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Restoration impacts on rates of denitrification and greenhouse gas fluxes from tropical coastal wetlands

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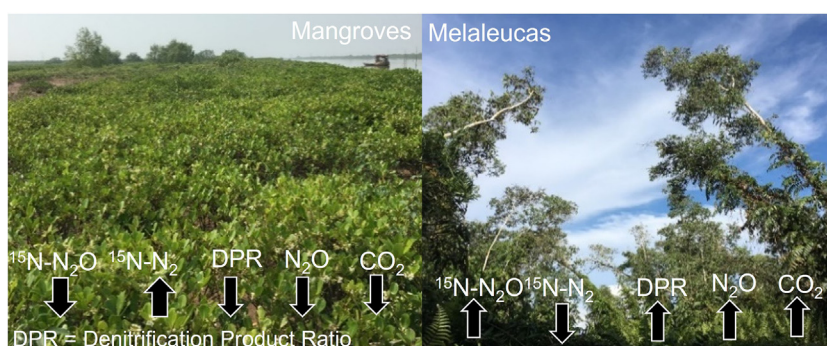
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HIGHLIGHTS

- Coastal wetlands sequester carbon, attenuate nitrogen and are targets of restoration.
- Potential rates of denitrification were higher in mangrove than Melaleuca forested wetland soils.
- Potential denitrification was more complete in mangrove than Melaleuca forested wetland soils.
- Potential greenhouse gas emissions were lower from mangrove than Melaleuca forested wetland soils.
- Restoration had little effect on nitrogen biogeochemistry.

GRAPHICAL ABSTRACT



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ABSTRACT

Forested coastal wetlands are globally important systems sequestering carbon and intercepting nitrogen pollution from nutrient-rich river systems. Coastal wetlands that have suffered extensive disturbance are the target of comprehensive restoration efforts. Accurate assessment of restoration success requires detailed mechanistic understanding of wetland soil biogeochemical functioning across restoration chrono-sequences, which remains poorly understood for these sparsely investigated systems. This study investigated denitrification and greenhouse gas fluxes in mangrove and Melaleuca forest soils of Vietnam, using the ^{15}N -Gas flux method. Denitrification-derived N_2O was significantly higher from Melaleuca than mangrove forest soils, despite higher potential rates of total denitrification in the mangrove forest soils ($8.1 \text{ ng N g}^{-1} \text{ h}^{-1}$) than the Melaleuca soils ($6.8 \text{ ng N g}^{-1} \text{ h}^{-1}$). Potential N_2O and CO_2 emissions were significantly higher from the Melaleuca soils than from the mangrove soils. Disturbance and subsequent recovery had no significant effect on N biogeochemistry except with respect to the denitrification product ratio in the mangrove sites, which was highest from the youngest mangrove site. Potential CO_2 and CH_4 fluxes were significantly affected by restoration in the mangrove soils. The lowest potential CO_2 emissions were observed in the mid-age plantation and potential CH_4 fluxes decreased in the older forests. The mangrove system, therefore, may remove excess N and improve water quality with low

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greenhouse gas emissions, whereas in Melaleucas, increased N_2O and CO_2 emissions also occur. These emissions are likely balanced by higher carbon stocks observed in the Melaleuca soils. These mechanistic insights highlight the importance of ecosystem restoration for pollution attenuation and reduction of greenhouse gas emissions from coastal wetlands. Restoration efforts should continue to focus on increasing wetland area and function, which will benefit local communities with improved water quality and potential for income generation under future carbon trading.

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1. Introduction

Forested coastal wetlands provide globally important ecosystem services including carbon and nitrogen (N) sequestration and removal of N to the atmosphere (Adame et al., 2019a; Barbier, 2019; Chmura et al., 2003; Krauss et al., 2018). Mangrove forests are well known for their high rates of carbon sequestration, however, other coastal forested wetlands may also sequester carbon at high rates (Chmura et al., 2003; Krauss et al., 2018; Lovelock and Duarte, 2019; McLeod et al., 2011). Melaleuca swamps are forested wetlands extending from riverine floodplains to coastal zones, where intermittent tidal flooding causes moderately saline soils (Adame et al., 2019a; Boland et al., 2006; Mensforth and Walker, 1996).

Soil biogeochemical processes in forested coastal wetlands influence N and carbon cycling, which may produce or consume greenhouse gases (GHGs: CO_2 , CH_4 and N_2O) and remove N from the system (Adame et al., 2019b; Bartlett and Harriss, 1993; Kreuzwieser et al., 2003; Reddy and DeLaune, 2008). Nitrification is the oxidation of NH_4^+ to NO_3^- while producing N_2O as a by-product (Ward, 2013). Denitrification is the reduction of NO_3^- to N_2 , which is returned to the atmosphere. Denitrification produces NO_2^- and N_2O as intermediate reactant products, and may result in N_2O release when denitrification is incomplete (Kendall, 1998; Seitzinger, 1988). Coastal wetlands may, therefore, prevent nutrient pollution from reaching coastal waters while being sources or sinks of GHGs (Wang et al., 2016). Rates of denitrification from mangrove and Melaleuca systems have been observed between 0.0 and 6.6 $\text{mg m}^{-2} \text{h}^{-1}$ or 0.05 to 6.3 $\text{mg N g}^{-1} \text{h}^{-1}$, with coupled nitrification-denitrification ranging between 0.05 and 6.5 $\text{mg m}^{-2} \text{h}^{-1}$ (Adame et al., 2019c; Adame et al., 2019b; Alongi et al., 2002; Alongi et al., 2004; Fernandes et al., 2012; Rivera-Monroy et al., 1995). Rates of nitrification from mangrove systems have been observed between 0 and 0.82 $\text{mg m}^{-2} \text{h}^{-1}$ or 0.6 to 57.1 $\text{ng N g}^{-1} \text{h}^{-1}$ (Alongi et al., 2002; Xiao et al., 2018).

Globally, forested coastal ecosystems are severely affected by global environmental change. Widespread deforestation and conversion for agriculture and aquaculture (Adame et al., 2019a; Alongi, 2002; Livesley and Andrusiak, 2012; Van Lavieren et al., 2012), as well as N pollution from rivers and atmospheric deposition (National Research Council, A., 2000), threaten these ecosystems. Forested coastal wetlands were once expansive throughout the deltas of Vietnam (90% of the Mekong River Delta comprised biodiverse, carbon-rich wetlands) but rapid, widespread losses of Melaleuca and mangrove forests have occurred in recent decades. While excess N inputs may cause further disturbance (Reef et al., 2010), coastal wetlands could represent an important natural N removal mechanism. Despite forests being responsible for 60% of the global N_2O emissions, how denitrification in coastal forested wetlands may be optimised to reduce nutrient pollution without increasing atmospheric N_2O emissions is poorly understood (Adame et al., 2019a; Cabezas et al., 2018; Lovelock and Duarte, 2019).

The global decline of coastal wetlands has led to restoration efforts to conserve and restore wetlands with the Vietnamese government increasing restoration of Melaleucas and mangroves. Restoration focusses on returning the hydrology, biota and soil to a pristine state through multiple strategies, which may include planting vegetation, restoring tidal flow, removing invasive species and improving water quality (Zhao et al., 2016). This results in complex gradients of forested coastal

wetlands ranging from highly disturbed to mature, protected systems. This leads to a critical need to understand biogeochemical and hydrological functioning across gradients of restored and mature wetlands, and in particular the implications for GHG emissions and nutrient attenuation.

Environmental stressors affect carbon and N processing either directly or by altering soil properties. For example, nutrient loading (Chen et al., 2016; Goreau and de Mello, 2007; Paerl, 1997), vegetation coverage (Duke et al., 2007; Lovelock et al., 2017), soil moisture and salinity (Ellison, 2000; IPCC, 2007) may all affect N removal and GHG fluxes (Kristensen, 2007; Oertel et al., 2016). A mosaic of varying N removal hotspots and GHG emissions is, therefore, expected across land-use and restoration gradients in coastal wetlands, especially as GHG fluxes from forested coastal wetland soils are highly variable and dependent on anthropogenic influences (Bouillon et al., 2008; Goreau and de Mello, 2007; Kristensen, 2007).

Investigations of GHG fluxes across different restoration stages of mangrove forests have revealed variable responses of CO_2 fluxes to deforestation. Higher, lower and no difference from deforested/degraded or bare mangrove sediment compared to forested mangrove sediment have been observed (Alongi et al., 1998; Bulmer et al., 2017; Castillo et al., 2017; Hien et al., 2018a; Senger et al., 2021). Lower CH_4 fluxes and emissions have been observed from deforested mangrove sediment and mudflats compared to forested mangrove sediments (Castillo et al., 2017; Soper et al., 2019). Deforestation and nutrient pollution have been observed to increase N_2O production (Castillo et al., 2017; Fernandes et al., 2010; Hershey et al., 2021). Higher rates of denitrification have been observed in a nutrient polluted mangrove forest compared to a pristine forest, and in vegetated versus unvegetated mangrove sediments (Fernandes et al., 2010; Kristensen et al., 1998). There has, however, been significantly less research on carbon and N cycling in Melaleuca forest soils. The effect of land-use change and restoration on GHG fluxes or N removal in Melaleucas remains unquantified (Adame et al., 2019a; Adame et al., 2019b; Adame et al., 2019c; Greenway and Jenkins, 2004; Jeffrey et al., 2020; Jeffrey et al., 2021; Tran and Dargusch, 2016; Tran et al., 2015). The current lack in understanding of carbon and N cycling across land-use and restoration gradients in coastal ecosystems prevents the responses of nutrient attenuation and GHG fluxes to global change from being assessed and consequently, prevents maximisation of ecosystem services through effective management (Allen et al., 2011).

