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Pristine mangrove creek waters are a sink of nitrous oxide

Damien T. Maher¹, James Z. Sippo^{1,2}, Douglas R. Tait^{1,2}, Ceylena Holloway^{1,2} & Isaac R. Santos^{1,2}

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Nitrous oxide (N₂O) is an important greenhouse gas, but large uncertainties remain in global budgets. Mangroves are thought to be a source of N₂O to the atmosphere in spite of the limited available data. Here we report high resolution time series observations in pristine Australian mangroves along a broad latitudinal gradient to assess the potential role of mangroves in global N₂O budgets. Surprisingly, five out of six creeks were under-saturated in dissolved N₂O, demonstrating mangrove creek waters were a sink for atmospheric N₂O. Air-water flux estimates showed an uptake of $1.52 \pm 0.17 \mu\text{mol m}^{-2} \text{d}^{-1}$, while an independent mass balance revealed an average sink of $1.05 \pm 0.59 \mu\text{mol m}^{-2} \text{d}^{-1}$. If these results can be upscaled to the global mangrove area, the N₂O sink ($\sim 2.0 \times 10^8 \text{ mol yr}^{-1}$) would offset $\sim 6\%$ of the estimated global riverine N₂O source. Our observations contrast previous estimates based on soil fluxes or mangrove waters influenced by upstream freshwater inputs. We suggest that the lack of available nitrogen in pristine mangroves favours N₂O consumption. Widespread and growing coastal eutrophication may change mangrove waters from a sink to a source of N₂O to the atmosphere, representing a positive feedback to climate change.

Nitrous oxide is a long lived greenhouse gas with an atmospheric lifetime of 118–131 years¹, a global warming potential about 300 times that of CO₂, and it is the major contributor to ozone destruction in the stratosphere². Microbial processes within soils and surface waters produce most atmospheric N₂O through nitrification and denitrification. The largest portion of global denitrification from natural sources is thought to occur within coastal waters ($\sim 45\%$)³, with these areas considered globally significant N₂O sources⁴. However global estimates lack empirical data from some key environments such as mangrove waterways.

Mangrove forests cover about 138,000 km² of coastline and are recognised to contribute disproportionately to global biogeochemical cycles⁵. Mangrove ecosystems are thought to be an important atmospheric N₂O source^{6–8}. Yet, N₂O observations in mangroves are highly variable and often focus on soil emissions from eutrophic systems influenced by freshwater inputs^{9,10}. The methodology typically used to estimate soil emissions (i.e. soil chambers) only captures N₂O fluxes from exposed soils. To date, little work has been done on the rates of N₂O exchange between surface waters and the atmosphere in mangroves. One study revealed high dissolved N₂O concentrations, and suggested mangroves were a significant source of N₂O to the atmosphere¹¹. This study was undertaken over two 24-h periods in a mangrove tidal creek influenced by upstream freshwater inputs. However, because rivers are often enriched in N₂O¹², upstream riverine inputs of N₂O may lead to a misinterpretation of the N₂O source. As a result, it is unclear whether high dissolved N₂O was related to upstream freshwater inputs or N₂O production within the mangrove forest.

To understand the role of mangroves within global N₂O budgets, we investigated dissolved N₂O dynamics in six pristine mangrove tidal creeks covering a latitudinal gradient in Australia (Fig. 1, Table 1). We initially hypothesised that mangrove waters are a source of N₂O to the atmosphere, which may play a disproportionately large role in global N₂O budgets per unit area. To test this hypothesis we used novel, automated, quasi-continuous measurement technology that allowed for high temporal resolution, high precision dissolved N₂O observations to be made *in situ*. Sites were selected to ensure the N₂O observations were only related to mangrove sinks and sources. The sites had mangrove dominated catchments and no obvious upstream riverine input or anthropogenic nitrogen sources (Table 1).

High temporal resolution observations over about 5 days in each creek revealed dissolved N₂O concentrations ranging from 3.4–9.1 nM (50–123% saturation, Table 2, Figs 2 and 3). Nitrate + nitrite (NO_x) concentrations in all the creeks were low (Fig. 3) and approached oceanic values, which average between 0.04 and 3.52 μM

¹School of Environment, Science and Engineering, Southern Cross University, Lismore, 2480 Australia.

