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Effects of a nutrient enrichment pulse on blue carbon ecosystems

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ABSTRACT

Coastal ecosystems are under increasing pressure from land-derived eutrophication in most developed coastlines worldwide. Here, we tested for 277 days the effects of a nutrient pulse on blue carbon retention and cycling within an Australian temperate coastal system. After 56 days of exposure, saltmarsh and mangrove plots subject to a high-nutrient treatment (~20 g N m^{-2} yr^{-1} and ~2 g P m^{-2} yr^{-1}) had ~23% lower superficial soil carbon stocks. Mangrove plots also experienced a ~33% reduction in the microbe Amplicon Sequence Variant richness and a shift in community structure linked to elevated ammonium concentrations. Live plant cover, tea litter decomposition, and soil carbon fluxes (CO_2 and CH_4) were not significantly affected by the pulse. Before the end of the experiment, soil carbon- and nitrogen-cycling had returned to control levels, highlighting the significant but short-lived impact that a nutrient pulse can have on the carbon sink capacity of coastal wetlands.

1. Introduction

Coastal vegetated ecosystems, such as mangroves, saltmarshes, and seagrass meadows (i.e., blue carbon ecosystems), are some of the world's most efficient natural carbon sinks (Serrano et al., 2019). They can sequester atmospheric carbon 30–50 times faster than terrestrial forests and store organic carbon in the sediments for millennial timescales (Duarte et al., 2013; Serrano et al., 2019). Given the increasing need to draw down atmospheric carbon emissions and mitigate climate change, coastal ecosystems are valuable assets capturing carbon, but also protecting the coastline, boosting fish production, and enhancing natural biodiversity (Mcleod et al., 2011).

Coastal wetlands, along with the ecosystem services they provide, are under threat by human and climatic change impacts. The nutrient overload of coastal waters is one of the major impacts on coastal ecosystems resulting from human activities in the Anthropocene (Smith and Schindler, 2009). Nutrient concentrations are elevated around much of the world's developed coastline due to the runoff of fertilisers from agricultural croplands, the discharges of aquaculture farms, and the release of untreated urban wastewater into coastal watersheds (Howarth and Marino, 2006). For instance, in Australia's Great Barrier Reef, the mean annual load of nitrogen and phosphorus to the coastline has increased 5.7- and 8.9-fold since European settlement, respectively (Kroon et al., 2012). These values may be significantly higher during wet years or extreme weather events (e.g., cyclones or monsoons) when intense rainfall can enhance atmospheric deposition, soil erosion, water infiltration, and the runoff of nutrients into coastal ecosystems (Zhang et al., 2009; Yang et al., 2012; Wu et al., 2018). Currently, synthetic fertilisers are one of the main drivers of coastal eutrophication worldwide given the high concentration of reactive nitrogen they contain and their increased application in agricultural croplands (Lu and Tian, 2017; Food and Nations, 2019).

Eutrophication's net effect on coastal wetlands is a highly debated topic that remains far from clear (Macreadie et al., 2017). This uncertainty is mostly driven by the simultaneous effect that nutrients can have on soil carbon inputs (i.e., enhanced primary production quality and quantity) and outputs (i.e., enhanced in microbial decomposition of organic matter). Some studies suggest the addition of nutrients can enhance the carbon storage capacity of coastal wetlands by increasing the production of aboveground and belowground plant biomass that results in the accumulation of sediments (Morris et al., 2002; Vivanco et al., 2015; Pastore et al., 2017). Others indicate a net neutral effect due to the lack of measurable responses or because eutrophication simultaneously stimulates both the production and decomposition of organic matter (Feller et al., 1999; Keuskamp et al., 2015b; Simpson et al., 2020). Conversely, a growing number of manipulations report that nutrient enrichment can reduce the carbon drawdown of coastal wetlands by boosting the microbial decomposition of organic matter (Morris and Bradley, 1999; Wigand et al., 2009; Deegan et al., 2012; Lovelock et al., 2014; Liu et al., 2017; Bulseco et al., 2019; Liu et al., 2020),

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enhancing the mortality of plants and roots (Lovelock et al., 2009), or triggering changes in plant species composition (Langley et al., 2013).

The nutrient-driven transformation of coastal wetlands to carbon sources is often driven by changes in the microbiome (community structure and/or activity) and its cycling of both carbon and nitrogen within the system (Holguin et al., 2001). In some coastal zones, microbe decomposers are not nitrogen-limited, so additional enrichment has a weaker effect on organic matter turnover (Feller et al., 2009; Keuskamp et al., 2015b). However, considering that many pristine coastal ecosystems are nutrient-depleted (Ryther and Dunstan, 1971; Howarth and Marino, 2006), an increase in reactive nitrogen (nitrate; NO_3^-) can stimulate the microorganisms and the ecosystem functions they mediate (Wigand et al., 2009; Deegan et al., 2012; Langley et al., 2013; Kearns et al., 2016). For example, a long term ecosystem-level experiment in saltmarshes revealed microbial respiration can nearly double when using nitrate to fuel the aerobic decomposition of organic matter (Deegan et al., 2012). Further, Bulseco et al. (2019) showed that nitrogen fertilisation can shift microbial community structure, decreasing alpha diversity and selecting for taxa that reduce nitrate and can easily oxidise complex forms of organic matter into more bioavailable forms. Although coastal wetlands can play a key role in nitrogen removal (Valiela and Cole, 2002; Zhao et al., 2019), these studies suggest nitrogen loading can threaten their ability to serve as carbon sinks by lowering the stability and burial of organic-rich sediments. Considering that higher microbial organic matter turnover can limit the ability of blue carbon ecosystems to serve as long-term carbon stores and, hence, mitigate climate change (Macreadie et al., 2017; Bulseco et al., 2019), it is critical to identify how nutrient addition affects multiple facets of the carbon cycle, including living biomass, greenhouse gas emissions, decomposition, and the microbiome.

