ullah (2017). Potential GHG fluxes were calculated from the linear portion or where the highest production was observed in the concentration–time series (White and Reddy 2003). If fluxes were below the MDCD value of the GC they were set to zero (Sgouridis and Ullah 2017). Potential annual fluxes were calculated using 365 days for Louisiana and 244 days for Quebec, as the salt marsh is frozen with assumed negligible GHG exchanges with the air from November to March. These fluxes were estimated to compare with potential elevated daily fluxes and demonstrate potential impacts of elevated temperature and N loading, not to represent an accurate estimate of in-situ N₂O fluxes.

Isotopic composition

The ¹⁵N content of the N₂ and N₂O in the 12-mL exetainer vials was determined using a continuous flow isotope ratio mass spectrometer (Elementar Isoprime PrecisION; Elementar Analysensysteme

GmbH, Hanau, Germany) coupled with a trace-gas pre-concentrator inlet with autosampler (isoFLOW GHG; Elementar Analysensysteme GmbH, Hanau, Germany). Gas samples in 12 ml exetainers were purged into a He stream through the autosampler and after passing through a CO₂ and an H₂O scrubber, then entered an initial liquid N₂ trap isolating and cryofocusing the N₂O. Following the initial N₂O trapping, N₂ was subsampled through a 7 μ l sub-sampling loop and after O₂ reduction through a Cu reduction furnace at 600 °C, the N₂ was directed to the Isotope Ratio Mass Spectrometer (IRMS) where the N₂ isotopologues (²⁸N₂, ²⁹N₂, and ³⁰N₂, respectively) were measured at a trap current of 100 μ A in both enriched (t₂₄ samples) and reference samples (t₀ samples). The rest of the gas sample was further concentrated in a second liquid N₂ trap and the isolated N₂O was further separated from any residual CO₂ by passing through a Poraplot Q gas chromatography column before being directed to the IRMS where the N₂O isotopologues (⁴⁴N₂O, ⁴⁵N₂O, and ⁴⁶N₂O, respectively) were measured at a trap current of 600 μ A in both enriched (t₂₄ samples) and reference samples (t₀ samples). Instrument stability checks were performed prior to each analysis by running a series of 10 reference pulses of pure N₂ and N₂O (BOC special gases) until a standard deviation of $\delta^{15}\text{N}$ better than 0.05‰ was achieved. Potential fluxes were then calculated using the isotopic ratios in the ‘non-equilibrium’ equations described in detail elsewhere (Mulvaney 1984; Bergsma et al. 2001).

Nutrient content

Nitrate, ammonium and phosphorus samples were processed and analysed by the Soil Ecology Research Group Lab, McGill University. Field-wet soils (5 g) were extracted with 25 ml of 2 M KCl for nitrate/ammonium analysis on a shaker at 200 rpm for 1 h. Samples were then centrifuged at 4000 rpm for 20 min, filtered (0.45 μ m) and analysed on a microplate reader with a limit of detection (LOD) and accuracy of 0.1 ppm and $\pm 5\%$, respectively (Sims et al. 1995). Soluble reactive phosphate was measured using the Olsen-P method (Olsen and Sommers 1982; Prokopy 1995). 2.5 g of dry soil were extracted with 25 ml Olsen-P solution (0.5 M NaHCO₃, pH 8.5), shaken for 20 min at 120 rpm and immediately filtered (without centrifuging or settling) over Q5

Fisherbrand filter paper. Extractant was analysed on a microplate reader with a LOD and accuracy of 1 mg P l⁻¹ and $\pm 6\%$, respectively (Jeannotte et al. 2004).

For dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analysis, field-wet soils (5 g) were extracted with 35 ml of ultrapure H₂O (18.2 M Ω), shaken at 200 rpm for 2 h and centrifuged for 20 min at 4000 rpm. Extractant was then filtered (0.45 μ m) and preserved at pH < 2 with 2 N HCl or analysed within 24 h. DOC and TDN were analysed on a TOC/TDN analyser (TOC VCSn+TMN-1, Shimadzu, Kyoto, Japan) with 50 mg C l⁻¹ and 10 mg l⁻¹ standards resulting in accuracy and precision of 0.3 and ± 0.3 mg C l⁻¹, and 0.5 and ± 0.3 mg N l⁻¹, respectively.

Soil C and N.