²National Marine Science Centre, Southern Cross University, PO Box 4321, Coffs Harbour, NSW 2450, Australia. Correspondence and requests for materials should be addressed to D.T.M. (email: damien.maher@scu.edu.au)

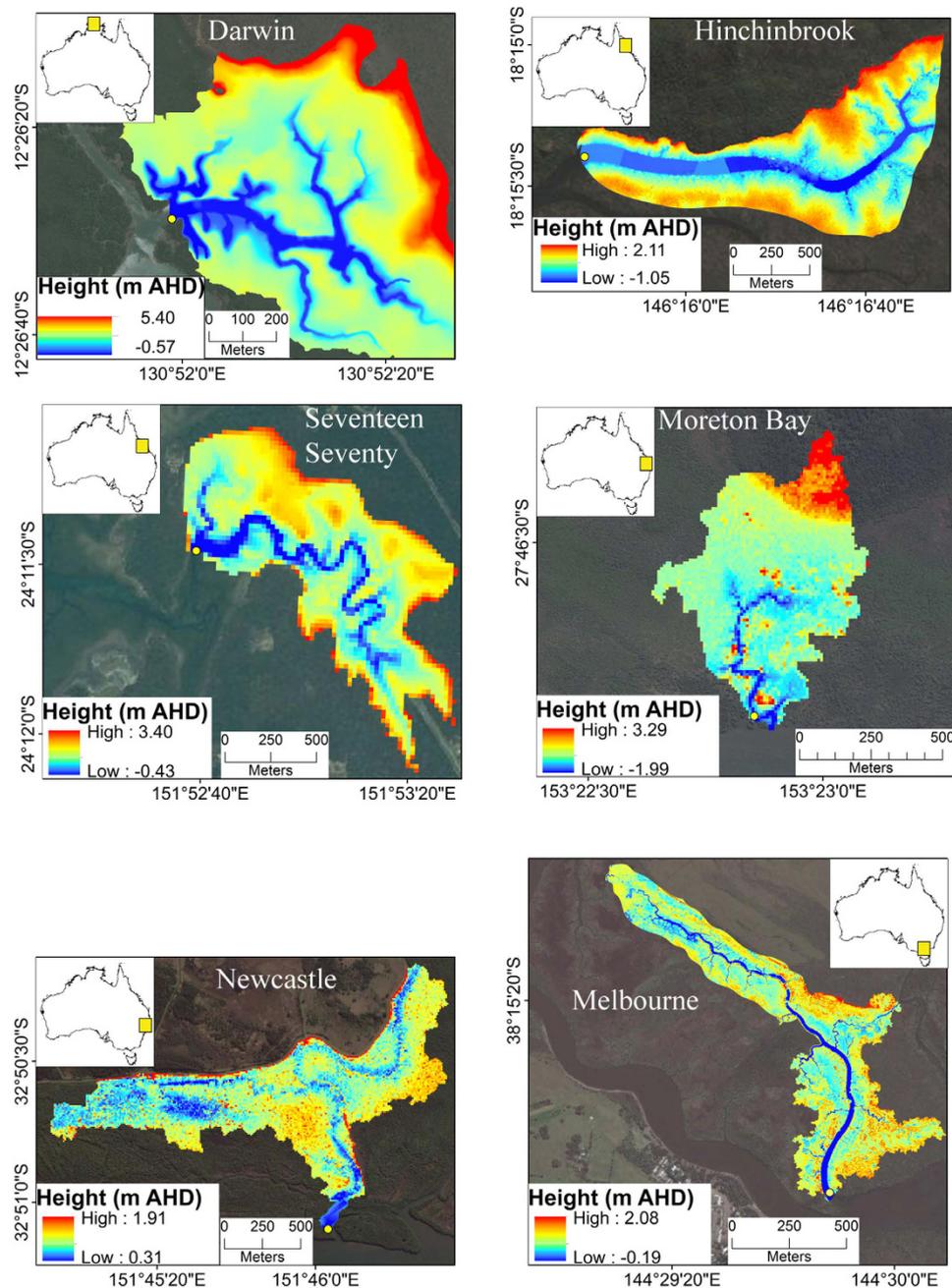


Figure 1. Location of six mangrove creeks on the North and East coast of Australia and the associated digital elevation models (DEM). Map and DEMs created with ESRI ArcGIS version 10.3 (<https://www.arcgis.com>). Image modified from Tait *et al.* (ref. 35).