The lack of consensus from nutrient-enrichment experiments is underpinned by the complex and multivariate effects of eutrophication on plants and microbes. As such, the nature and magnitude of the net response is known to vary according to the biogeochemical conditions of the environment (vegetation zone; season; latitude; nature of nutrient-limitation; Feller et al., 1999, Feller et al., 2009, Moseman-Valtierra et al., 2011, Keuskamp et al., 2015a, Hayes et al., 2017) and the characteristics of the nutrient load (nutrient type, form, and concentration; enrichment duration; Vivanco et al., 2015, Crowther et al., 2019). For example, in Australia the root decomposition of *Avicennia marina* mangroves from the scrub zone was enhanced by nutrient addition, but not on the taller mangroves from the fringe zone (Hayes et al., 2017).

Despite the need to better understand the response of coastal ecosystems to eutrophication, many experimental choices have hindered obtaining a full picture of nutrient-driven ecosystem effects. Most nutrient-enrichment studies have tested the effects of high levels of eutrophication (>100 g N m^{-2} yr^{-1}; e.g., Morris and Bradley, 1999, Hayes et al., 2017) without considering the effect of lower, but more common, levels of land-derived nitrogen present in the worlds' coastlines (<30 g N m^{-2} yr^{-1}; Valiela and Cole, 2002). For example, as little as 1.4 g N m^{-2} of nitrogen fertilisation was enough to shift temperate marshes from N_2O sinks to sources (Moseman-Valtierra et al., 2011). Further, research has greatly focussed on long-term or chronic exposures to nitrogen loads (e.g., Deegan et al., 2012; Keuskamp et al., 2015a; Keuskamp et al., 2015b), with less emphasis on episodic nutrient inputs (e.g., those driven by rare flooding events) that can highlight the ecosystem's resilience and recovery potential following a sporadic disturbance event. Given that single low-nutrient pulses have been largely understudied, it is unclear the magnitude of their environmental repercussions and the ability of coastal ecosystems to recover.

In this study, we experimentally tested the effects of nutrient enrichment on blue carbon soils and cycling within a temperate mangrove and saltmarsh ecosystem in Australia. Specifically, we examined the effect of a fertiliser pulse on blue carbon by monitoring the response of *superficial soil carbon stocks*, *litter decomposition*, *greenhouse gas fluxes*, *microbial community composition*, and *plant cover* over the course of nine months. We predicted that nutrient enrichment would increase plant cover, but also enhance carbon turnover in the soil, thereby leading to a net weakening of blue carbon capacity. Our results will help to manage nutrient loading (especially from agriculture run-offs) in coastal areas with rich blue carbon stocks.

2. Materials and methods

2.1. Study site

Towra Point Nature Reserve is situated within Botany Bay in New South Wales, Australia (Fig. 1a). The site consists of 380 ha of coastal vegetated ecosystems including ~40% of the mangrove forests and ~60% of the saltmarsh communities in the Sydney region (Dyall et al., 2004). *Avicennia marina* is the dominant mangrove species at the site, with individuals of the river mangrove *Aegiceras corniculatum* present in low numbers. The saltmarsh community in the low intertidal zone is dominated by the marine couch *Sporobolus virginicus* and the beaded glasswort *Sarcocornia quinqueflora*, while the rush *Juncus kraussii* is highly abundant in the higher grounds (Clarke and Hannon, 1967; Adam et al., 1988). The mangrove and saltmarsh communities have a sharp zonation extending into two clear bands along the coastline. Coastal wetlands in the Botany Bay estuary are spatially dynamic with mangroves rapidly encroaching into the saltmarsh during the last 70 years (Kelleway et al., 2016).

Within the southern shoreline of Sydney's Botany Bay, Towra Point receives from the catchments a nutrient load of ~11,586 kg TN yr^{-1} and ~1333 kg TP yr^{-1} mainly originating from urban run-off (SMCMA, 2011). Towra Point has a mean salinity of 32 in nearby open water (Geoscience Australia, 2015) and water infiltration rates increasing progressively with distance from the coast (Clarke and Hannon, 1967; Adam et al., 1988). Superficial soils have different grain sizes across ecosystems, with mangroves having coarser size fractions (47% sand, 47% silt) than saltmarsh soils (silt and clay >70%; Palacios & Kelleway unpublished data). Soil depth microprofiles of oxygen also show a pronounced distinction between ecosystems, with oxygen only reaching a penetration depth of 0.8 mm in mangroves, but down to 5.3 mm in saltmarsh (Brodersen et al., 2019). Finally, the mangrove soil acidifies more rapidly than the saltmarsh, with pH dropping from 8.2 (at the surface) to 6.5 at 4.5 mm depth in the mangroves, but at 17 mm depth in the saltmarsh (Brodersen et al., 2019). During the study, air temperature 15 cm above the soil surface ranged between 18 and 33 °C in the mangroves and between 21 and 39 °C in the saltmarsh (recorded with an Ultra-portable Greenhouse Gas Analyser; see Soil carbon fluxes below).

2.2. Nutrient experiment

Changes in superficial soil carbon parameters were compared among six experimental levels crossed with two ecosystem types (mangrove, saltmarsh) and three nutrient treatments (high-nutrient level, low-nutrient level, control without nutrient addition). For each ecosystem type, fifteen replicate 1 m^2 plots were constructed in a single row parallel to the coastline. Three consecutive plots were grouped into an experimental 'block'. Within each block, plots were systematically assigned to one of the three nutrient treatments and situated 2 m apart. Distance between blocks was approximately 5 m. A total of 30 plots were constructed, with five replicates plots being prepared for each of the six experimental levels (Fig. 1b). Plots within the mangrove ecosystem did not include any seedlings, saplings, nor trees to facilitate all the manipulations and monitoring required in the plot.