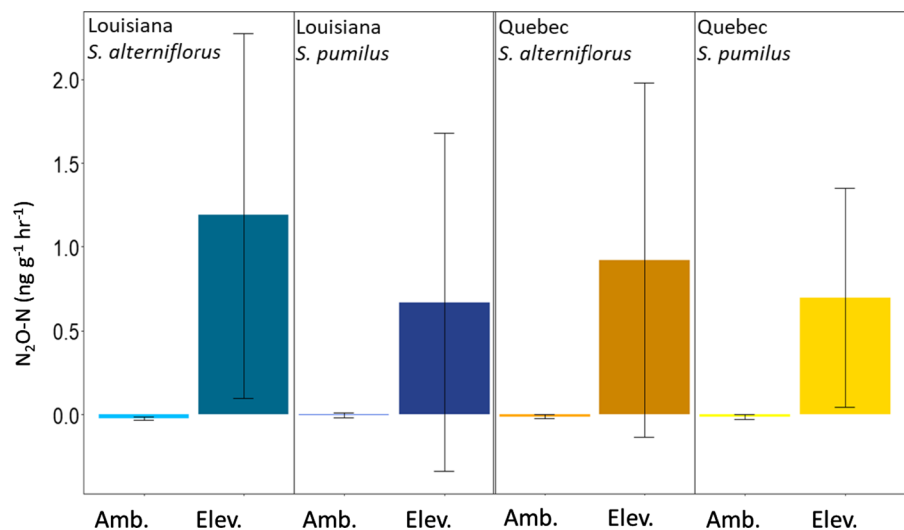
Subsamples of the bulk cores were dried at 60 °C, ground and analysed for TC, TN, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Ground dry samples (< 1 mm) were subsequently analysed for elemental C & N contents as well as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes via a continuous flow isotope ratio mass spectrometer (Elementar Isoprime PreciSION; Elementar Analysensysteme GmbH, Hanau, Germany) coupled with an elemental analyser (EA) inlet (vario PYRO cube; Elementar Analysensysteme GmbH, Hanau, Germany). The EA was calibrated with sulfanilamide (N: 16.26%, C: 41.81%) and the precision as a relative standard deviation (RSD) was < 5% for both C & N. The IRMS was calibrated against international reference standards (caffeine: USGS61 ($- 2.87\%$ $\delta^{15}\text{N}$, $- 35.05\%$

$\delta^{13}\text{C}$ VPDB), USGS62 (20.17‰ $\delta^{15}\text{N}$, $- 14.79\%$ $\delta^{13}\text{C}$ VPDB), USGS63 (37.83‰ $\delta^{15}\text{N}$ $- 1.17\%$ $\delta^{13}\text{C}$ VPDB) and the precision as a standard deviation (SD) was < 0.06‰ for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes.

Statistical analysis

Due to the relatively small sample size (n=5 per treatment per site), non-parametric statistical tests were used. With small sample sizes both normality and parametric tests lose their power, therefore, non-parametric tests were used to avoid incorrect assumptions of normality creating false positives or negatives by using an inappropriate parametric test with greater statistical power (Sullivan et al., 2016; Team 2011). Differences between ambient and elevated treatments were tested for significance using Wilcoxon signed rank test. Differences between climatic region and vegetation type under ambient and elevated conditions were tested for significance using Kruskal–Wallis rank sum test ($p < 0.05$). Where significant differences were found, a post-hoc Dunn test with Bonferroni correction was performed to identify which groups were significantly different. Spearman's R correlation, which is a non-parametric correlation analysis able to find linear or non-linear relationships, was used to investigate potential relationships between biogeochemical reaction rates and environmental variables. Correlations were performed separately on ambient versus elevated flux datasets as many variables are the same at each site between

Fig. 2 Potential rates of N₂O fluxes in ng N g dry soil⁻¹ h⁻¹ across the four salt marsh sites (Louisiana *S. alterniflorus*, Louisiana *S. pumilus*, Quebec *S. alterniflorus* and Quebec *S. pumilus*) under ambient (amb., in-situ soil temperature and nutrient concentration) and elevated (elev., soil temperature + 5 °C, double nutrient concentration) treatments. N = 5 and all error bars represent 1 standard deviation