Here, we investigate N transformation processes and CO_2 , CH_4 , N_2O and N_2 fluxes in two tropical, forested coastal wetlands. We focuss on tropical estuaries to address knowledge gaps resulting from research bias toward temperate estuaries (Vieillard et al., 2020). We sampled mangrove and Melaleuca soils across various degrees of land-use and restoration in Vietnam. We used stable isotopic (^{15}N) tracers to determine potential rates of nitrification and denitrification, which are more reliable than traditional techniques, such as the acetylene block method. We, therefore, utilise this method to produce estimates of potential N reduction and contributions of denitrification to N_2O fluxes in forested coastal wetlands (Seitzinger et al., 1993; Yu et al., 2010). There have been no comparative studies in mangroves or Melaleucas that used isotopic tracers to investigate both gaseous products of denitrification (N_2O and N_2) to provide more complete estimates of the N removal potential of these ecosystems. We aimed to determine

the relative importance of denitrification in N_2O production and the ability of coastal wetlands to reduce N pollution. We measured GHG fluxes and determined whether N_2O or N_2 was the dominant product of denitrification and therefore, if these systems are capable of reducing N pollution without increasing N_2O emissions. We hypothesise that N biogeochemistry and soil-atmosphere GHG fluxes will vary between land-use and restoration classes, in both magnitude and direction, as well as between wetland types.

2. Methods and materials

2.1. Study sites

The study was conducted in two forested coastal wetlands representing the diversity of wetland types with a variety of restoration and land-use found in Vietnam. Mangrove soil samples were collected from the mangrove forest in Xuan Thuy National Park (NP), Red River Delta, Northern Vietnam, which was the first designated Ramsar site in South East Asia (Fig. 1a). Mangrove forests cover 11 km² of the 71 km² of wetland area within Xuan Thuy NP. Melaleuca soil samples were collected from a freshwater Melaleuca forest in U Minh Thuong NP, Mekong River Delta, Southern Vietnam (Fig. 1a), which was designated a Ramsar site in 2016 (Quan et al., 2018). U Minh Thuong NP is located in the upper region of U Minh forest, a peat swamp ecosystem covering 600 km² (CARE, 2004). The forest is dominated by *Melaleuca cajuputi* ranging from semi-natural Melaleuca forests to monoculture Melaleuca plantations (Buckton et al., 1999). U Minh Thuong NP contains the largest area (55.8 km²) of Melaleuca swamp in the U Minh forest (CARE, 2004).

The climate in Xuan Thuy and U Minh Thuong NP's is tropical monsoon with average annual temperatures of 24.0 and 26.5 °C, respectively

(Hien et al., 2018b; Phan and Subasinghe, 2018). In Xuan Thuy NP the wet and dry seasons occur between May and October, and November and April, respectively. In U Minh Thuong NP the wet and dry seasons occur between June and November, and December to May, respectively. The average annual rainfall is 1775 mm in Xuan Thuy NP and 2360 mm in U Minh Thuong NP (Nam Dinh Province Statistics Office, 2016; Phan and Subasinghe, 2018). Xuan Thuy NP is flooded at high tide and has an irregular diurnal tide with a large amplitude between 0.37 and 3.54 m. Canal systems around U Minh Thuong NP regulate water level and the groundwater level was approximately 10 to 50 cm below the soil surface across sites during sampling (Center for Oceanography, 2016).

Xuan Thuy NP is extensively used for aquaculture resulting in significant mangrove losses. Mangrove restoration projects, such as seedling planting, have been undertaken leading to a mosaic of restoration and land-use in the NP. U Minh Thuong NP is located in an area of the Mekong River Delta where Melaleuca wetlands have been extensively drained for agriculture and is surrounded by agricultural land, with a 131 km² buffer zone (Tran et al., 2015). In 2002, a large fire in the NP burnt 32.1 km² of vegetation and peat (Tran et al., 2015; Vietnam Environment Protection Agency, 2003). These disturbances have created a gradient of different land-use and restoration stages, from the converted agricultural land outside the NP to the mature Melaleuca forest in the centre of the NP, which survived the 2002 fire.

We sampled forested coastal wetland soils from eight land-use/restoration zones within the two NPs (Fig. 1b and Table 1). The study area within Xuan Thuy NP was located on Tra Creek, approximately four kilometres upstream of where the creek meets the Red River in the Ba Lat estuary. This area contains a variety of restoration stages with varying tree species, planted or natural growth and bare soil. Four sites were identified that represent different restoration stages: A) A degraded area with no vegetation, B) An approximately five to

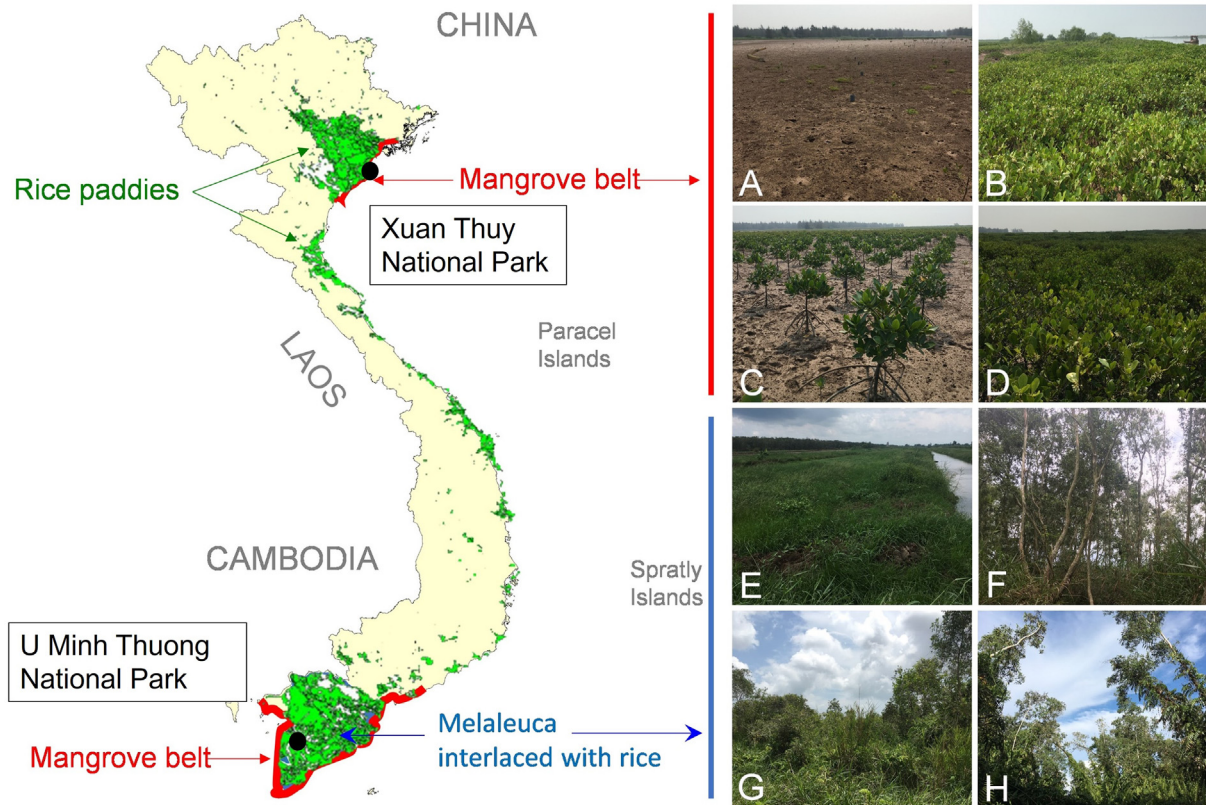


Fig. 1. A map of the wetland area and type in Vietnam, including the locations of the field sites (black dots). The field sites are the mangrove forest in Xuan Thuy National Park, northern Vietnam and the melaleuca forest in U Minh Thuong National Park, southern Vietnam. The map contains data from the Environmental Systems Research Institute. Photographs of the eight sampling sites across two gradients of land-uses, one in the mangrove forest of Xuan Thuy National Park (A–D) and one in the melaleuca forest of U Minh Thuong National Park (E–H) are also included. The sampling sites are detailed in the text.

Table 1

Details of the eight restoration classes investigated including site name, site description, wetland ecosystem and national park.