Nutrient enrichment was achieved by adding a single dose of Osmocote all-purpose landscape fertiliser to the surface of the experimental plots [Scotts™: (N)21.2, (P)1.9, (K)5.7]. The low-nutrient plots received 45.25 ± 1.6 g m^{-2} yr^{-1} (mean ± SE) of the granular fertiliser, equivalent to 9.5 ± 0.3 g N m^{-2} yr^{-1} and 0.85 ± 0.03 g P m^{-2} yr^{-1}. Instead, high-nutrient plots were exposed to 99.3 ± 2.1 g m^{-2} yr^{-1} of

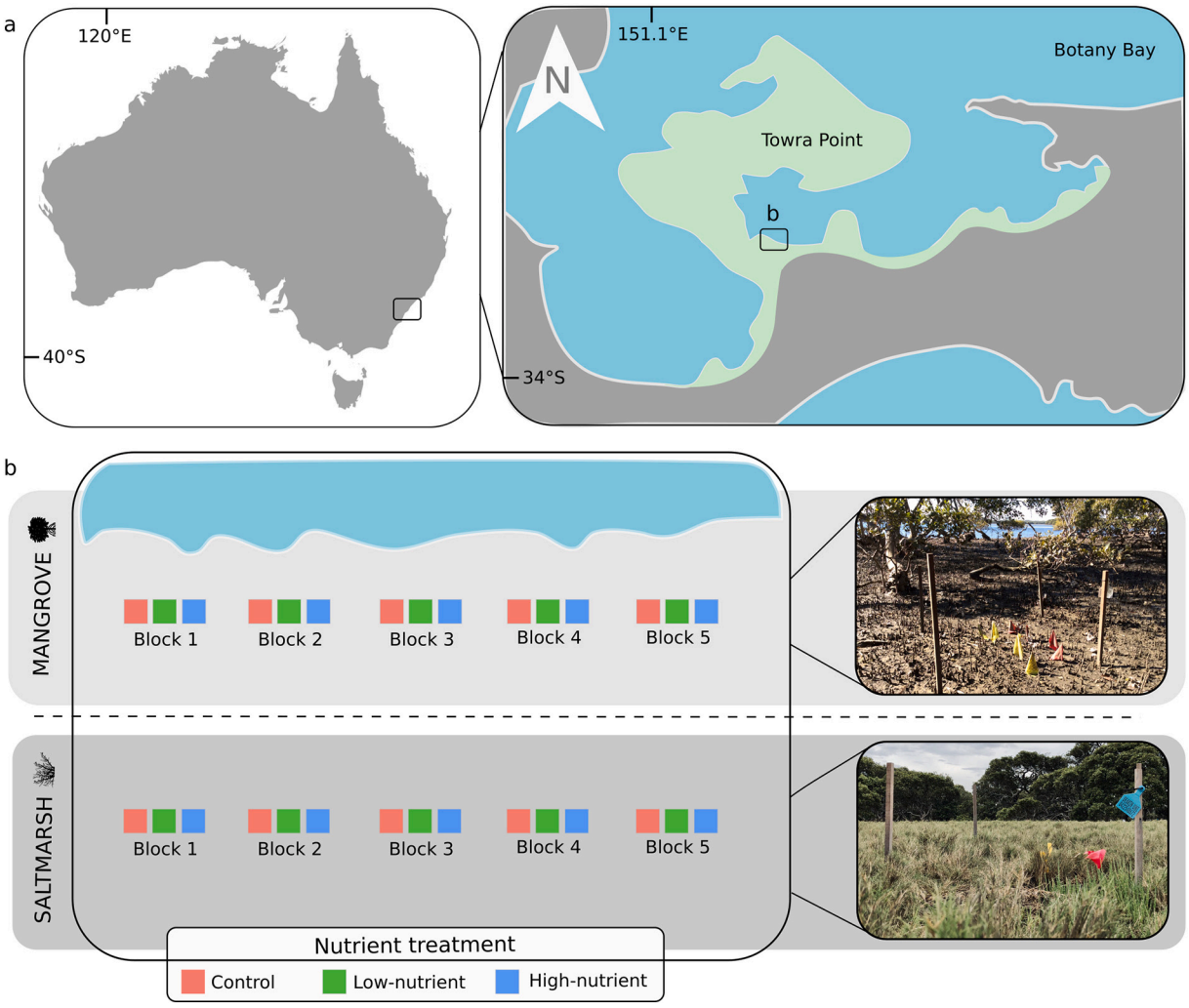

Fig. 1. (a) Setup of the nutrient experiment conducted in the coastal ecosystems of Towra Point, Botany Bay, Australia. Green shading in the map denotes the coastal wetlands of Towra Point Nature Reserve. (b) For each ecosystem type (mangrove, saltmarsh), fifteen replicate 1 m^2 plots were constructed in a single row parallel to the coastline. Three consecutive plots were grouped into an experimental 'block'. Within each block, plots were systematically assigned to one of the three nutrient treatments (control, low-nutrient load, high-nutrient load). A total of 30 plots were constructed. Nutrient enrichment was achieved by adding a single dose of Osmocote all-purpose landscape fertiliser to the surface of the experimental plots. Photos taken at low tide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertiliser, equivalent to 21.06 ± 0.4 g N m^{-2} yr^{-1} and 1.89 ± 0.04 g P m^{-2} yr^{-1}. These values are within the fertiliser input range of 5–30 g N m^{-2} yr^{-1} and 0.5–1.5 g P m^{-2} yr^{-1} reported in developed croplands (Lu and Tian, 2017), but on the lower end of the range of experimental nitrogen manipulations conducted on coastal wetlands (10–400 g N m^{-2} yr^{-1}; Vivanco et al., 2015). The highest nitrogen loads reported on natural coastal wetlands are ~100 g N m^{-2} yr^{-1} (Valiela and Cole, 2002).

Research commenced on June 15th 2018 (T0; addition of the fertiliser to the plots) and was monitored on August 10th 2018 (T1; 56 days), September 27th 2018 (T2; 104 days), November 23rd 2018 (T3; 161 days) and March 19th 2019 (T4; 277 days). Sampling at T0 and T1 took place during the austral winter, T2 in spring, and T3 and T4 at the beginning and end of summer, respectively. All sampling occurred during low tide and daylight hours. Data was collected with the help of the #BlueCarbonArmy; a citizen science program ran by Deakin University's Blue Carbon Lab, HSBC and Earthwatch Australia. Participants provided support in the collection of soil samples (for carbon stock and microbial analysis), the survey of vegetation, and the deployment or retrieval of tea bags (see next section).

2.3. Porewater nutrient concentration

At T1 and T4, superficial soil samples (<2 cm deep) were collected from three experimental blocks (Block #1, #3, #5) on each ecosystem and kept in cool conditions (4 °C) until analysis in the laboratory. Filtered pore water samples were analysed for nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), and phosphate content (PO_4^{3-}) following Rice et al. (2017).