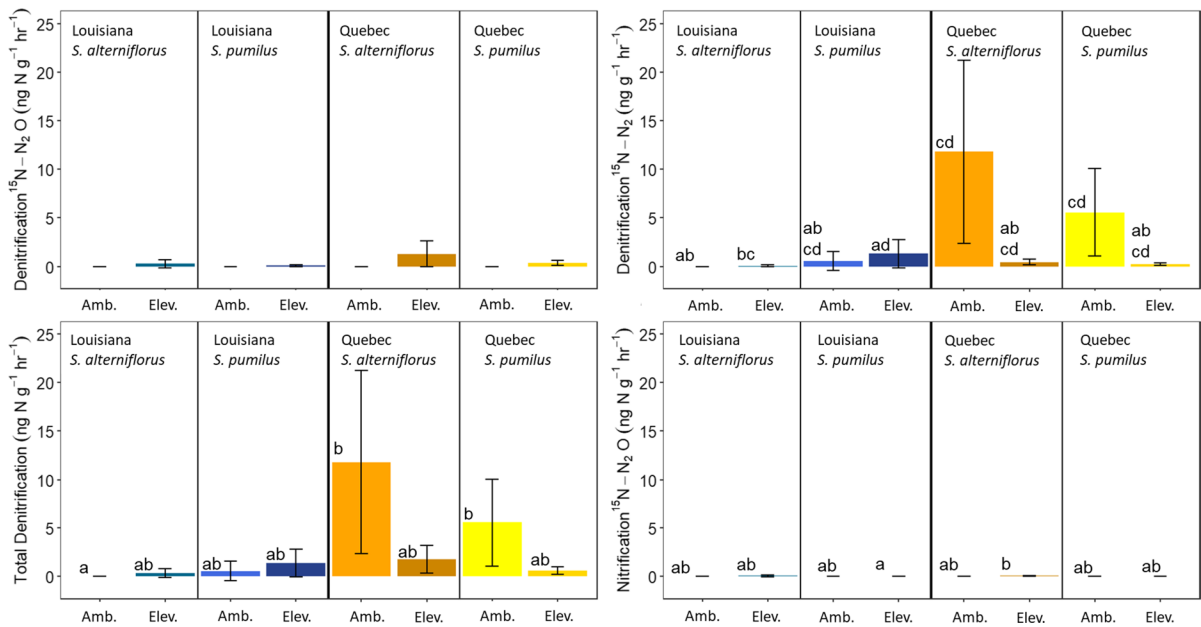


Fig. 3 Potential rates of denitrification-derived N_2O fluxes, denitrification-derived N_2 fluxes, total denitrification (denitrification-derived N_2O and denitrification-derived N_2) and nitrification-derived N_2O fluxes in $\text{ng N g dry soil}^{-1} \text{h}^{-1}$ across the four salt marsh sites (Louisiana *S. alterniflorus*, Louisiana *S. pumilus*, Quebec *S. alterniflorus* and Quebec *S. pumilus*) under

ambient (in-situ soil temperature and nutrient concentration) and elevated (soil temperature +5 °C, double nutrient concentration) treatments. $N=5$ and all error bars represent 1 standard deviation. Statistically significant differences are shown with different letters

Table 2 Statistically significant correlations, given by Spearman's correlations adjusted p-values, between environmental variables and potential biogeochemical reaction rates.

Variable	Elev. DNT- N_2O	Amb. DNT- N_2	Amb. total DNT	Elev. total DNT	Elev. DNT- N_2
Soil moisture	-0.62/0.03	-0.77/<0.01	-0.72/0.01		
DOC		0.68/0.01	0.65/0.02		
NO_3^-			0.62/0.03		
SRP	0.62/0.03	0.65/0.02	0.58/<0.05		
TC		-0.69/0.01	-0.65/0.02		
TN	-0.59/0.04	-0.73/<0.01	-0.67/0.01		
$\delta^{15}\text{N}$		0.74/<0.01	0.72/0.01		
Elev. NO_3^-					0.61/0.03
Amb. DNT- N_2O		0.58/<0.05	0.68/0.01		
Elev. DNT- N_2				0.79/<0.01	
Amb. DNT- N_2			0.98/<0.01		

Variable abbreviations are: dissolved organic carbon (DOC), soluble reactive phosphate (SRP), total carbon (TC), total nitrogen (TN), ambient treatment (Amb.), elevated treatment (Elev.) and denitrification (DNT).

treatments and so the large difference in fluxes when elevating temperature and Nr may mask some controlling effects.