Site	Description	Wetland Ecosystem	National Park
A	A degraded area with no vegetation	Mangrove	Xuan Thuy
B	An approximately five to seven year old, naturally-regenerated forest of <i>Aegiceras corniculatum</i> , <i>Derris trifoliata</i> Lour and <i>Acanthus ebracteatus</i>	Mangrove	Xuan Thuy
C	An eight year old re-planted forest of <i>Bruguiera gymnorrhiza</i> and <i>Sonneratia apalata</i>	Mangrove	Xuan Thuy
D	An approximately 15 year old, naturally-regenerated forest of <i>Avicennia marina</i> , <i>Sonneratia caseolaris</i> and <i>Kandelia obovate</i>	Mangrove	Xuan Thuy
E	Agricultural land adjacent to the national park	Melaleuca	U Minh Thuong
F	Restoring Melaleuca forest on clay	Melaleuca	U Minh Thuong
G	Restoring Melaleuca forest on shallow peat underlain by clay	Melaleuca	U Minh Thuong
H	Mature Melaleuca forest on peat	Melaleuca	U Minh Thuong

seven year old, naturally-regenerated forest of *Aegiceras corniculatum*, *Derris trifoliata* Lour and *Acanthus ebracteatus*, C) An eight year old re-planted forest of *Bruguiera gymnorrhiza* and *Sonneratia apalata*, and D) An approximately 15 year old, naturally-regenerated forest of *Avicennia marina*, *Sonneratia caseolaris* and *Kandelia obovate*. In U Minh Thuong NP, samples were taken along a gradient from agricultural to mature Melaleucas resulting in four sites: E) Agricultural land adjacent to the NP, F) Restoring Melaleuca forest on clay, G) Restoring Melaleuca forest on shallow peat underlain by clay and H) Mature Melaleuca forest on peat.

2.2. Soil sampling

Soils for incubation were sampled using a five cm diameter augur. Samples were taken at 0–15 cm depth in the mangrove sites at low tide on the 24th of April 2019 (five cores per site). Samples were taken at 0–10 and 10–20 cm depths in the Melaleuca sites on the 13th of May 2019 (three cores per site). Three 50 cm long cores, divided into 10 cm intervals, were collected using a five cm diameter augur on the 10th of May 2019 in the mangrove sites and the 13th of May 2019 in the Melaleuca sites. Each soil sample was placed into an air-tight bag and the equipment cleaned prior to the next sample being taken. It was not always possible to sample with the augur in the Melaleuca wetland, in these cases a square of peat was cut and the subsamples taken from this.

2.3. Soil properties

Gravimetric soil moisture content was determined from 0 to 15 cm depth mangrove samples, and from 0 to 10 and 10–20 cm depth Melaleuca samples. Approximately 30 g of wet soil was dried in an oven at 105 °C for 24 h. The samples were then re-weighed and gravimetric soil moisture content was determined from the dry weight and the mass loss during drying.

Core subsamples up to 50 cm depth were dried at 105 °C. The dry soil was then sieved (2 mm) and pulverised with a pestle and mortar. Duplicate subsamples of each sample depth were analysed for total carbon (TC), total N (TN), $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ on an Elemental Analyser (vario PYRO cube, Elemental Analysensysteme GmbH, Hanau, Germany), coupled with an isotope ratio mass spectrometer (Elementar Isoprime PreciSION, Elemental Analysensysteme GmbH, Hanau, Germany). The Elemental Analyser was calibrated with sulphanilamide (N: 16.26%, C: 41.81%) and the isotope ratio mass spectrometer was calibrated against international reference standards (caffeine: USGS61, USGS62, USGS63). The relative standard deviation of the in-house soil reference standard was 1.7, 3.0, 9.7 and -2.1‰, respectively, for TC, TN, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

Sub-samples of each soil sample were taken for NO_3^- , DOC and TDN analysis. Soil was extracted in a 1:5 ratio with deionised H_2O and with 0.1 M K_2SO_4 for the Melaleuca NO_3^- analysis. Samples were shaken at

200 rpm for one hr, centrifuged at 4000 rpm for 20 min and filtered at 0.45 μm . Extracts were preserved at pH 2 with 5 N HCl for DOC and TDN analysis.

NO_3^- was determined colourimetrically from mangrove extracts at 430 nm on a spectrophotometer (L-VIS-400, Labnics Company, Fremont, USA) and from the Melaleuca extracts at 540 nm on a spectrophotometer (Jenway 6850). Mangrove samples were prepared for analysis using the disunphophenic method and Melaleuca samples were prepared using the vanadium method. A 0.5 mg $\text{NO}_3^- \text{ l}^{-1}$ standard gave an accuracy of 0.009 mg $\text{NO}_3^- \text{ l}^{-1}$ and precision of ± 0.001 mg $\text{NO}_3^- \text{ l}^{-1}$ for the mangrove samples.

DOC and TN were measured on a total organic carbon – TN analyser (multi N/C® 2100/2100S, Analytik Jena AG, Jena, Germany), with a LOD of 0.05 mg C l^{-1} . A 10 mg C l^{-1} standard gave an accuracy of 0.2 mg C l^{-1} and a precision of ± 0.2 mg C l^{-1} , and a 1 mg N l^{-1} standard gave an accuracy of 0.01 mg N l^{-1} and a precision of ± 0.04 mg N l^{-1} for the mangrove samples. A 20 mg C l^{-1} standard gave an accuracy of 0.9 mg C l^{-1} and a precision of ± 0.3 mg C l^{-1} , and a 3 mg N l^{-1} standard gave an accuracy of 0.05 mg N l^{-1} and a precision of ± 0.3 mg N l^{-1} for the Melaleuca samples.

2.4. Potential flux estimates

Potential fluxes of soil GHG were determined during laboratory incubation experiments. Incubation soil samples were stored air-tight on ice in the dark or in a refrigerator until incubation experiments were performed, except during transport of Melaleuca soils from Vietnam to the U.K. Mangrove soils were sieved (2 mm) and homogenised. 20 g of field-moist soil was added to a 100 ml glass incubation jar and stored cold until incubation experiments began.

K^{15}NO_3 (98 at.%) and $\text{NH}_4^{15}\text{Cl}$ (98 at.%) were used as tracers to determine rates of denitrification and nitrification, respectively, and the dominant product of denitrification (N_2O versus N_2) was also determined (Ambus et al., 2006; Matson et al., 2009; Sgouridis and Ullah, 2015, 2017). For mangrove samples, twenty incubation experiments were performed with $^{15}\text{N}\text{-NO}_3^-$ enrichment (five soil samples from each of sites A–D) and sixteen incubation experiments were performed with $^{15}\text{N}\text{-NH}_4^+$ enrichment (four soil samples from each of sites A–D). For Melaleuca samples, eleven incubation experiments were performed (three soil samples from each of sites F–H and two soil samples from site E) with samples homogenised from the 0–10 cm and 10–20 cm depth.

The volume and concentration of the tracer added varied between sites so that the volumetric soil moisture content was adjusted to approximately 60% for the mangrove samples and the ^{15}N enrichment was at 20% the ambient concentration of NO_3^- or NH_4^+ depending on the tracer.

Gas samples were taken immediately after the tracer was added and the jars closed with a rubber septum, seven ml were subsampled for GHG concentration and 20 ml were subsampled for isotopic analysis of ^{15}N . Samples were immediately injected into pre-evacuated exetainers

(three and 12 ml, respectively, Labco, UK). The incubation jars were then wrapped in tin foil and gas samples for GHG concentrations were taken at five and 24 h, and gas samples for ^{15}N isotope analysis were taken at 24 h. Laboratory temperature was measured using a PyroScience Firesting optical oxygen meter (PyroScience, Aachen, Germany).

2.5. GHG concentration analysis

Concentrations of CO_2 , CH_4 and N_2O in all samples were measured simultaneously on an Agilent 7890A Gas Chromatograph (GC). This was fitted with both a Flame Ionisation Detector (FID) for CO_2 and CH_4 analysis, and a micro electron capture detector (μECD) for N_2O analysis. The CO_2 was methanised prior to passing into the FID. The GC had a splitless, 1 ml sample loop and an oven temperature of 60°C , the FID temperature was 250°C and the μECD temperature was 350°C . The FID had a make-up N_2 flow of 2 ml min^{-1} with gas flows of 48 ml min^{-1} of hydrogen and 500 ml min^{-1} air. The μECD had a make-up argon and methane flow of 2 ml min^{-1} . The run time was nine minutes with CH_4 , CO_2 and N_2O eluted at 3.6, 5.8 and 7.0 min, respectively. The GC had an accuracy of 21.26, 0.07 and 0.02 ppm and a precision of ± 30.82 , 0.08 and 0.03 for CO_2 , CH_4 and N_2O , respectively, from 600, 5 and 1 ppm standards. Potential GHG fluxes were calculated using the linear portion of the incubations or where the highest production was observed (White and Reddy, 2003). Where fluxes were below the minimum detectable concentration difference for the GC, fluxes were set to zero (Sgouridis and Ullah, 2017).