[Table 1, oceanic data was sourced from the Integrated Marine Observing System (IMOS)]. Ammonium (NH_4) was higher in the two southern sites which also had lower salinity suggesting a freshwater source of NH_4 to these systems. When data were averaged over the five days of the time series, five out of the six mangrove creeks were under-saturated in dissolved N_2O , resulting in atmospheric N_2O uptake by these waters (Fig. 3, Table 2, Supplementary Information). The only creek that was on average supersaturated in N_2O (Newcastle) had the lowest salinity (Figs 2 and 3, Supplementary Information), suggesting freshwater inputs were the likely source of N_2O supersaturation, or freshwater supplied the nitrogen fuelling N_2O production.

The average N_2O concentrations in each of the six mangrove creeks were lower than in the only previous study measuring dissolved N_2O over a tidal cycle in mangrove waters which found mean concentrations of $9.0 \pm 2.3 \text{ nmol L}^{-1}$ (~170% saturation) (dry season) and $8.6 \pm 1.3 \text{ nmol L}^{-1}$ (~120% saturation) (wet season) over two 24 hour time series in the Andaman Islands¹¹. Relatively high dissolved N_2O concentrations are often found in rivers and inner estuaries, where catchment nitrogen inputs fuel N_2O production through nitrification and

	Darwin	Hinchinbrook Island	Seventeen Seventy	Moreton Bay	Newcastle	Melbourne
Latitude (°S)	12.4°	18.3°	24.2°	27.8°	32.9°	38.3°
No. of Mangrove species	36	29	13	7	3	1
Tide Height						
Max (m)	7.1	3.0	3.6	2.5	1.5	2.1
Min (m)	1.7	0.9	0.7	0.3	0.6	0.4
Range (m)	5.4	2.1	2.9	2.2	0.9	1.7
Average (m)	4.3 ± 1.7	1.8 ± 0.5	1.7 ± 0.8	1.2 ± 0.6	1.0 ± 0.3	1.1 ± 0.4
Air Temperature						
Max (°C)	27.9	23.2	20.0	28.6	26.4	21.2
Min (°C)	25.2	16.4	7.2	19.6	19.8	15.2
Average (°C)	26.5 ± 0.5	21.4 ± 0.7	16.8 ± 2.1	22.9 ± 1.7	23.6 ± 0.9	18.1 ± 1.4
Ave yr temp (°C)	32.0 ± 0.4	28.9 ± 0.5	25.7 ± 0.5	25.2 ± 1.0	21.6 ± 0.6	20.3 ± 0.6
Ave rain (mm yr ⁻¹)	1729 ± 377	2216 ± 677	1180 ± 326	1336 ± 389	1120 ± 268	621 ± 139
Salinity						
Max	35.5	35.8	39.4	39.5	34.8	36.2
Min	34.7	35.0	35.7	35.4	28.1	27.6
Average	35.1 ± 0.2	35.3 ± 0.1	37.7 ± 1.1	37.0 ± 4.2	31.8 ± 1.6	34.4 ± 1.1
Submerged area						
Max (ha)	72.6	286.3	109.0	37.0	126.6	29.8
Min (ha)	15.9	224.5	3.1	0.8	7.9	0.9
Range (ha)	56.7	61.8	105.9	36.2	118.7	28.9
Average (ha)	57.9 ± 11.1	275.6 ± 10.9	37.2 ± 37.8	25.8 ± 13.9	111.3 ± 31.0	4.8 ± 5.2
Oceanic nutrient concentrations (µM) ¹						
IMOS station	NRS DAR	NRS YON	**	NRS NSI	NRS PHB	NRS MAI
NO ₃	0.43	0.04	0.87	1.69	3.52	2.39
NH ₄	0.22	0.09	0.17	0.24	0.29	0.18

Table 1. Study site characteristics during the 4 to 7 day time series deployments. ¹Data are average from nearest IMOS stations over the preceding 5 years, **Data are averaged from stations NRSYON and NRSNSI.

denitrification^{13–15}. With salinity ranging from 0 to 28 in the Andaman Island study, the higher N₂O concentrations observed may have been driven by freshwater nitrogen loads, rather than mangrove related processes.