Results

GHG fluxes, rates of denitrification-derived N_2O and N_2 , rates of nitrification-derived N_2O and environmental parameters across marsh sites under ambient and elevated conditions are presented in Table 1, Figs. 1, 2 and 3; correlations and statistical analyses are provided in Table 2, S1, S2 and Fig. S2. All fluxes are calculated per gram of dry soil. Higher fluxes typically had higher absolute variability, which often resulted in no significant difference between the ambient and elevated treatments (p values > 0.06 , Table S1). Fluxes generally increased with increased temperature and N, and thus the absolute variability also increased, making predictions of future N reduction and GHG fluxes less certain.

Under ambient conditions, all marsh sites represented minor sinks of N_2O , while under elevated conditions all sites became relatively large sources of N_2O (Table 1 and Fig. 2). This represented a switch from minor sink of N_2O to major source of N_2O under ambient to elevated temperature and Nr loading. GHG fluxes, rates of denitrification and rates of nitrification-derived N_2O often varied between climatic region and vegetation type (Fig. 3 and S1), however, the differences were usually not statistically significant (p values > 0.08 , Table S2). Statistically significant differences in N processing were observed in denitrification-derived N_2 and total denitrification (p values < 0.02 , Table S2) where fluxes from Louisiana *S. alterniflorus* soils were lower than those from both Quebec vegetation type soils under ambient treatments, denitrification-derived N_2 where fluxes from Louisiana *S. alterniflorus* soils were greater than those from Louisiana *S. pumilus* soils under elevated treatments (p value = 0.02, Table S2) and nitrification-derived N_2O where fluxes from Louisiana *S. pumilus* soils were lower than those from Quebec *S. alterniflorus* soils under elevated conditions (p value = 0.02, Table S2). Statistically significant differences in GHG fluxes were observed in CH_4 where fluxes from Quebec *S. pumilus* soils were lower than those from both Louisiana vegetation type soils under ambient conditions (p value < 0.02 , Table S2), CH_4

where fluxes from Louisiana *S. alterniflorus* soils were greater than those from Quebec *S. pumilus* soils under elevated conditions (p value = 0.01, Table S2) and CO_2 where fluxes from Louisiana *S. pumilus* soils were lower than those from Quebec *S. pumilus* soils under elevated conditions (p value = 0.04, Table S2). The elevated emissions of N_2O trended higher from *S. alterniflorus* than *S. pumilus* (44%: Louisiana soils and 24%: Quebec soils), with *S. alterniflorus* fluxes 23% higher from low latitude soils than high latitude soils, however, these differences were not statistically significant (p value = 0.64, Table S2).

The higher N_2O fluxes under elevated conditions were driven by greater denitrification-derived N_2O fluxes than under ambient conditions. Discrepancies between N_2O fluxes and N_2O production (denitrification-derived N_2O + nitrification-derived N_2O) under elevated conditions (Table 1) may be due to large sample-to-sample variation or due to other N_2O sources not investigated here, such as dissimilatory nitrate reduction to ammonium (DNRA), which may occur at faster rates than denitrification in salt marshes and may produce N_2O with a linear relationship with nitrate concentration (Giblin et al., 2013; Sun et al., 2018). Denitrification-derived N_2O fluxes increased due to a combination of less complete (i.e., higher $\text{N}_2\text{O}:\text{N}_2$) denitrification and increases in rates of denitrification in the low latitude soils. In the low latitude soils, total denitrification (denitrification-derived N_2O plus denitrification-derived N_2) slightly increased in the *S. alterniflorus* soil under elevated conditions, whereas in the *S. pumilus* soil there was a larger increase in total denitrification (Fig. 3). Despite these increases, rates of denitrification in ambient high latitude soils dwarfed all other fluxes, therefore, the elevated fluxes remained relatively low. Denitrification in Louisiana *S. pumilus* soils was more complete under elevated treatment than in the Louisiana *S. alterniflorus* soils, therefore, while small increases in denitrification-derived N_2O were observed in both vegetation types, this increase was greater from the *S. alterniflorus* soils (Fig. 3). However, the difference between ambient and elevated treatment fluxes was not significantly different (p values > 0.06 , Table S1). The largest increase in denitrification-derived N_2O fluxes was observed in Quebec *S. alterniflorus* soils. Nitrification-derived N_2O fluxes had little effect on total N_2O fluxes, despite increased emissions from

S. alterniflorus under elevated conditions, due to the relatively low contribution to N_2O emissions (Fig. 3).

Under ambient conditions, total denitrification was complete so that rates of total denitrification equalled rates of denitrification-derived N_2 fluxes (Table 1, Fig. S3 1:1 ratio). Total denitrification was higher in Quebec soils than in Louisiana soils, but vegetation effects varied between regions. In the elevated treatments, there was less complete denitrification resulting in increased denitrification-derived N_2O fluxes, which were not consistent across region and vegetation type. A large decrease in total denitrification was observed in the Quebec soils, alongside a relatively smaller increase in rates in Louisiana soils under elevated conditions.