2.6. Isotopic analysis

Stable isotopes of $^{15}\text{N}\text{-N}_2\text{O}$ and $^{15}\text{N}\text{-N}_2$ were analysed at the Stable Isotope Facility, UC Davis, USA. Gas samples were purged into a helium carrier stream then N_2 and N_2O were isolated and concentrated (ThermoScientific GasBench + Precon, Bremen, Germany). N_2 was sampled through a 5–100 μl sampling loop and passed into an isotope ratio mass spectrometer (IRMS; ThermoScientific Delta V Plus, Bremen, Germany) for analysis. The rest of the gas sample was passed through a CO_2 scrubber and N_2O was trapped, concentrated, and passed into the IRMS for analysis. The N_2 was calibrated against an Oztech N_2 standard (Oztech Trading Company, $\delta^{15}\text{N}$ vs. air = 0.61). N_2O was calibrated by converting N_2O to N_2 and O_2 in a heated gold tube (800°C) and calibrating the subsequent N_2 and O_2 with the Oztech N_2 standard and an Oztech O_2 standard ($\delta^{18}\text{O}$ vs. VSMOW = 27.48). Potential fluxes were then calculated using the methods described in Bergsma et al. (2001) and Mulvaney (1984).

2.7. Statistical analysis

Statistical analysis was performed using R (R Core Development Team, 2011). Significant differences in parameters between the wetlands and sites were determined using the non-parametric Wilcoxon Signed Rank test and Kruskal-Wallis rank sum test, respectively, where $n = 31$. Where the Kruskal-Wallis showed significant differences a Dunn post-hoc test with Bonferroni correction was performed to identify which pairs of sites were significantly different. The non-parametric Kruskal-Wallis rank sum test was used for the sediment data with depth and where significant differences were found a post-hoc Dunn test with Bonferroni correction was performed.

3. Results

3.1. Potential $^{15}\text{N}\text{-N}_2\text{O}$ fluxes

$^{15}\text{N}\text{-N}_2\text{O}$ fluxes produced from $^{15}\text{N}\text{-NO}_3^-$ enriched experiments represent potential denitrification-derived N_2O . $^{15}\text{N}\text{-NH}_4^+$ enriched experiments represent potentially nitrification-derived N_2O .

Potential fluxes of denitrification-derived N_2O were highest from Melaleuca soils and this difference was significant ($p\text{-value} = 0.02$, Wilcoxon, Fig. 3a). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2\text{O}$ fluxes were highest from Melaleuca than mangrove soils, but this difference was not significant ($p\text{-value} = 0.73$, Wilcoxon, Fig. S1).

Across mangrove restoration sites, potential fluxes of denitrification-derived N_2O were highest from site B and lowest from site A, however, this was not significant ($p\text{-value} = 0.10$, Kruskal-Wallis, Fig. 3a). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2\text{O}$ fluxes followed the same pattern with no significant difference observed ($p\text{-value} = 0.07$, Kruskal-Wallis, Fig. S1).

Across Melaleuca restoration sites, potential fluxes of denitrification-derived N_2O were highest from site G and lowest from site E, however, this was not significant ($p\text{-value} = 0.14$, Kruskal-Wallis, Fig. 3a). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2\text{O}$ fluxes were highest from site F and lowest from site H, with no significant difference observed ($p\text{-value} = 0.41$, Kruskal-Wallis, Fig. S1).

Potential fluxes of nitrification-derived $^{15}\text{N}\text{-N}_2\text{O}$ from mangrove soils were generally lower than those derived from denitrification. Potential fluxes were lowest from site C (Fig. 3b) but were not significantly different ($p\text{-value} = 0.60$, Kruskal-Wallis). When normalised to soil carbon, potential nitrification-driven $^{15}\text{N}\text{-N}_2\text{O}$ fluxes from mangrove soils were highest from site B and lowest from site D (Fig. S1) but were not significantly different ($p\text{-value} = 0.68$, Kruskal-Wallis).

3.2. Potential $^{15}\text{N}\text{-N}_2$ fluxes

Potential fluxes of denitrification-derived $^{15}\text{N}\text{-N}_2$ were highest from mangrove soils and this difference was significant ($p\text{-value} = 0.02$, Wilcoxon, Fig. 3c). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2\text{O}$ fluxes were highest from mangrove soils and this difference was significant ($p\text{-value} < 0.01$, Wilcoxon, Fig. S1).

Across mangrove restoration sites, potential fluxes of denitrification-derived N_2 were highest from site A and lowest from site B, however, this was not significant ($p\text{-value} = 0.10$, Kruskal-Wallis, Fig. 3c). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2$ fluxes were highest from site C and lowest from site D, and were significantly different ($p\text{-value} = 0.01$, Kruskal-Wallis, Fig. S1). Significant differences were observed between mangrove sites A and D, and C and D ($p\text{-value} = 0.01$ and 0.01 , respectively, Dunn).

Across Melaleuca restoration sites, potential fluxes of denitrification-derived N_2 were highest from site G and lowest from site E, however, this was not significant ($p\text{-value} = 0.10$, Kruskal-Wallis, Fig. 3c). When normalised to soil carbon, potential denitrification-driven $^{15}\text{N}\text{-N}_2$ fluxes were highest from site E and lowest from site H, with no significant difference observed ($p\text{-value} = 0.18$, Kruskal-Wallis, Fig. S1).

The denitrification product ratio was highest in Melaleuca soils and the difference was significant ($p\text{-value} < 0.01$, Wilcoxon, Fig. 3d). Across mangrove restoration sites, the denitrification product ratio was highest from site B and lowest from site A, and was significantly different ($p\text{-value} = 0.04$, Kruskal-Wallis, $p\text{-value} = 0.01$, Dunn, Fig. 3d).

Across Melaleuca restoration sites, the denitrification product ratio was highest from site F and lowest from site E, however, this was not significant ($p\text{-value} = 0.27$, Kruskal-Wallis, Fig. 3c). All patterns were the same when normalised to soil carbon.

3.3. Potential GHG fluxes from $^{15}\text{N}\text{-NO}_3^-$ enriched incubation experiments

3.3.1. N_2O

Potential N_2O fluxes were always positive (Fig. 4a). Potential N_2O fluxes were highest from Melaleuca soils and this difference was significant ($p\text{-value} = 0.01$, Wilcoxon, Fig. 4a). When normalised to

soil carbon, potential N₂O fluxes were similar from mangrove and Melaleuca soils (p -value = 0.10, Wilcoxon, Fig. S2).

Across mangrove restoration sites, potential N₂O fluxes were highest from site B and lowest from site C, however, this was not significant (p -value = 0.13, Kruskal-Wallis, Fig. 4a). When normalised to soil carbon, potential N₂O fluxes were highest from site B, however differences were not significant (p -value = 0.15, Kruskal-Wallis, Fig. S2).

Across Melaleuca restoration sites, potential N₂O fluxes were highest from sites F and G, and lowest from site E, however, this was not significant (p -value = 0.11, Kruskal-Wallis, Fig. 4a). When normalised to soil carbon, potential N₂O fluxes were highest from site F and lowest from site H, with no significant difference observed (p -value = 0.44, Kruskal-Wallis, Fig. S2).

3.3.2. CO₂ from microbial respiration

Potential CO₂ fluxes were always positive (Fig. 4b). Potential CO₂ fluxes were highest from Melaleuca soils and the difference was significant (p -value <0.01, Wilcoxon, Fig. 4b). When normalised to soil carbon, potential CO₂ fluxes were highest from mangrove soils and the difference was significant (p -value <0.01, Wilcoxon, Fig. S2).

Across mangrove restoration sites, potential CO₂ fluxes were highest from site B and lowest from site C, and were significantly different (p -value <0.01, Kruskal-Wallis, Fig. 4b). Significant differences were found between mangrove sites B and C (p -value <0.01, Dunn), C and D (p -value = 0.01, Dunn). When normalised to soil carbon, potential CO₂ flux patterns were the same and were also significantly different (p -value <0.01, Kruskal-Wallis, Fig. S2). Significant differences were observed between mangrove sites B and C (p -value <0.01, Dunn).

Across Melaleuca restoration sites, potential CO₂ fluxes were highest from site G and lowest from site E, and were significantly different (p -value = 0.03, Kruskal-Wallis, Fig. 4b). However, no significant difference between Melaleuca sites were found during post-hoc testing. When normalised to soil carbon, potential CO₂ fluxes were highest from sites E and G, and lowest from site F, with no significant difference observed (p -value = 0.18, Kruskal-Wallis, Fig. S2).

3.3.3. CH₄

Potential CH₄ fluxes were positive, negative or below the limit of detection depending on the site (Fig. 4c). Positive potential CH₄ fluxes were observed from sites A, B, E and F and were highest at site F, whereas large, negative potential fluxes were observed from sites G and H (Fig. 4c). Potential CH₄ fluxes from sites C and D were below the limit of detection.

Potential CH₄ fluxes were small and positive from the mangrove soils, and large but negative from the Melaleuca soils but were not significantly different (p -value = 0.70, Wilcoxon, Fig. 4c). When normalised to soil carbon, potential CH₄ fluxes were positive from both wetland types and were highest from the mangrove soils, although this difference was not significant (p -value = 0.43, Wilcoxon, Fig. S2).