A complex combination of drivers may influence dissolved N₂O production and consumption within mangrove ecosystems. Previous studies have found either nitrification^{6,11,16} or denitrification^{7,8,17} to be the dominant N₂O production pathway in mangroves. N₂O cycling is coupled to NO_x and ammonium (NH₄⁺) availability¹⁷, and a number of environmental factors such as denitrification and nitrifier bacteria abundance, redox potential, temperature, porewater or groundwater exchange, sulphur cycling, and local conditions such as topography, biomass, species composition and root structure⁷. For example, N₂O concentration has been found to increase with; increasing denitrifier abundance¹⁸, increasing temperature¹³, inputs of groundwater/porewater¹⁹ or through N₂O reduction inhibition by H₂S²⁰, and reduced N₂O production has been linked to low redox potentials in soils⁶.

Pristine mangrove waters generally have low inorganic nitrogen concentrations²¹. Mangroves may conserve nitrogen through nitrate reduction via dissimilatory nitrate reduction to ammonium (DNRA) rather than denitrification²². This may enhance the N₂O sink capacity of mangroves via two ways. First, N₂O production through denitrification is inhibited due to competition for nitrate by DNRA. Second, DNRA bacteria and archaea possess the enzyme system required to catalyse the reduction of N₂O to N₂²³. Previously it was thought that denitrifiers were the only group capable of N₂O reduction. In addition, some plants can produce and exude compounds that inhibit nitrification such as cyclic diterpenes²⁴. Similar compounds have been found in the bark of mangrove roots²⁵. These compounds may act as a chemical defence limiting nitrogen loss from highly productive mangrove ecosystems with limited nitrogen.

Overall, N₂O atmospheric fluxes for individual systems ranged from -3.43 to $0.71 \mu\text{mol m}^{-2} \text{d}^{-1}$ (mean $-1.52 \pm 0.17 \mu\text{mol m}^{-2} \text{d}^{-1}$) when using well established flux models (Table 2). Using an independent mass balance approach, mangroves were estimated to have an atmospheric flux of -3.20 to $0.03 \mu\text{mol m}^{-2} \text{d}^{-1}$ (mean $-1.05 \pm 0.59 \mu\text{mol m}^{-2} \text{d}^{-1}$). Overall, these two independent approaches are consistent in that 5 out of the 6 systems were a sink for atmospheric N₂O, giving confidence in our observations. The only system that released N₂O to the atmosphere on average over the five day time series (Newcastle) also had the greatest input of freshwater (Figs 2 and 3).

Upscaling the N₂O flux from mangrove waters in this study provides a first order estimate of fluxes from pristine mangrove systems. The mean atmospheric flux from the six sites was $-1.52 (\pm 0.17) \mu\text{mol m}^{-2} \text{d}^{-1}$ and the global mangrove area is estimated to be $0.36 \times 10^6 \text{ km}^2$ ¹¹ using the global forested area from Borges *et al.*²⁶ ($\sim 0.2 \times 10^6 \text{ km}^2$), and the average wetland forest area from Selvam²⁷ ($0.16 \times 10^6 \text{ km}^2$), as done in the only other