2.4. Plant cover

Percentage cover of aboveground plant biomass was measured on each experimental plot using 25 × 25 cm quadrats. Surveys in saltmarsh plots took into account the percent cover of saltmarsh species (i.e., *Sporobolus virginicus*, *Sarcocornia quinqueflora*), while in the mangrove plots it included the cover of *Avicennia marina*'s pneumatophores, as none of the mangrove plots included mangrove trees. Surveys were undertaken at T1, T2, T3, and T4.

2.5. Soil carbon and nitrogen stocks

Superficial soil cores were collected from the plots using 50 mL plastic syringes (2.6 cm diameter). The protocol involved removing any above-ground vegetation, positioning the syringe perpendicular to the soil, and slowly pushing the syringe 4.5 cm deep. The suction generated by the syringe allowed the removal of a soil sample. Soil cores were transported to the laboratory where they were dried in a fan-forced oven (60 °C for one week), weighed to estimate dry bulk density (g cm^{-3}), and ground into powder using a Retsch RM200 Electric Mortar Grinder. Soil samples included both sediments and belowground live biomass within the core (Howard et al., 2014). The proportion of organic carbon and nitrogen was analysed using a EuroVector MicroElemental C:N Analyser. All the samples tested negative for the presence of inorganic carbon as per the effervescence test described by Schlacher and Connolly (2014). Statistical analyses were run on log carbon stocks (tonnes ha^{-1}), *log* nitrogen stocks (tonnes ha^{-1}) and *log* C:N ratio [((C%*1000)/12)/((N %*1000)/14)]. Sampling was undertaken at T1, T2, T3, and T4.

2.6. Soil carbon fluxes

Greenhouse gas fluxes (CH_4 and CO_2) were measured at low tide using an Ultra-portable Greenhouse Gas Analyser (UGGA; Los Gatos Research, Model 915- 0011). Carbon dioxide and methane (parts per million or ppm) were measured using a chamber (0.04 m^3 volume, 0.07 m^2 surface area), with two air hoses connected to the roof (influx and efflux) created a closed circuit with a cavity enhanced laser absorption based UGGA. Air temperature and greenhouse gas measurements were logged every 5 s during a 5 min contact period, and the diffusive gas flux from the soil surface to the atmosphere (mg m^{-2} d^{-1}) (***F***) was calculated using equation:

$$\boldsymbol{F} = \left[s\left(\frac{\boldsymbol{V}}{\boldsymbol{R}\times\boldsymbol{T}\times\boldsymbol{A}}\right)\right]\boldsymbol{t} \quad (1)$$

where ***s*** represents the slope of change in chamber gas concentrations over time (ppm/s), ***V*** is the chamber volume (m^3), ***R*** is the universal gas constant (8.2 × 10–5 m^3 atm K^{-1} mol^{-1}), ***T*** is the temperature in the chamber (K), ***A*** is the surface area of the chamber (m^2), and ***t*** is the conversion from seconds to day and μmol to mmol (Lambert and Fréchette, 2005). Statistical analyses were run on log-transformed CO_2 and CH_4 (mg m^{-2} d^{-1}). Sampling was undertaken at T1, T2, T3, and T4.

2.7. Litter decomposition

Using a modified tea bag index protocol (Keuskamp et al., 2013), we examined litter decomposition rate by monitoring the biomass loss of standardised litters: green tea (Lipton; EAN 87 22700 05552 5) and rooibos tea (Lipton; EAN 87 22700 18843 8). The green tea contains a higher proportion of water soluble compounds (simple sugars and phenolics), while the rooibos tea consists of a higher proportion of acid insoluble compounds (e.g., lignin; Keuskamp et al., 2013). At T0, eight pre-weighted tea bags were buried on each plot (4 green and 4 rooibos) at approximately 10 cm depth using spades. At each of the subsequent monitoring times, two tea bags per plot were manually retrieved (1 green and 1 rooibos). Tea bags were processed by removing all attached soil (via a distilled water rinse), drying in a fan-forced oven (60 °C for 3 days), and re-weighing. The proportion of mass remaining at each time point was used to calculate an exponential decay rate for each tea type according to each Ecosystem*Treatment combination by fitting a model with a fix exponential decay, as:

$$\boldsymbol{proportion}remaining \sim \boldsymbol{e}^{-\boldsymbol{k}\times\boldsymbol{time}} \quad (2)$$

where ***time*** is the number of days since deployment and ***k*** is the rate of exponential decay in proportion d^{-1} (Wieder and Lang, 1982), using least-square techniques (function nls in R).

2.8. Microbial community

In alignment with the sampling of porewater nutrient concentration, we collected surface soil samples for microbial analyses at T1 and T4 to capture the early and later communities shifts. Sampling was carried out in Blocks #1, #3, #5 of each ecosystem by inserting 3 mL plastic syringes (1 cm diameter) in the top 1 cm of wetland soil. Soil samples were kept in 1 mL of RNA later solution (Malmstrom, 2015) and transported to the laboratory for storage at −80 °C until processing. Sediments (0.20–0.26 g) of soil were extracted using the Qiagen DNA PowerSoil extraction kit. The gDNA was normalised to 5 ng μL^{-1} before running triplicate PCRs using 16S rRNA modified V4 515f–806r primers (Walters et al., 2016) and the following PCR methods: 94 °C for 3 min, 25 cycles [94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s] and 72 °C for 5 min. The PCR products were pooled and cleaned using AmPure beads, before indexing and sequencing on the Illumina MiSeq. Bioinformatics was performed using QIIME2 v2019.4 and v2019.7 with DADA2 pipeline (Callahan et al., 2016). After normalising to 42,000 reads, alpha diversity (Amplicon sequence variants -ASV- richness, Shannon Index, Pielou Evenness) and beta diversity (weighted UniFrac distance matrix) were calculated. ASVs were classified using the Silva 99% 515-806 v 132 database.