Across mangrove restoration sites, potential CH₄ fluxes were highest from site A and lowest from sites C and D, and were significantly different (p -value = 0.01, Kruskal-Wallis, Fig. 4c). Significant differences were observed between mangrove sites A and C (p -value = 0.02, Dunn), and A and D (p -value = 0.02, Dunn). When normalised to soil carbon, potential CH₄ flux patterns were the same and were also significantly different (p -value = 0.01, Kruskal-Wallis, Fig. S2). Significant differences were observed between mangrove sites A and C (p -value = 0.02, Dunn), and A and D (p -value = 0.02, Dunn).

Across Melaleuca restoration sites, the largest positive potential CH₄ flux was from site F and the largest negative potential CH₄ flux was from site H, however, no significant differences were observed (p -value = 0.05, Kruskal-Wallis, Fig. 4c). When normalised to soil carbon, the largest positive potential CH₄ flux was from site E and the largest negative potential CH₄ flux was from site H, with no significant difference observed (p -value = 0.07, Kruskal-Wallis, Fig. S2).

3.4. Soil properties

The soil properties of each site are shown in Tables 2 and 3. Soil moisture was highest in the Melaleuca soils, with the highest soil moisture in site G and the lowest in site E. Soil moisture was similar across mangrove restoration sites. Where soil moisture >100% indicates the soils were fully saturated. The concentration of extractable nitrate was highest in the mangrove soils, where concentrations were similar across restoration sites. Across the Melaleuca restoration sites, extractable nitrate was highest in site H and lowest in site F. Extractable DOC showed greater variation between sites than extractable nitrate, with concentrations highest in Melaleuca soils. Across mangrove restoration sites, extractable DOC was highest in site B and lowest in site C, and across Melaleuca restoration sites was highest in site H and lowest in site E. Extractable TDN was lowest in the mangrove soils, with lowest concentrations in site C and highest in site B. Across Melaleuca restoration sites, the extractable TDN was highest in site H and lowest in site E. Soil TC and TN followed similar trends and were highest in the Melaleuca soils. Across mangrove restoration sites, TN was similar across sites and TC was highest in site D and lowest in site C. Across Melaleuca restoration sites, TC and TN were highest in site H and lowest in site E. The C:N ratio was similar between mangrove restoration sites A to D and across Melaleuca restoration sites was highest in site H and lowest in site E. Patterns of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were not clear between sites, however, in general $\delta^{15}\text{N}$ was lightest in the mangrove soils and $\delta^{13}\text{C}$ was lightest in the Melaleuca soils.

Fig. 2 summarises chemical soil parameters with depth. TN, TC and C:N were highest in Melaleuca soils and $\delta^{13}\text{C}$ were lightest in Melaleuca soils. TN and TC were relatively low at all depths at sites A to E and decreased with depth at sites F to H, except at site H where TC slightly increased with depth. $\delta^{13}\text{C}$ ratios were lightest at sites F to H where they became heavier with depth. C:N ratio and $\delta^{15}\text{N}$ did not show consistent patterns with depth.

Across all depths, TN and TC were highest at sites H and G, all other sites were low except F with intermediate values at 0–10 and 10–20 cm depths. The C:N ratio was highest at site H and then decreased from G to B to D, with other sites having similar or low ratios. $\delta^{15}\text{N}$ values were lightest at site B and heaviest at site E. $\delta^{13}\text{C}$ values were lightest at sites F–H, then increased from E to D to B to A to C.

All measured parameters were significantly different between sites (p -values all <0.01, Kruskal-Wallis) with differences between many sites significantly different, see Table S1 for details. All measured parameters, except $\delta^{15}\text{N}$ (p -value = 0.08, Kruskal-Wallis, Table S2), were significantly different between wetlands (mangrove and Melaleuca forest soils) (p -values <0.01 for TN, TC and $\delta^{13}\text{C}$ and p -value = 0.01 for C:N, Kruskal-Wallis).

Table 2

The sediment properties of each site.

Site	Depth (cm)	Soil moisture (Gravimetric, %)	NO ₃ (mg N g ⁻¹ wet soil)	DOC (mg C g ⁻¹ wet soil)	TDN (mg N g ⁻¹ wet soil)
A	0–15	41.0	1.1	19.6	0.7
B	0–15	42.1	1.2	25.4	2.8
C	0–15	34.5	1.2	18.2	0.0
D	0–15	45.0	1.2	20.3	0.6
E	0–20	35.9	0.8	13.1	0.8
F	0–20	106.7	0.6	27.8	11.6
G	0–20	586.5	0.9	67.6	11.2
H	0–20	388.0	1.3	109.8	16.5

Presented are the soil moisture as gravimetric water content in percent, nitrate concentration in micrograms of nitrogen per gram of wet soil, DOC concentration in micrograms of carbon per gram of wet soil and total dissolved nitrogen concentration in microgram of nitrogen per gram of wet soil. A. Deforested mangroves, B. 5–7 year old natural mangrove regrowth, C. 8 year old mangrove plantation, D. 15 year old natural mangrove regrowth, E. Melaleuca converted to agriculture, F. Restoring Melaleuca on clay, G. Restoring Melaleuca on peat underlain with clay and H. Mature Melaleuca on deep peat.

Table 3

Sediment total nitrogen (%), total carbon (%), C:N ratio, $\delta^{15}\text{N}$ (Air standard) and $\delta^{13}\text{C}$ (Vienna Pee Dee Belemnite standard) in the top 20 cm of each of the sites studied.

Site	Depth (cm)	N (%)	C (%)	C/N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
A	0-20	0.06	0.79	13.71	-7.00	-20.03
B	0-20	0.05	0.76	15.23	-8.82	-23.03
C	0-20	0.05	0.52	11.03	-10.30	-17.85
D	0-20	0.07	0.98	13.58	0.99	-22.96
E	0-20	0.10	1.14	11.85	1.82	-26.36
F	0-20	0.56	11.72	18.17	0.98	-30.41
G	0-20	2.01	43.20	21.78	-2.23	-29.97
H	0-20	2.08	49.49	23.97	-1.40	-30.32

A. Deforested mangroves, B. 5–7 year old natural mangrove regrowth, C. 8 year old mangrove plantation, D. 15 year old natural mangrove regrowth, E. Melaleuca converted to agriculture, F. Restoring Melaleuca on clay, G. Restoring Melaleuca on peat underlain with clay and H. Mature Melaleuca on deep peat.

4. Discussion

4.1. Controls and mechanisms of carbon and N cycling

Potential denitrification played a more dominant role in N_2O production in Melaleuca than in mangrove soils. This appears to be due to potential denitrification being more complete in mangrove than Melaleuca soils, which was evidenced by the higher potential denitrification-driven $^{15}\text{N}-\text{N}_2$ fluxes and lower denitrification product ratio observed in the mangrove soils. In mangrove soils, potential nitrification played a minor role in N_2O production.

The total rate of potential denitrification was determined from the potential $^{15}\text{N}-\text{N}_2\text{O}$ flux due to denitrification and the potential $^{15}\text{N}-\text{N}_2$ flux due to denitrification. The total rate of potential denitrification was higher in the mangrove soils ($8.1 \text{ ng N g}^{-1} \text{ h}^{-1}$) than in Melaleuca soils ($6.8 \text{ ng N g}^{-1} \text{ h}^{-1}$), indicating that mangrove ecosystem had a higher capacity of N removal, while also emitting less N_2O . A higher capacity for N removal from the Melaleucas might be expected given the more saturated, organic-rich soils found in these systems, which are favourable for denitrifier activity (Cardenas et al., 2017; Tomasek et al., 2019). The mangrove soils, however, have higher nitrate availability, more labile carbon and lower C:N, which all maintain high rates of denitrification (Klemetsson et al., 2005; Luo et al., 1999; Pérez et al., 2010). The large variation in soil moisture content between the mangrove and Melaleuca soils used in the incubation experiments likely had a large effect on biogeochemical processing, however, the soil moisture was reflective of the expected higher saturation in Melaleuca soils in natural systems.

Higher rates of denitrification from the mangrove soils contradict the only previous study on denitrification from Melaleucas and mangroves, which found rates of denitrification were higher in Melaleuca soils than in mangrove soils in Australia (Adame et al., 2019b). This difference may be due to the inclusion of multiple restoration sites in this study, if only forested mangrove sites and Melaleuca with peat soil sites are considered, then the rate of denitrification would be higher in the Melaleuca system.