Site	Latitude	NO _x Concentration (μM)		NH ₄ concentration (μM)		N ₂ O concentration (nM)		N ₂ O saturation (%)		Average atmospheric N ₂ O flux ^a (μmol m ⁻² d ⁻¹)	Weighted atmospheric N ₂ O flux ^b (μmol m ⁻² d ⁻¹)	N ₂ O mass balance ^c (μmol m ⁻² d ⁻¹)
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Daily integrated	Daily integrated
Darwin	-12.45	0.59	<0.1–1.07	0.53	<0.1–0.79	6.3	6.0–6.8	98.9	94.7–104.3	-0.12 ± 0.01 ^d	-0.07 ^d	-0.11
Hinchinbrook	-18.25	0.75	0.29–1.14	1.56	<0.1–5.93	6.1	5.6–6.9	83.3	75.4–90.5	-3.43 ± 0.06 ^d	-3.26 ^d	-3.20
Seventeen Seventy	-24.17	0.56	<0.1–1.07	0.75	0.29–1.86	7.7	7.1–8.9	94.3	87.6–106.2	-1.75 ± 0.11 ^d	-0.48 ^d	-0.35
Moreton Bay	-27.78	ND	ND	ND	ND	5.1	3.4–6.6	77.4	50.3–104.7	-3.19 ± 0.08 ^d	-1.52 ^d	-2.61
Newcastle	-32.85	0.11	<0.1–0.50	12.69	0.5–40.14	7.5	6.4–8.9	106.4	92.1–123.4	0.71 ± 0.03 ^d	0.69 ^d	0.03
Melbourne	-38.26	0.11	<0.1–0.42	25.50	<0.1–142.86	7.9	6.9–9.1	96.6	85.9–114.8	-1.33 ± 0.07 ^d	-0.12 ^d	-0.08
Average		0.42	<0.1–1.14	8.20	<0.1–142.86	6.8	3.4–9.1	92.9	50.3–123.4	-1.52 ± 0.17	-0.79 ± 0.57	-1.05 ± 0.59

Table 2. Field observations of inorganic nitrogen, N₂O and estimates of air-water fluxes in six pristine Australian mangrove creeks. ^aPer m² water area, ±95% confidence interval ^bweighted for changes in water area and flux rates over the study period and normalised to total catchment area, ^ccalculated as a function of discharge and dissolved N₂O concentration integrated at 1 minute intervals, normalised to catchment area, ^dFlux rates determined using empirical transfer velocity equation of Ho *et al.*³³.