2.9. Data analyses

We used a split-plot design to quantify the effects of acute nutrient enrichment on the plant cover and soil carbon of saltmarsh and mangrove ecosystems. Linear mixed effect models (LMM) with 'Ecosystem', 'Treatment', 'Time', and all 2- and 3-way interactions as fixed factors and 'Block' as a random effect were run independently for each response variable: superficial soil carbon stock, soil C:N ratio, CO_2 flux, CH_4 flux, and microbe alpha diversity. Decay rates (k) and proportion mass remaining at T2 and T4 from green and rooibos tea decomposition were analysed using 'Ecosystem', 'Treatment', and their interaction as fixed factors. All variables satisfied the assumptions of normality and homoscedasticity from parametric tests. LMM and exponential decay rate analyses were performed in R version 3.6.1 (R Core Team, 2020).

Multivariate permutational analyses (PERMANOVA) were used to analyse beta diversity at using "Ecosystem", "Treatment", "Time" and all 2- and 3-way interactions as fixed factors, and "Block" as a random factor for the soil microbiome communities. A SIMPER analysis on the 0.1% filtered taxonomic table was used to identify which taxonomic groups were driving the significant differences. Data were visualised with nMDS plots. The potential links between environmental parameters (soil porewater nutrients, soil carbon, soil C:N ratios, GHG flux and air temperature recorded by the UGGA) and the community beta diversity were tested with Distance-based linear modelling (DistLM), using BEST selection procedure and AIC criterion, and visualising with dbRDA. A draftsman plot showed that there were no significant co-correlations over 0.8 for the variables. An alpha of 0.05 was used for all analyses. In cases where permutation tests had <200 permutations, a Monte Carlo correction was used [P(MC)]. Software PRIMER + Permanova (v7; Anderson et al., 2008) were used for statistical analyses.

3. Results

3.1. Porewater nutrient concentration

Fifty-six days after the start of the experiment (T1), mangrove and saltmarsh plots that received high-nutrient loads had significantly higher total nitrogen (ammonia+nitrate+nitrite) and phosphorous concentrations than control (N: $t_{15} = -2.275$, $p = 0.038$; P: $t_{15} = -2.532$, $p = 0.023$) and low-nutrient plots (N: $t_{15} = -2.176$, $p = 0.046$; P: $t_{15} = -2.614$, $p = 0.02$; Fig. 2). These nutrient effects had disappeared after 277 days (T4), when the nitrogen and phosphorous concentrations

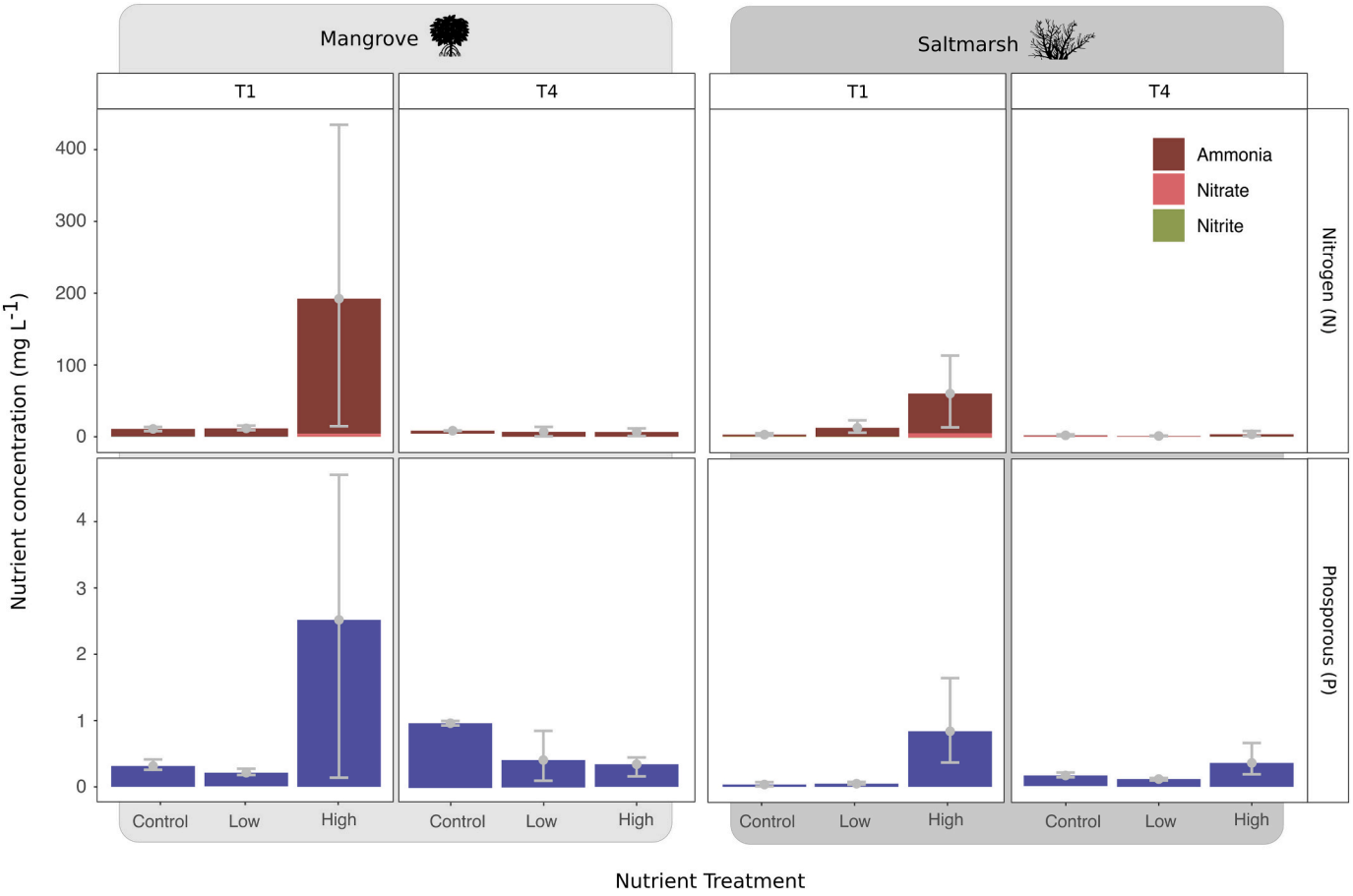

Fig. 2. Soil porewater concentration of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3) and phosphate (PO_4^{3-}) for the three experimental treatments (control, low-, high-nutrient loads) and two ecosystems (mangroves, saltmarsh). Nutrient concentrations were measured at 56 days (T1) and 277 days (T4) after the addition of nutrients. Values denote mean ± 95% confidence intervals.

from high- and low-nutrient plots were similar to the controls ($p > 0.05$; Fig. 2). Although we applied the same nutrient treatments to both coastal ecosystems (see Materials and methods, Nutrient experiment), after 56 days the nutrient retention differed between them. High-nutrient plots in the mangroves seemed to reach higher values than high-nutrient plots in the saltmarsh ($p > 0.05$; Fig. 2).