Potential GHG emissions were higher from Melaleuca soils than in mangrove soils despite Melaleuca soils having negative potential CH_4

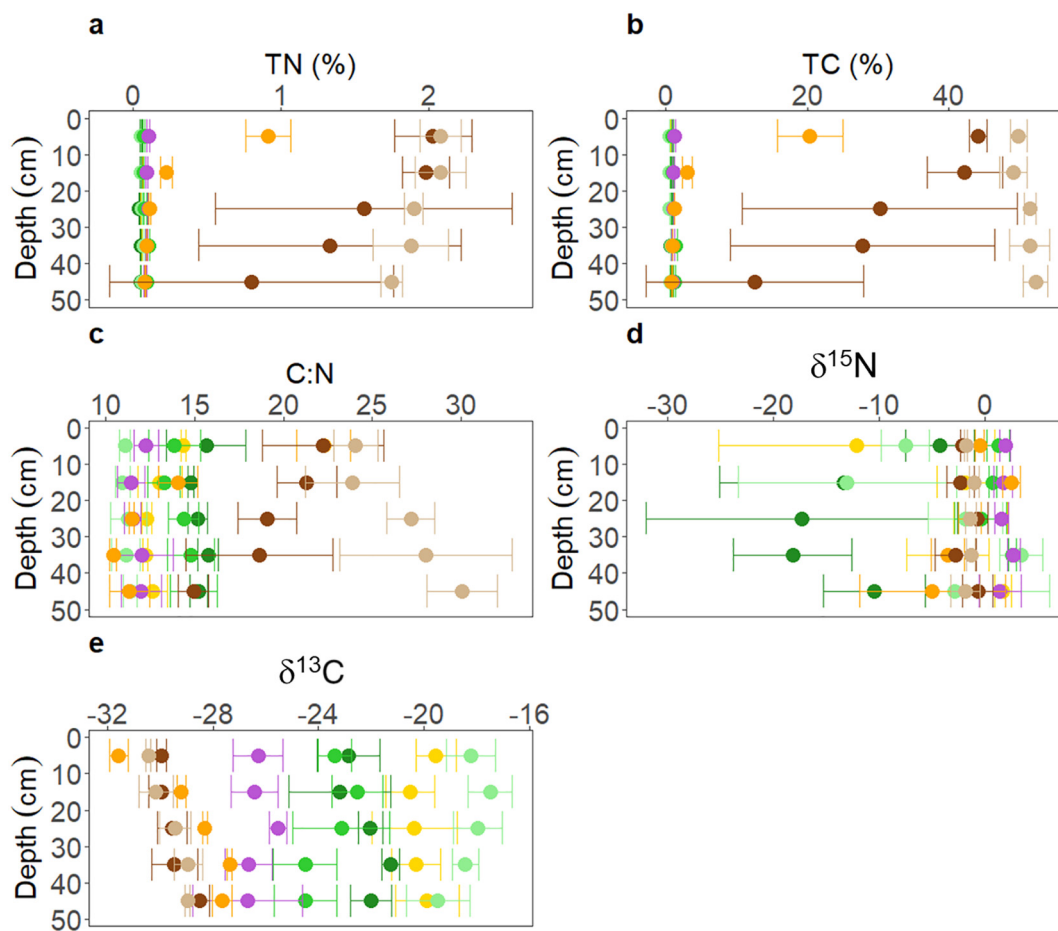


Fig. 2. Sediment properties, with depth, of the sites. a. total nitrogen (%), b. total carbon (%), c. C:N ratio, d. $\delta^{15}\text{N}$ (Air standard) and e. $\delta^{13}\text{C}$ (Vienna Pee Dee Belemnite standard). A. Deforested mangroves, B. 5–7 year old natural mangrove regrowth, C. 8 year old mangrove plantation, D. 15 year old natural mangrove regrowth, E. Melaleuca converted to agriculture, F. Restoring Melaleuca on clay, G. Restoring Melaleuca on peat underlain with clay and H. Mature Melaleuca on deep peat.

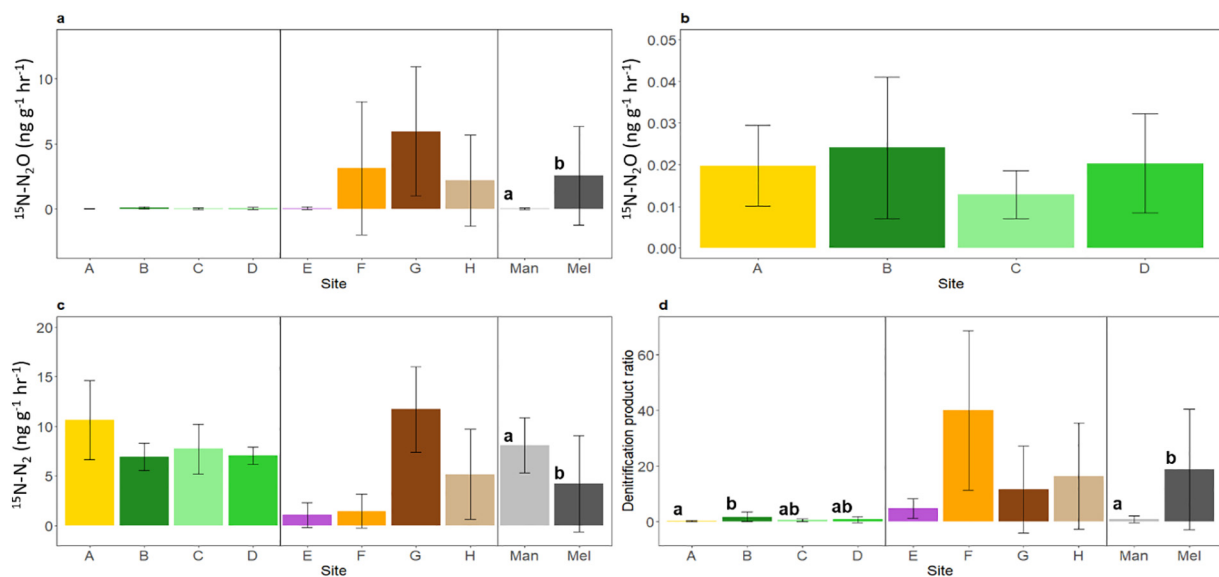


Fig. 3. Denitrification-derived $^{15}\text{N}-\text{N}_2\text{O}$ fluxes, expressed in $\text{ng g}^{-1} \text{hr}^{-1}$, b. Nitrification-derived $^{15}\text{N}-\text{N}_2\text{O}$ fluxes, expressed in $\text{ng g}^{-1} \text{hr}^{-1}$, c. $^{15}\text{N}-\text{N}_2$ fluxes derived due to denitrification from laboratory incubations spiked with $^{15}\text{N}-\text{NO}_3$, expressed in $\text{ng g}^{-1} \text{hr}^{-1}$, and d. the denitrification product ratio ($^{15}\text{N}-\text{N}_2\text{O}/^{15}\text{N}-\text{N}_2$). Significantly different pairs are indicated with different letters. Error bars represent one standard deviation from the mean. A. Deforested mangroves, B. 5–7 year old natural mangrove regrowth, C. 8 year old mangrove plantation, D. 15 year old natural mangrove regrowth, E. Melaleuca converted to agriculture, F. Restoring Melaleuca on clay, G. Restoring Melaleuca on peat underlain with clay and H. Mature Melaleuca on deep peat.

fluxes. Negative potential CH_4 fluxes from the saturated, peat-dominated Melaleuca soils were unexpected and may result from the soils being incubated in aerobic environments. High leaf litter volumes in the upper soil layers were less saturated and the groundwater level was >10 cm below the surface. This may lead to aerobic conditions dominating the upper soil layers and promoting methanotrophy over methanogenesis. Additionally, here we only consider soils with no influence of trees. It is possible that in-situ CH_4 fluxes would be positive not negative due to transport of CH_4 through tree stems, from the soil to the

atmosphere, without possibility of oxidation (Pangala et al., 2013). Melaleucas have been observed in-situ as CH_4 sinks, which was attributed to low soil moisture (Livesley and Andrusiak, 2012).

Differences in potential GHG fluxes between ecosystems were due to higher carbon lability in the mangrove soils. Higher carbon lability was indicated by potential CO_2 and CH_4 emissions being higher from mangrove soils when normalised by soil carbon and mangrove soils having lower TC than the Melaleuca soils. Only the difference in potential CO_2 emissions, however, was significant. Higher potential CO_2 and