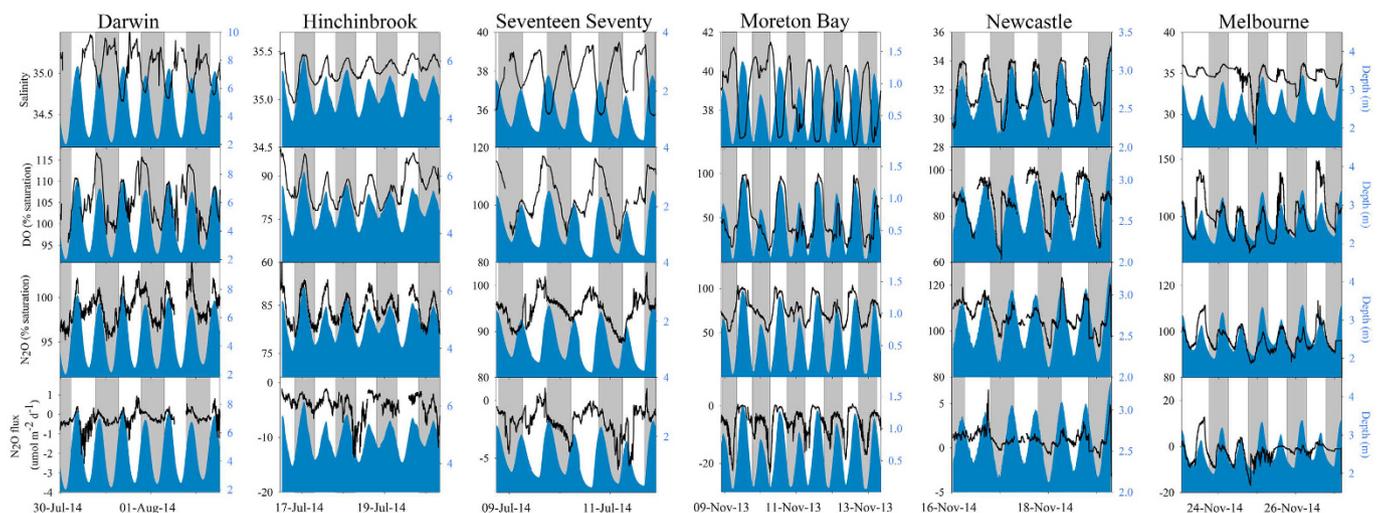


Figure 2. Time series observations in the six mangrove tidal creeks. Vertical grey bars represent night time period. Note the different Y axes scales to highlight temporal gradients. Solid blue shaded area represents tidal height. Salinity and depth data were originally reported in Tait *et al.* (ref. 35).

previous estimate of the global mangrove creek N₂O flux¹¹. The uncertainty associated with this estimate of creek area is unknown, but likely large. Assuming all global mangrove systems are in a pristine state is unrealistic, yet provides a first order estimate of the potential sink capacity of mangrove waters and allows for an update from the only previous estimate based on fluxes measured from one site during two 24 hour measurement campaigns¹¹. Further, seasonal variability could not be determined during the current study. Adequate assessment of seasonality in N₂O fluxes would be required to better constrain these estimates. In spite of these obvious caveats, if our estimates are representative of the N₂O flux from pristine mangroves, the global N₂O sink in pristine mangrove systems would be 2×10^8 mol yr⁻¹. Previous estimates of N₂O fluxes from mangrove systems reported a net source to the atmosphere, with global extrapolations of 2.7×10^9 mol yr⁻¹ from mangrove soils and waters¹¹, and $\sim 3.2 \times 10^8$ to 13×10^{10} mol N₂O yr⁻¹ from soil emissions alone¹⁰. In contrast to our investigation, previous studies focused on systems with upstream freshwater inputs and therefore presumably some associated nitrogen inputs, which may fuel N₂O production via nitrification and denitrification.

The sink nature of N₂O within pristine mangroves is a rarity for aquatic systems, with most waterbodies being a source of N₂O to the atmosphere. Estuaries and rivers are thought to contribute approximately 5% of the natural global N₂O emissions from aquatic systems²⁸. If our estimates are representative of mangroves globally, our results imply that pristine mangroves may offset about 3% of estuarine emissions ($\sim 7.14 \times 10^9$ mols yr⁻¹)²⁸, or 6% of N₂O outgassing from rivers ($\sim 3.57 \times 10^9$ mols yr⁻¹)²⁸ and therefore play a relatively minor role within the global N₂O budget from natural waterways. We suggest that the pristine mangrove N₂O sink behaviour is related to nitrate limitation as well as microbial and plant inhibitory processes (Fig. 4). Combined with previous studies that indicate significant N₂O releases from mangroves that are higher in nitrate¹¹, our observations imply that