3.2. Plant cover

Aboveground plant cover was not significantly altered by the nutrient treatments in neither mangrove nor saltmarsh plots ($p > 0.05$; Fig. S1). All saltmarsh plots maintained a 50–70% cover independent of the treatment or sampling time. Similarly, mangrove plots had a consistent 15–30% pneumatophore cover throughout the experiment.

3.3. Soil carbon stocks, nitrogen stocks, and C:N ratio

Nutrient enrichment had a significant effect on surface soil carbon stock (Treatment, $\chi^2 = 6.51$, Df = 2, $p = 0.038$) and C:N ratio (Treatment, $\chi^2 = 8.54$, Df = 2, $p = 0.013$), with plots that received high-nutrient loads having approximately 23% lower carbon stock and 14% lower C:N ratio than control plots after 56 days of exposure (T1; Fig. 3a, b,e,f). Soil nitrogen stocks did not differ between nutrient treatments (T1; Treatment, $\chi^2 = 1.47$, Df = 2, $p > 0.05$; Fig. 3c,d), suggesting changes in the C:N ratio were mainly driven by the reduction in organic carbon. The effect of the nutrient overload was not detected at subsequent sampling times (T2–T4) when all experimental plots recorded relatively similar values for soil carbon stock, nitrogen stock, and C:N ratio (Figs. S2–S4).

Surface soil carbon stocks were relatively similar in saltmarsh and mangrove ecosystems with all plots holding between 10 and 20 tonnes ha^{-1} (T1; Ecosystem, $\chi^2 = 1.39$, Df = 2, $p = 0.23$). However, saltmarsh plots had significantly higher soil nitrogen stocks (T1; Ecosystem; $\chi^2 = 23.85$, Df = 2, $p < 0.001$; Fig. 3c,d) and therefore significantly lower C:N ratios than mangrove plots (T1; Ecosystem, $\chi^2 = 70.91$, Df = 2, $p < 0.001$; Fig. 3e,f). These patterns between ecosystems remained consistent throughout the entire experiment (T2–T4; Figs. S2–S4).

3.4. Soil carbon fluxes

Soil carbon fluxes (CO_2 and CH_4) were not significantly affected by the nutrient treatments after 56 days of exposure (T1; Treatment, CO_2, $\chi^2 = 1.37$, Df = 2, $p = 0.5$; CH_4, $\chi^2 = 0.12$, Df = 2, $p = 0.93$; Fig. 3g–j), nor at subsequent sampling times (T2–T4; Figs. S5–S6). Mangrove and saltmarsh plots had similar CO_2 emissions of 4,000–10,000 mg m^{-2} d^{-1}, but CH_4 fluxes in the saltmarsh were significantly lower than those from mangroves (T1; Ecosystem; $\chi^2 = 13.41$, Df = 2, $p < 0.001$). Soil carbon fluxes significantly differed between sampling times (Time; CO_2, $\chi^2 = 8.14$, Df = 3, $p = 0.042$; CH_4, $\chi^2 = 24.67$, Df = 3, $p < 0.001$), with relatively higher values recorded during the austral summer months (T3 = November 23rd 2018 and T4 = March 19th 2019; Fig. S5–S6).

3.5. Litter decomposition

There was no effect of nutrients on the decay rates of neither rooibos ($k = 0.0038$ d^{-1}; Treatment, $F_{2,26} = 1.23$, $p = 0.31$) nor green tea (k =