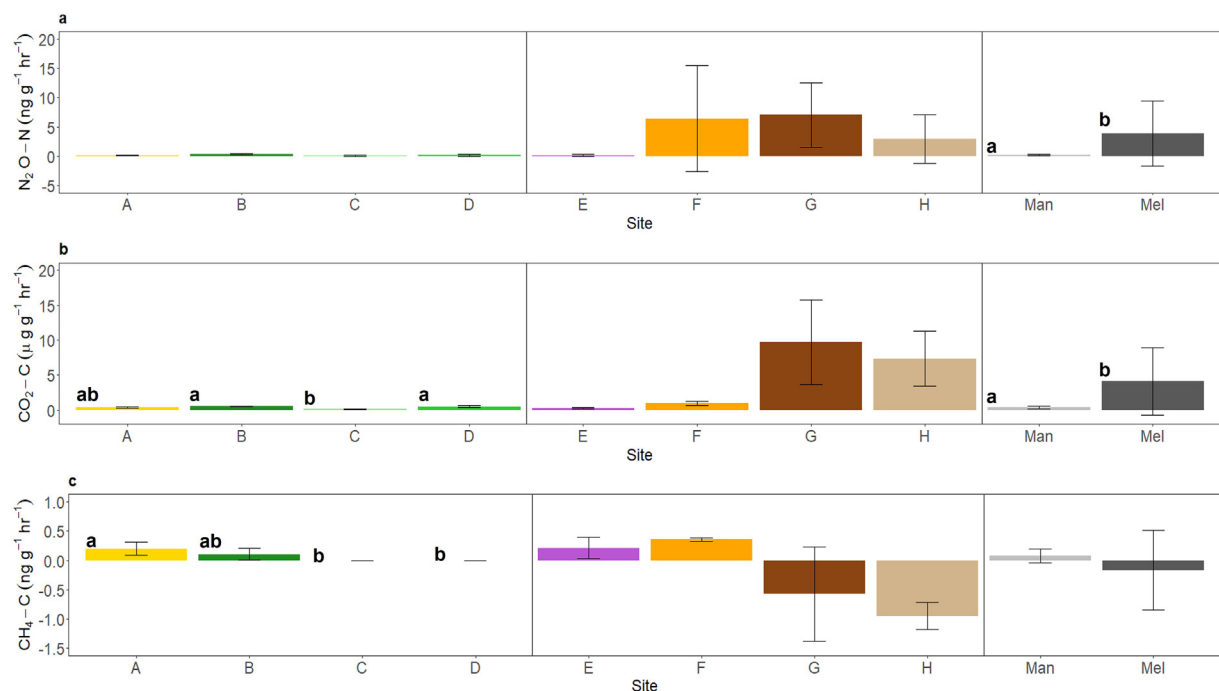


Fig. 4. Average greenhouse gas fluxes (CO_2 , N_2O , CH_4) per site expressed per gram of dry sediment per hour from laboratory incubations spiked with $^{15}\text{N}-\text{NO}_3$. Significantly different pairs are indicated with different letters. Error bars represent one standard deviation from the mean. A. Deforested mangroves, B. 5–7 year old natural mangrove regrowth, C. 8 year old mangrove plantation, D. 15 year old natural mangrove regrowth, E. Melaleuca converted to agriculture, F. Restoring Melaleuca on clay, G. Restoring Melaleuca on peat underlain with clay and H. Mature Melaleuca on deep peat.

N₂O emissions from Melaleuca soils may be further explained by higher soil TC and TN in those soils. However, the potential GHG flux patterns contradicted other measured soil properties such as higher C:N, higher soil moisture and lower nitrate concentration observed in Melaleuca soils. These soil properties typically result in low N₂O and CO₂ emissions, and CH₄ emissions not uptake (Blain et al., 2006; Moore and Knowles, 1989; Weier et al., 1993).

The soil data indicates that Melaleuca soils store more carbon and N than mangrove soils as well as processing less carbon. This may be due to lower availability of labile carbon, despite Melaleucas also showing increased carbon and N cycling (higher potential CO₂ and N₂O emissions). The stocks of carbon and N in Melaleuca soils may be large enough to experience some losses due to processing, while maintaining relatively high soil concentrations, which supports the high carbon and N stocks previously found in Melaleuca ecosystems (Adame et al., 2019a; Tran et al., 2015). Further evidence with respect to carbon is seen in the forested Melaleuca sites, which had $\delta^{13}\text{C}$ values that became heavier with depth, indicating high rates of decomposition (Nadelhoffer and Fry, 1988). Long-term soil carbon and N accumulation with some organic decomposition has been observed previously in Melaleucas and further supports the findings presented here (Adame et al., 2019a). The Melaleuca soils were characterised by higher soil moisture and substrate availability, which increased N and carbon cycling, alongside higher $\delta^{15}\text{N}$, which indicates increased N processing. These soil properties explain the higher potential GHG fluxes, and potential denitrification-derived N₂O observed from the Melaleuca soils. The $\delta^{13}\text{C}$ in the Melaleuca soils was within the range of -28 to -35‰, which indicates uptake of atmospheric CO₂ by plants with C₃ photosynthesis before burial through roots (Boutton et al., 1998). The biogeochemistry here reflects processes found in temperate peatlands where CH₄ emissions are balanced by substantial carbon storage belowground, which may also modulate the relatively high potential N₂O emissions from the Melaleuca soils.

4.2. Comparison of N removal potential to other ecosystems

The potential rate of denitrification measured in the mangrove soils (8.1 ng N g⁻¹ h⁻¹) was within the range of that previously found for mangroves in China and India (5.6 to 1307.3 ng N g⁻¹ h⁻¹) (Fernandes et al., 2010; Fernandes et al., 2016; Fernandes et al., 2013; Xiao et al., 2018; Zhang et al., 2020). The rate was at the low end of this range showing that this mangrove system does not have a high N removal capacity through denitrification. This may be due to the mangrove sites here being drained at low tide or differences in soil texture, whereas other systems may retain more water promoting denitrification. Additionally, experiments were performed under aerobic conditions, which could further decrease denitrification. The potential rates of denitrification in both the mangrove and Melaleuca soils studied here were at the lower end of ranges or lower than rates found in other ecosystems including peatlands in France, Slovenia and Alaska (0.0 to 8208.3 ng N g⁻¹ h⁻¹) (Francez et al., 2000; Kane et al., 2013; Pal et al., 2010), salt marshes (20 to 38.75 ng N g⁻¹ h⁻¹) (Yang et al., 2015), riverine wetlands (168.1 to 232.5 ng N g⁻¹ h⁻¹) (Johnston et al., 2001) and riparian wetlands (24.0 to 604.0 ng N g⁻¹ h⁻¹) (Maître et al., 2005). Adame et al. (2019c) found denitrification rates in Australian Melaleucas similar to those from other tropical and temperate wetlands, therefore, the Melaleuca system studied here also appears to have a low N removal capacity through denitrification. The rates could not be compared to other studies using the same methodology due to unit conversion constraints; therefore, the methodology may also be affecting this comparison.

4.3. Restoration-age chrono sequence - mangrove

N processing varied between restoration sites but was only significantly different with respect to the denitrification product ratio. Potential denitrification-driven N₂ fluxes were much larger than potential denitrification-derived N₂O fluxes, therefore, patterns of potential total

denitrification between sites reflected potential N₂ fluxes. Potential denitrification was highest in the deforested mangrove site and lowest in the areas of natural regrowth, maybe due to increased oxygenation associated with root zones (Chiu and Chou, 1993), but was not significantly different between sites indicating that N removal was unaffected by restoration processes. Potential denitrification-driven ¹⁵N—N₂ fluxes normalised by soil C showed similar patterns to those without normalisation which were significant. The highest potential flux was from site C not A so differences were not fully explained by carbon availability in the sites and there was available labile carbon that was not limiting denitrifying activity.

Potential denitrification-derived fluxes of ¹⁵N—N₂O were also site-dependent and were highest in the youngest natural regrowth (B), where the highest denitrification product ratio was observed, and lowest in the deforested soils (A). This indicates that overall potential denitrification was more complete in the deforested and plantation sites, and less complete in the areas of natural regrowth. Although potential denitrification was close to completion in the mangrove sites as evidenced by the low denitrification product ratios and high potential denitrification-derived N₂ fluxes. The deforested and plantation areas may have been similar to each other due to the relatively low density of trees in the plantation resulting in areas of bare soil.

Potential N₂O emissions decreased from the younger area of natural regrowth to the older area of natural regrowth showing more complete denitrification over time. Although there were some differences in potential GHG emissions between restoration classes all of these potential fluxes were low. Potential N₂O and CO₂ fluxes were highest in both areas of natural regrowth and lowest in the mangrove plantation. Potential CH₄ fluxes were highest from the deforested area and negligible from the plantation and oldest natural regrowth. CH₄ patterns indicate that a reduction in potential CH₄ emissions occurs with increasing forest age. CH₄ flux patterns contradicted previous findings that pneumatophores led to five-fold increases in CH₄ emissions (He et al., 2019), considering sites C and D contained *Sonneratia* and *Avicennia* species, which are known to be the mangrove species with best developed pneumatophores (Spalding, 2001). The differences between sites were significant for CO₂ and CH₄ indicating that potential soil carbon emissions are affected by changes in restoration, although the higher CO₂ in areas of natural regrowth may be explained by higher concentrations of DOC.

N and carbon cycling across the mangrove sites show interesting patterns in relation to age. Potential denitrification was lowest in the natural regrowth sites where the denitrification product ratio, and potential N₂O and CO₂ fluxes were highest. This was further evidenced in the soil data where %N and %C were similar and low between sites. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, however, indicate N and carbon turnover were low in site B and show sites of natural regrowth stored more soil N and carbon. The sites of natural regrowth tended to have higher soil moisture, N and carbon concentrations and C:N, which all facilitate biogeochemical cycling as further evidenced by the high $\delta^{15}\text{N}$ observed in the oldest area of natural regrowth. This is similar to previously observed relationships in mangroves of higher potential GHG fluxes and denitrification in areas of higher N and carbon (Hernández and Junca-Gómez, 2020; Hien et al., 2018a; Shiao et al., 2016). Soil moisture is more complex with both decreases in GHG flux (Cameron et al., 2019) and increases in CH₄ fluxes observed at higher soil moisture or water levels (Hernández and Junca-Gómez, 2020).