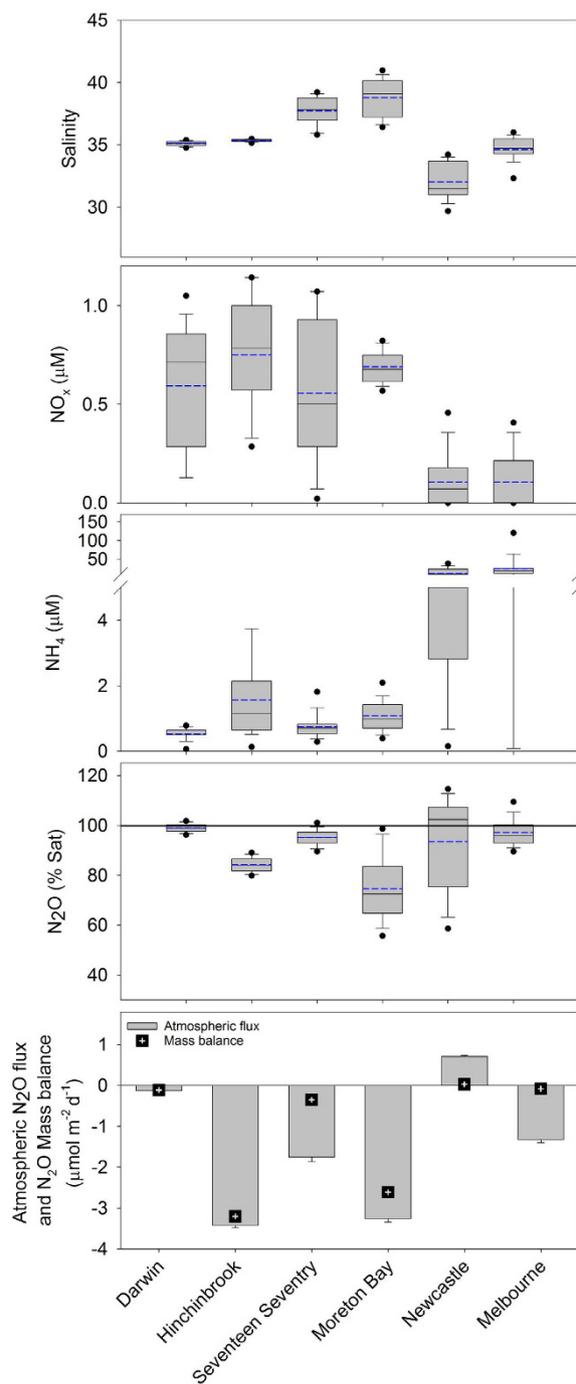


Figure 3. Box plots of salinity, nutrients and N₂O data from time series observations (5th and 95th percentile, mean = solid black line, median is dashed blue line). Nutrient data for Moreton Bay were from Gleeson *et al.* (ref. 22). Atmospheric N₂O flux and N₂O mass balance were mean \pm 95% confidence interval for each tidal cycle measured, corrected for tidal changes in creek area, and normalised to mangrove intertidal area.

human induced eutrophication, and in particular increased nitrogen loading may shift mangrove waters from a sink to a source of N₂O to the atmosphere.

Methods

This study was undertaken in six pristine mangrove tidal creeks on the Eastern and Northern Australian coast (Fig. 1; Table 1). Importantly, creeks were selected from low lying areas with small catchments in order to prevent significant upstream freshwater inputs that could mask processes occurring within intertidal mangroves. Therefore, freshwater input was assumed to occur only as a result of direct rainfall over the creek and the small intertidal areas.

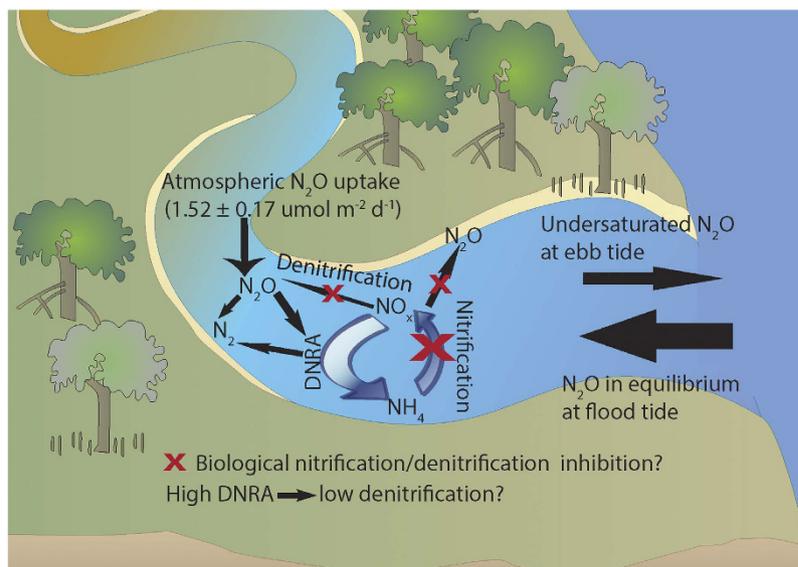


Figure 4. Conceptual model summarizing the potential processes controlling N₂O dynamics in pristine mangrove creek waters. Figure constructed using symbols courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/).