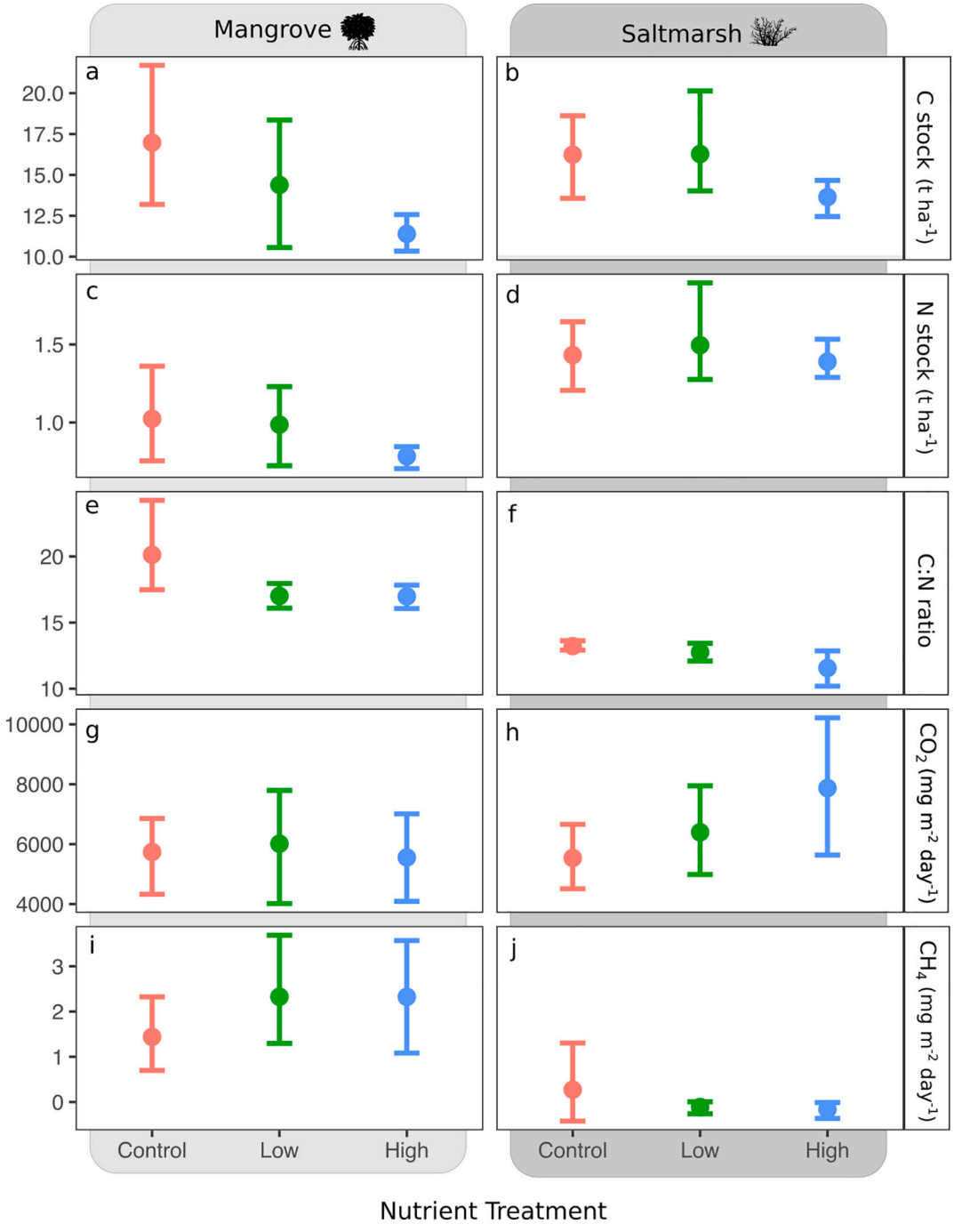

Fig. 3. Soil stocks and fluxes of mangrove (a,c,e,g,i) and saltmarsh plots (b,d,f,h,j) exposed to either control (red bars), low- (green bars) or high-nutrient treatments (blue bars). Results for soil organic carbon stocks (t ha^{-1} ; a,b), soil nitrogen stocks (t ha^{-1}; c,d), C:N ratio (e,f), CO_2 fluxes (mg m^{-2} day^{-1}; g,h), and CH_4 fluxes (mg m^{-2} day^{-1}; i,j) taken after 56 days (T1) of nutrient addition. Values denote mean ± 95% confidence intervals. Results for subsequent monitoring times available on Supplementary Materials (Figs. S2–S6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.0177 d^{-1}; Treatment, $F_{2,26} = 0.81$, $p = 0.45$; Fig. 4). While not statistically significant, high-nutrient plots systematically recorded slightly faster decay rates (by only 0.02–0.08%; Table S1) for each combination of ecosystem type and tea type, possibly suggesting a trend of nutrient enrichment accelerating litter decay. Finally, there were no significant differences in the mass remaining at T2 or T4 for any of the teas across nutrient treatments.

The decay rate of rooibos tea was significantly slower than the decay rate of green tea (Fig. 4) and this difference was greater in the mangrove ecosystem than in the saltmarsh (Ecosystem*Tea; $F_{1,56} = 11.8$, $p = 0.001$; Fig. 4, see Table S1 for decay rates). Throughout the incubation period, the decomposition of green tea remained relatively similar in both ecosystems, while that of the rooibos tea did not. During the first 100 days of the experiment (T2), the mass loss of rooibos tea was significantly higher in the mangrove plots than in the saltmarsh (36% vs 33% mass lost, respectively, $F_{1,25} = 5.25$, $p = 0.03$; Fig. 4). However, after this initial period of leaching (passive loss of soluble cell contents) the decomposition process was more rapid in the saltmarsh plots. At the end of the experiment (T4, 277 days), the mass loss of rooibos tea was 12% higher in the saltmarsh compared to the mangrove plots ($F_{1,47} = 33.51$ $p < 0.001$, Fig. 4).