CH_4 fluxes and their responses to the elevated treatments were inconsistent across sites. CH_4 emissions were relatively small for all samples except the high latitude *S. pumilus* where fluxes were zero (Fig. S1). Ambient CO_2 fluxes resulting from microbial respiration were similar across all sites, however, they were slightly higher from Quebec *S. pumilus*. CO_2 fluxes (microbial respiration) decreased under elevated treatments in Louisiana marsh sites but increased under elevated treatments in Quebec marsh sites, however, these changes were not significant. Under elevated conditions, CO_2 fluxes (microbial respiration) were higher from high latitude than low latitude *S. pumilus* marsh soils, with a statistically significant difference in fluxes between the two *S. pumilus* sites.

Several environmental variables were significantly correlated with reaction rates (Fig. S2, Table 2). DOC, TC and TN concentrations were positively related to denitrification-derived N_2 and total denitrification (which was primarily driven by N_2 due to completeness of denitrification, p value ≤ 0.02). The significant relationship between $\delta^{15}N$ and ambient denitrification (p value ≤ 0.01) indicates increased soil N transformation rates due to denitrification. In addition, the NO_3^- concentration was significantly related to ambient total denitrification (p value = 0.03). Soluble reactive phosphate (SRP) was also significantly correlated with ambient denitrification-derived N_2 , ambient total denitrification and elevated denitrification-derived N_2O (p values < 0.05). Soil moisture was significantly correlated with ambient and elevated denitrification reaction rates (elevated denitrification-derived N_2O , ambient denitrification-derived N_2

and ambient total denitrification, p values ≤ 0.03). Elevated denitrification-derived N_2O was significantly, negatively correlated with TN (p value = 0.04) and elevated denitrification-derived N_2 was significantly, positively correlated with elevated NO_3^- (p value = 0.03). Some processes (N_2O fluxes, ambient denitrification-derived N_2O , elevated total denitrification and nitrification-derived N_2O) were not significantly related to any environmental parameters.

Discussion

The investigated influence of short-term concurrent increases in both temperature and Nr is representative of observed long-term trends of increasing surface water temperatures, predicted increases in future sea surface temperature and short-term increases in Nr. Increasing temperatures over time have been observed in both the Gulf of Mexico and Gulf of St. Lawrence, which have high rates of warming above the global ocean average (Wang et al., 2023, Galbraith et al., 2023) and predicted increases in future sea surface temperatures (specifically ocean heatwaves) associated with the Intergovernmental Panel on Climate Change representative concentration pathways 8.5 projection (Alexander et al., 2018) could both lead to higher temperatures in salt marsh soils. Short-term increases in Nr may be caused by pulses of nitrogen inputs, for example from overbank flooding during extreme flooding events or fertiliser application in the watershed.

If we compare daily elevated N_2O fluxes with potential annual ambient N_2O fluxes, we find that 15–60% (depending on marsh vegetation and climate zone) of the potential annual ambient N_2O sink may be offset by N_2O emissions in one day, under elevated conditions. Therefore, the combined predicted increases in temperature and Nr in the future may have large implications for the value of salt marshes for GHG reductions, even if they co-occur infrequently and for short periods as these short-term combined increases in temperature and Nr produce N_2O emissions which are significant when compared to potential annual fluxes (upscaled from ambient conditions), under both climate regimes. There are limitations to upscaling from single measurement, laboratory fluxes to potential annual fluxes, these are discussed in the following section.

Experimental design considerations

The current study was designed as a laboratory, microcosm experiment as this allows greater control over process measurements and manipulation of environmental conditions than *in-situ* studies. We used intact cores to minimise some of the disturbance associated with sampling and subsequent laboratory experiments. Drivers of reaction rates and their responses to global change are key gaps in our knowledge of salt marsh biogeochemistry and using laboratory incubation experiments allowed us to investigate effects of predicted increases in future temperature and Nr on N cycling in salt marsh soils.