Previous studies of the effects of deforestation and restoration on soil biogeochemistry show a variety of responses. Higher and lower CO₂ fluxes in deforested or bare soil compared with mangroves have been observed (Bulmer et al., 2017; Castillo et al., 2017; Hien et al., 2018a). Here, deforested soil had similar potential CO₂ emissions to areas of natural regrowth but higher potential fluxes from the plantation, showing the effect of deforestation is confounded by other environmental factors. Similar CO₂ emissions between mangroves of varying stand age have been observed (Alongi et al., 1998), which is

reflected here with similar potential fluxes from both natural regrowth sites. CH₄ fluxes are generally low in mangroves explaining the low potential fluxes found here across all sites (He et al., 2019; Strangmann et al., 2008; Zheng et al., 2018). These fluxes, however, have been shown to increase with anthropogenic nutrient inputs (Strangmann et al., 2008; Zheng et al., 2018), which may explain higher CH₄ from site B, located along a creek. Mangrove species may affect CH₄ fluxes with presence of pneumatophores decreasing fluxes, which was not observed here, potentially due to other vegetation characteristics such as differing tree age and density (He et al., 2019; Hernández and Junca-Gómez, 2020). Here, deforestation resulted in higher potential CH₄ soil emissions than from the forested sites, which contradicts previously observed lower CH₄ fluxes at deforested sites (Castillo et al., 2017). Mangrove species did not affect potential soil N₂O emissions here, supporting previous findings, however, deforestation has been shown to increase N₂O fluxes, which was not observed here (Castillo et al., 2017; Hernández and Junca-Gómez, 2020). Castillo et al. (2017) investigated various deforested sites, including abandoned aquaculture and salt ponds, coconut plantations and cleared mangroves, which would have affected soil properties and substrate availability beyond the effect of clearing mangroves alone.

4.4. Restoration age/chrono sequence - Melaleuca

The Melaleuca sites covered a gradient of restoration that was reflected in N and carbon processing. Potential total denitrification was highest in the sites with Melaleucas underlain by peat but decreased from the restoring to mature site. This indicates a need for further detailed field studies to track changes in N₂O:N₂ emission ratios with age, under varying soil moisture and reactive N loading rates, especially as these differences were not significant here. This will inform restoration of these critical ecosystems to optimise carbon capture and reduce GHG emissions. Potential denitrification-derived N₂O emissions were highest in the forested Melaleuca sites, which was likely due to the high soil moisture at these sites resulting in high potential rates of denitrification when spiked with nitrate tracer. The denitrification product ratio was lowest in the agricultural site where denitrification-driven ¹⁵N-N₂ was also lowest, due to a relatively small potential rate of denitrification at this site, presumably due to oxygenated soil conditions due to drainage (Ullah and Faulkner, 2006). In the forested Melaleuca sites, the denitrification product ratio decreased from clay to peat systems as potential denitrification-derived N₂ and denitrification rates increased. This indicates that upon restoration, denitrification is more complete and occurs at higher rates. Potential denitrification was fueled by available carbon in the soils of the Melaleuca systems as potential denitrification-derived N₂ normalised by soil carbon was highest from the agricultural site (Ullah and Faulkner, 2006).

Potential emissions of N₂O and CO₂ decreased from restoring to mature peat systems, with potential N₂O emissions also higher in the restoring clay system than the mature peat system. The decrease in potential N₂O emissions is likely from the higher C:N of the mature peat system as a low C:N tends to produce N₂O (Klemetsson et al., 2005). Potential CH₄ fluxes were more complex, and indicated that Melaleuca soils may act as either relatively high CH₄ sources or as CH₄ sinks. The Melaleuca soils converted from sources to sinks once restored to peat systems and the sink increased as the Melaleuca peat system matured. Potential CO₂ emissions were higher from the peat systems than the restoring clay system, and so some soil carbon may be lost on restoration of Melaleucas. The difference in GHGs between sites is likely due to the high carbon content of the peat Melaleuca soils, which would produce CO₂ via aerobic respiration. Additionally, potential GHG fluxes normalised by soil carbon produced potential GHG fluxes relatively lower than those not normalised by soil carbon. No differences in GHG emissions between sites were significant.

Higher potential rates of denitrification, N₂O and CO₂ emissions and CH₄ sinks, therefore, indicate that increased N and carbon turnover

were associated with both the restoring and mature Melaleucas on peat. These sites also had high soil moisture, carbon and N concentrations and C:N, therefore, facilitating nutrient cycling. Conversely, soil δ¹⁵N and δ¹³C were lightest in these Melaleuca peat sites, which indicates less N and carbon turnover.

Further work is required to resolve differences in biogeochemical cycling, including the effect of primary production, between coastal wetlands and restoration gradients. This should include longer-term, in-situ studies and target multiple restoration classes over larger geographical areas. Fully understanding the response of soil biogeochemistry to future global change will enable more effective management strategies and improvements in ecosystem services, which may benefit the local and wider communities.

4.5. Ecosystem service provisions

Both mangrove and Melaleuca forested coastal wetlands showed the potential to remove excess reactive N, which may result from local intensive agricultural and aquacultural practises, or riverine and atmospheric inputs. Removal of excess reactive N is crucial to improve water quality and reduce eutrophication, both of which have large negative effects on local livelihoods and ecosystems. The source of nitrate used in denitrification is important to determine if nitrate is removed from overlying waters or produced locally by nitrification, which in turn affects the ability to influence N pollution (Kristensen et al., 1998).

The mangrove ecosystem showed the potential for more complete denitrification, releasing harmless N₂ into the atmosphere rather than N₂O, which has many negative effects including climate warming (Jaffe, 2000). However, the Melaleuca soils had potentially higher capacity for carbon and N storage (as evidenced by higher soil %C and %N), which may offset the increased potential emissions of N₂O and CO₂ from these soils relative to the mangrove soils.

Mangrove soils may retain their capacity for water quality improvements during degradation and restoration as there was no significant effect of restoration on N removal or potential N₂O fluxes. Although primary production was not measured, denser tree growth in the areas of natural regrowth is expected to uptake more carbon and balance the higher potential CO₂ emissions from the soils in these sites. As restored mangrove systems age carbon sequestration in the soil is likely to increase due to a decrease in potential CH₄ emissions.

Potential N removal via denitrification increased and was more complete as Melaleuca soils converted to peat-dominated systems. This indicates that restoration of Melaleuca wetlands may increase their capacity to reduce nutrient pollution and improve water quality, although differences observed between sites here were not significant. Potential CO₂ emissions from the Melaleuca restoration gradient were complex with higher potential emissions occurring once peat was formed but then decreasing with increased restoration. This, alongside a switch from a potential source to a sink of CH₄ once Melaleucas were restored to peat-dominated systems and the much higher TC of these soils, indicates that carbon sequestration will dominate once Melaleuca systems are fully restored. Again, these differences were not significant and require further investigation.

The mechanistic insights gained through this study highlight the importance of ecosystem restoration for pollution attenuation (e.g. denitrification-driven N₂ flux), carbon sequestration and reduction of GHG emissions. Restoration efforts should continue to focus on increasing wetland area and function, which will benefit local communities with improved water quality and potential for income generated by carbon trading.

5. Conclusions

Mangrove and Melaleuca forest soils provide crucial potential for the removal of reactive N through denitrification. Of the two ecosystems we studied, mangroves removed reactive N through denitrification while

maintaining low potential N₂O emissions. Melaleucas also attenuated pollutants and stored large quantities of carbon in their soils but with relatively higher potential N₂O emissions due to incomplete potential denitrification.

Disturbance and subsequent recovery or restoration of these coastal wetland ecosystems did not have a significant effect on N removal and potential GHG fluxes in the Melaleuca wetland. Conversely, in the mangrove wetland a significant effect was observed with higher denitrification product ratios in younger mangrove trees, lower potential CO₂ fluxes from the plantation and a decrease in potential CH₄ fluxes once mangroves were restored and increased in age. Coastal wetlands are able to reduce reactive nitrogen pollution to improve water quality, with mangroves in particular returning harmless N₂ to the atmosphere through complete denitrification. Continued restoration of coastal wetlands is crucial to reduce nitrogen pollution from coastal zones and reduce GHG emissions, therefore, increasing the negative climate feedback of these systems through C sequestration.

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CRediT authorship contribution statement

S.C-W: Conceptualization, Investigation, Writing, Visualisation **A.N:** Investigation **M.N:** Conceptualization, Writing, Supervision **M.W:** Investigation **A.T:** Investigation **H.L:** Conceptualization, Writing, Supervision **F.S:** Writing **S.K:** Conceptualization, Writing, Supervision, Funding Acquisition **N.K:** Conceptualization, Writing, Supervision, Funding Acquisition **N.N:** Conceptualization **R.L.H:** Investigation **S.U:** Conceptualization, Writing, Supervision, Funding Acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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