To measure dissolved N₂O, creek water was continually pumped from a depth of ~1 m into a showerhead air-water exchanger at a flow rate of approximately 3 L min⁻¹. Continuous N₂O concentrations (±2 ppb) were measured in the shower head exchanger at one second intervals for about five days at each site using a Picarro G2308 cavity ring-down spectrometer (CRDS) as described elsewhere^{29,30}. The CRDS was calibrated using a 350 ppb standard (Air Liquide, Australia), prior to, and upon completion of the field measurements. Instrument drift was less than 2 ppb between calibrations (i.e. over several weeks). This 2 ppb drift equates to <0.10 nM *in situ* temperature and salinity (assuming a total 4 ppb error in the concentration difference between the atmospheric and water column measurements). N₂O dry molar fractions were converted to dissolved N₂O concentrations as a function of pressure and solubility³¹ (1):

$$C_{\text{N}_2\text{O}} = \beta \cdot x' \cdot P \quad (1)$$

where β is the Bunsen solubility coefficient calculated from temperature and salinity³², x' is the dry molar fraction of N₂O and P is ambient pressure. Atmospheric pressure and temperature were measured with a weather station located on site (Davis Vanatge Pro II), and pressure and temperature within the equilibrator was measured with a temperature/pressure logger (Van Essen CTD logger).

At each site, estuarine current velocity and water depth were measured at fifteen minute intervals using an acoustic doppler current profiler (SonTek Argonaut). Salinity, water temperature, pH, and dissolved oxygen were measured at 15 minute intervals using a calibrated multi-parameter water quality sonde (Hydrolab DS5). Dissolved nitrogen concentrations were measured on discrete samples collected hourly during a 24 hour time series ($n = 25$ per site), and analysed using flow injection analysis (Lachat Quickchem 8000). The samples were filtered with 0.45 μm cellulose acetate filters and kept frozen until analysis within 1 month. NO_x detection limits were 0.07 μM (error ±3%) and NH₄ detection limits were 0.35 μM (error ±5%).

The flux of N₂O in mangrove waters was estimated using two different approaches. First, we applied a gas exchange model. The transfer of N₂O between the water and atmosphere was estimated as a function of the concentration in water and atmospheric concentration, solubility and gas transfer velocity at one minute intervals (2):

$$F = k(C_w - \alpha C_a) \quad (2)$$

where k is the gas transfer velocity, C_w and C_a are the water and air phase concentrations respectively and α is the solubility coefficient.

Gas transfer velocity was calculated using an empirical relationship developed specifically for mangroves³³ (3):

$$k_{600} = 1.58e^{0.3u} + 1.539v^{0.5}h^{-0.5} \quad (3)$$

where k_{600} is the gas transfer velocity normalised to a schmidt number of 600, u is the wind speed at a height of 10 m, v is current velocity (cm s^{-1}) and h is water depth (m). Positive values indicate a flux of N₂O from the water to the atmosphere, while negative values indicate gas exchange from the atmosphere to water. Atmospheric concentrations were measured with the same CRDS at least once per day for a period of 5 to 10 minutes. There was no significant difference between the atmospheric dry molar fractions measured at each site (ANOVA $p > 0.05$), therefore we used the pooled mean of 326 ppb for our atmospheric endmember. Wind speed data was sourced

from the Australian Bureau of Meteorology for the nearest station near each site (all stations were within 20 km of the corresponding study site).

Second, we developed a mass balance approach that estimates the net water borne flux of N₂O in and out of the mangrove catchment. Volumetric water discharge was calculated at one minute intervals using a high resolution LIDAR derived digital elevation model (DEM) with 1 m grid, ±0.1 m elevation accuracy, as described by Maher *et al.*³⁴. The net exchange of N₂O was then calculated as a function of concentration and discharge, with a mass balance constructed incorporating water borne exchange and the net air water flux within the mangroves. The air water flux was calculated as a function of time specific wetted area at one minute intervals, calculated using depth and the DEM.

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Author Contributions

D.T.M., I.R.S. and D.R.T. designed the experiment, D.T.M., D.R.T., J.Z.S. and C.H. undertook the field work and analysis D.T.M., J.Z.S. and I.R.S. wrote the manuscript, all authors reviewed and edited the manuscript.

Additional Information

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