Nonetheless, there are limitations of laboratory incubation experiments, which are important to consider when interpreting the findings of this study. Firstly, all fluxes presented here are potential fluxes as reaction rates were measured under laboratory conditions, therefore, relative differences between sites and treatments can be compared but are not representative of *in-situ* rates. Secondly, sampling intact cores and incubating in the laboratory prevents some *in-situ* conditions and drivers from being represented. Once cores were sampled some characteristics were inevitably altered, such as pH and microbial community activity, therefore, *in-situ* experiments could be more representative of biogeochemical reaction rates. Additionally, some environmental drivers such as interacting plant effects could not be considered. Thirdly, we measured fluxes over 24 h, so there are limits to upscaling the ambient daily fluxes to potential ambient annual rates. These limitations stem from large uncertainties in taking one flux measurement as representative for a whole year, especially for gas fluxes which are typically highly variable, as drivers such as temperature, substrate availability, microbial activity are not fully accounted for and microbial N cycling is highly dynamic. Upscaling to ambient annual rates demonstrates the large influence of temporary pulses of temperature and nitrogen loading on climate feedback in salt marshes and is, therefore, a useful tool.

Despite these limitations, the intention of this study was to compare the effects of elevated temperature and Nr across climatic regions and vegetation types on salt marsh N cycling. The consistent handling of cores, comparable treatment levels and controlled environmental conditions within the

laboratory provides confidence that the relative reaction rates and differences between treatments and marsh sites can be considered to provide critical insights of changes in salt marsh N cycling under future global change.

Responses of N cycling to global change

Under ambient conditions, nutrient attenuation through complete denitrification (0% N₂O emissions) indicates the importance of salt marshes in removing Nr without decreasing the value of the carbon sink. Shifts to less complete denitrification under elevated conditions alongside large decreases in total denitrification from Quebec soils, indicate that this nutrient filtration capacity may be limited in some regions. Under elevated N loading, excess Nr may not be removed via dissimilatory reduction to N₂ in coastal wetlands, and any Nr that is removed will have an associated negative effect of increased N₂O emissions.

N₂O fluxes responded to elevated nitrogen by shifting from sink to source as expected (Murray et al. 2015; Chmura et al. 2016; Roughan et al. 2018). Under ambient treatments, N₂O sinks were consistent with many salt marshes that are typically sinks or small sources of N₂O (Tobias and Neubauer 2019). Under increased N supply, higher N₂O emissions and denitrification-derived N₂O emissions, alongside less complete denitrification, also followed expected trends (Smith et al. 1983; Tobias et al. 2001; Moseman-Valtierra et al. 2011; Roughan et al. 2018), with increased N₂O due to higher NO₃⁻ availability for organic carbon oxidation and increased NH₄⁺ supply for coupled nitrification–denitrification (Hamersley and Howes 2005; Tobias and Neubauer 2019). Despite a shift under increased NO₃⁻ to N₂O over N₂ production, higher fluxes of denitrification-derived N₂ in elevated treatments were observed and are likely due to higher rates of denitrification still producing more N₂ (Tobias and Neubauer 2019). Elevated N₂O fluxes had larger variation than the ambient fluxes, which suggests that future N₂O fluxes may also be less predictable, complicating estimates of C storage value of individual salt marshes. Without excess N loading, salt marsh environments tend to have saturated, anoxic soils where denitrification is almost complete (< 10% N₂O as end-product) (Tobias and Neubauer 2019) reflecting the complete, ambient

denitrification (0% N_2O as end-product) observed here.

Higher nitrogen (both NO_3^- and NH_4^+) inputs typically lead to increased rates of denitrification (Koch et al. 1992; Hamersley and Howes 2005; Tobias and Neubauer 2019), as observed in the Louisiana marsh soils in this study. Large decreases in denitrification in Quebec marsh soils were, therefore, unexpected. Substrate availability drove rates of denitrification, including SRP, which is not typically investigated in fertilisation experiments. Previous observations have shown that N_2O fluxes from Canadian salt marshes are higher when both N and P were elevated as compared to N alone (Chmura et al. 2016), highlighting the importance of considering SRP as well as Nr in future studies. Nitrification may also increase N_2O emissions under increased nitrogen loading (Tobias et al. 2001; Moseman-Valtierra 2012; Peng et al. 2021). However, here all nitrification-derived N_2O fluxes were negligible, even when increased fluxes under elevated conditions were observed. This may be due to low rates of nitrification in anoxic conditions such as are found in saturated soils, typical of wetlands (Mitsch and Gosselink 2015).

In the incubations presented here, N_2O fluxes and denitrification-derived N_2O fluxes increased under elevated temperatures and nitrogen loading, responding as expected to increasing temperature (Moseman-Valtierra 2012). Rates of total denitrification, denitrification-derived N_2 fluxes and nitrification-derived N_2O fluxes did not increase as expected across all marsh sites under elevated temperature treatments. Higher temperatures are widely known to increase rates of microbial respiration (Kirwan and Blum 2011), with rates of respiration, denitrification and N_2O fluxes all observed to increase with temperature in salt marshes (Kaplan et al. 1979; Bartlett et al. 1987; Ferrón et al. 2007; Hirota et al. 2007; Moseman-Valtierra et al. 2011). Yet, due to the complexity and high variability in biogeochemical reaction rates observed in salt marshes, the expected increases are not always observed, and responses to increased temperature and Nr can be complex. This complexity was evident in the responses to the elevated treatment, which combined increases in temperature and Nr, causing more complex interactive effects across differing climatic regions and vegetation types that we could not discern.

In cases where future increases in N loading occur with more extreme flooding, water level will also increase, however, in this study, water level was controlled between treatments to minimise the number of changing variables. Increasing water level is usually associated with increasing anaerobic conditions, higher rates of denitrification and increases in both N_2O and N_2 emissions (Reddy and Patrick 1984), therefore, our elevated flux estimates are likely to be conservative. This was reflected in the positive relationship between soil moisture and rates of denitrification with soil moisture being a well-known control on rates of denitrification (Bai et al. 2017) due to anoxic conditions (Blain et al. 2006). However, other responses have also been observed including no correlation between water table depth and N_2O fluxes (Chmura et al. 2016), only small increases in N_2O immediately after reflooding of a drained salt marsh (Wollenberg et al. 2018) and higher N_2O emissions during N enrichment in higher elevation zones with lower soil saturations where oxic and anoxic environments were coupled (Martin et al. 2018), which highlight the complexity of estimating salt marsh biogeochemical responses to global change.

Differences in biogeochemical processing rates between climatic regions and vegetation type

We expected different results between climatic regions and vegetation types, particularly in the magnitude of the response, even if the trend was the same. As we were not able to investigate temperature and Nr increases independently this prevented some of the expected responses to the individual drivers from being observed. Ambient soil temperature was higher in Louisiana (27–29.2 °C) than Quebec (15.2–17.2 °C), thus an increase of 5 °C was expected to produce a larger increase in biogeochemical rates in Quebec soils than in Louisiana soils, but this response was not observed. The combined elevated treatment may also have led to the inconsistent response of CH_4 fluxes across marsh sites due to the varied drivers of CH_4 production versus CH_4 consumption. The variability in CH_4 responses may have resulted from the large differences in salinity between marsh sites, however, salinity is not always a good proxy for CH_4 production and rates of methanogenesis may be high at high salinities (King and Wiebe 1980; Oremland et al. 1982). Further investigation of a priming effect

of sulphate concentration on responses of CH₄ fluxes to future global change thus is required.

N₂O fluxes observed here (− 21 to − 56 μg N m^{−2} d^{−1} for ambient and 1526 to 2965 μg N m^{−2} d^{−1} for elevated) were within the range of previous observations, which were between − 770 and 769,745 μg N m^{−2} d^{−1} (e.g., Hirota et al. 2007; Ford et al. 2012; Chmura et al. 2016; Martin and Moseman-Valtierra 2017; Martin et al. 2018). The large range of previously observed fluxes represents sinks from salt marshes with no nitrogen loading and sources from salt marshes with elevated nitrogen (Martin and Moseman-Valtierra 2017; Martin et al. 2018).

Higher soil temperature and lower salinities in the Louisiana marsh soils cause higher rates of biogeochemical activity than in the Quebec marsh soils (e.g., Rysgaard et al. 1999; Giblin et al. 2010; Kirwan and Blum 2011; Poffenbarger et al. 2011; Abdul-Aziz et al. 2018); however, soil C and N properties indicated less processing of C and N in the Louisiana marsh soils. This was reflected in rates of soil respiration (Fig. S1), particularly under elevated conditions, where soil respiration was higher in Quebec soils than Louisiana soils. Higher soil moisture in Louisiana soils likely had a larger effect on biogeochemical reaction rates, explaining less turnover but higher ambient CH₄ fluxes than in the Quebec soils (Zhao et al. 2020). The higher ambient denitrification in Quebec marsh soils was likely due to a potentially more dynamic, rapidly changing N pool (as indicated by much more enriched δ¹⁵N showing substantial fractionation) that could potentially support higher denitrification, unless these soils were receiving enriched N inputs from other sources. The soil moisture was low (<70%) so coupled nitrification–denitrification could have driven some of the denitrification measured here. Furthermore, DOC and SRP concentrations were higher in Quebec perhaps driving increased denitrification through more readily available substrate (He et al. 2016) and through phosphate accumulating organisms as has been shown in wastewater treatment plants e.g. (Cho et al. 2004), respectively.

When comparing vegetation zones in each climatic region, *S. alterniflorus* soils drove higher reaction rates or larger responses to global change than *S. pumilus* soils for most fluxes, but these differences were not statistically significant. This was likely due to differences in soil properties, potentially resulting

from *in-situ* plant influence, causing more favourable conditions for biogeochemical reactions in *S. alterniflorus*. Substrate availability (DOC, TDN and SRP) was higher in the *S. alterniflorus* soils relative to the *S. pumilus* soils in the same climatic region, thus driving higher biogeochemical reaction rates (Moseman-Valtierra 2012; He et al. 2016; Noyce and Megonigal 2021; Rosentreter et al. 2021). Lower TC, TN and soil moisture were found in the *S. alterniflorus* soils, indicating more carbon and nitrogen turnover, and more aerobic conditions, which support evidence of higher rates of biogeochemical reactivity (Blain et al. 2006). Despite the environmental data providing evidence for higher reactivity in *S. alterniflorus* soils, fluxes of denitrification-derived N₂ and total denitrification from Louisiana soils were higher from *S. pumilus*.

The higher salinity of the *S. alterniflorus* soil would be expected to lead to lower rates of denitrification (Rysgaard et al. 1999); however, as denitrification rates were not related to salinity, other factors such as substrate availability and soil moisture, were more important drivers (He et al. 2016). Differences in microbial community composition between the vegetation zones could also drive differences in reaction rates; despite similar microbial communities found in *S. alterniflorus* and *S. pumilus* zones in New England salt marshes (Barry et al. 2022), as changes in salinity (as found between these vegetation types) causes large variations in microbial reaction rates (Weston et al. 2006, 2011; Stagg et al. 2017).

Summary and conclusions

Potential N₂O fluxes increased, from minor sinks to major sources, in all marsh sites under elevated (nutrient and temperature) conditions as potential denitrification became less complete. This switch was, however, not statistically significant, likely due to higher variability in fluxes in elevated treatments. The response of potential total denitrification varied between climatic regions with rates in marsh soils from Louisiana increasing while rates in marsh soils from Quebec decreased. Potential biogeochemical reaction rates were usually higher in *S. alterniflorus* than in *S. pumilus*. Potential N₂O fluxes under elevated conditions may offset 15–60% of the annual

potential ambient N₂O sink in one day, indicating that short-term, infrequent, increases in reactive N and temperature may have large implications for future salt marsh GHG fluxes.

Nutrient filtration through complete potential denitrification (0% N₂O emissions) under ambient conditions shows the ability of salt marshes to reduce nitrogen without decreasing the value of the carbon sink. Shifts to less complete potential denitrification (higher N₂O:N₂) under elevated conditions, alongside large decreases in potential total denitrification from Quebec soils, indicate that the nutrient filtration capacity may be limited in some regions, and that there may be an associated negative effect of increased GHG emissions under future global change scenarios.

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Author contributions All authors contributed to the editing of the manuscript. SCW, AD, CLS, TQ, CMS collected soil samples, SCW and AD performed laboratory experiments, SCW and FS analysed samples, and SCW performed all data analysis and wrote the manuscript.

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Data availability The datasets generated during this study are available in the Zenodo repository, [<https://doi.org/10.5281/zenodo.7625434>].

Declarations

Conflict of interest The authors have no relevant financial or non-financial interested to disclose.

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