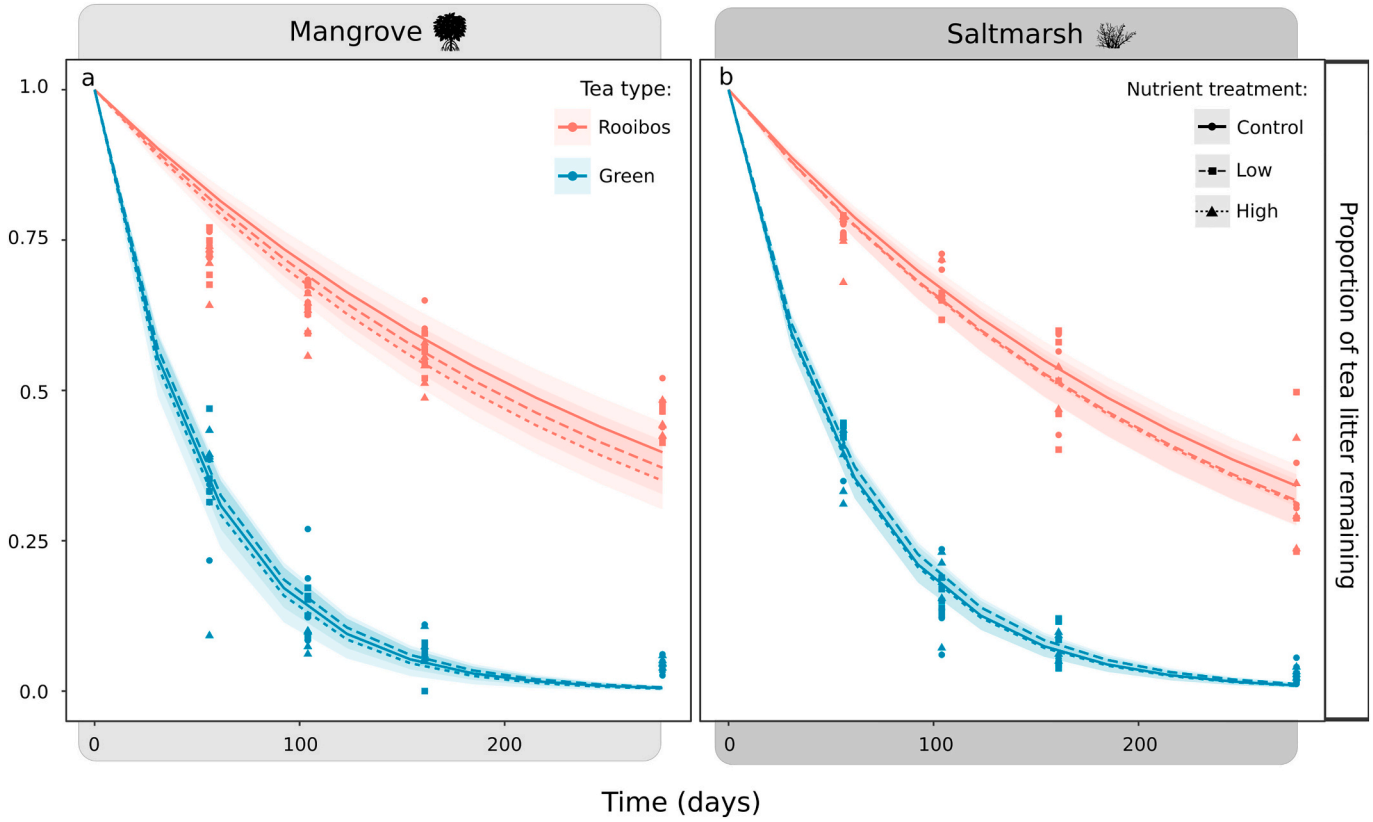

Fig. 4. Exponential decay model predictions (±95% CI) for changes in the proportion of green and rooibos tea in (a) mangrove and (b) saltmarsh plots across 277 days of incubation (T4). Plots were exposed to either control (continuous line), low- (dashed line) or high-nutrient treatments (dotted line). N = 240 tea bags (five tea bags per combination of Ecosystem, Treatment, Tea and Time). In this study, early decomposition is considered within the first 100 days of incubation.

3.6. Microbial community

The nutrient enrichment pulse mainly affected the microbial community from the mangrove ecosystem (Fig. 5). Mangrove plots exposed for 56 days to low and high-nutrient loads had 17.5% and 33.3% lower ASV richness than control plots, respectively (Ecosystem*Treatment at T1; $p < 0.001$). These plots also had lower microbe Shannon diversity, but this effect was weaker (<5% diversity loss; Ecosystem*Treatment at T1; $p = 0.06$). At the end of the experiment the effect of the nutrient addition on microbe alpha diversity (ASV richness and Shannon diversity) had mostly disappeared and there were no significant differences between control and fertilised plots (T4; 277 days after nutrient addition; Fig. S7).

The surface microbiomes of both blue carbon ecosystems were characterised by high relative abundances of Proteobacteria (up to 50%), consisting of Gamma-, Delta- and Alphaproteobacteria (Fig. S8). Soils were also abundant in Bacteroidia and Anaerolineae groups, with all samples containing 5–10% relative abundance of the archaea Nitrosophaeria. Most soil samples had algal reads (>2% Cyanobacteria) except those from the saltmarsh plots collected at T4 (Fig. S8). The beta diversity analysis indicated an Ecosystem*Treatment interaction (Psuedo-F = 2.951, P(perm) = 0.037; nMDS in Fig. S9). SIMPER analysis revealed that the highest average dissimilarity was found between the control and high-nutrient treatments within the saltmarsh (38.4%) and mangrove ecosystems (29.7%; Table S2). Within the saltmarsh soils, the high-nutrient plots had higher relative abundances of a halophilic archaeon *Halogranum* ASV, two putative methylotrophic Methylomirabilaceae ASVs, a *Nitrosopumilus* ASV and a Nitrososphaeraceae ASV, while the controls recorded higher densities of Desulfobulbaceae and a Nitrosopumilaceae ASV (Table S2). For the mangrove soils, high-nutrient treatments had higher relative abundances of *Nitrosopumilus* (3 ASVs) and a Nitrosococcaceae ASV, while the control plots contained a Desulfobulbaceae, Rhodospirillales and an uncultured Nitrosopumilaceae ASV (Table S2).

Superficial soil microbiomes greatly differed between ecosystems. First, the microbial communities from the mangroves were more diverse (Shannon diversity and ASV richness) than those from the saltmarsh (Figs. 5, S7). The community structures were also significantly different (pseudo-F = 10.136, P(perm) = 0.013) (Figs. S8–S9). These differences were driven by the saltmarsh microbiome having higher relative abundances of the Gamma- (including Nitrosococcaceae, *Woseia* sp. and Methylomirabilaceae (wb1-A12)) and Alphaproteobacteria (Rhodospirillales, Kiloniellaceae, Geminicoccaceae), as well as archaea from the Nitrososphaeraceae family (4 ASVs) and *Halogranum* genus (2 ASVs) (Fig. S8). In contrast, mangrove soil microbiomes were characterised by higher relative abundances of putative sulphate reducing families Syntrophobacteraceae, Desulfobulbaceae and 4 Desulfobacteraceae ASVs (Deltaproteobacteria) (Fig. S8). Mangrove communities also had higher relative abundances of Rhodospirillales, 3 ASVs from the archaea family Nitrosopumilaceae and 2 ASVs from cyanobacteria (Fig. S8).

During nutrient and microbial sampling at T4, we mistakenly sampled Block #2 instead of Block #3 in the saltmarsh ecosystem. Despite this oversight on our side, the saltmarsh microbial community resulted profoundly different to that in the mangrove soil (e.g., no overlap between circles and triangles at any of the sampling times; Fig. S9). This conclusion was robust and highly likely to be unaffected by sampling of the saltmarsh Block #3 at T4.

The distance-based linear modelling revealed that soil C:N ratio and ammonium concentration were the most important environmental parameters for microbiome composition. Specifically, the best-fitting model following AIC only included C:N ratio (24% of explained variability) and Ammonia (13% of explained variability; Fig. 6). Conversely,

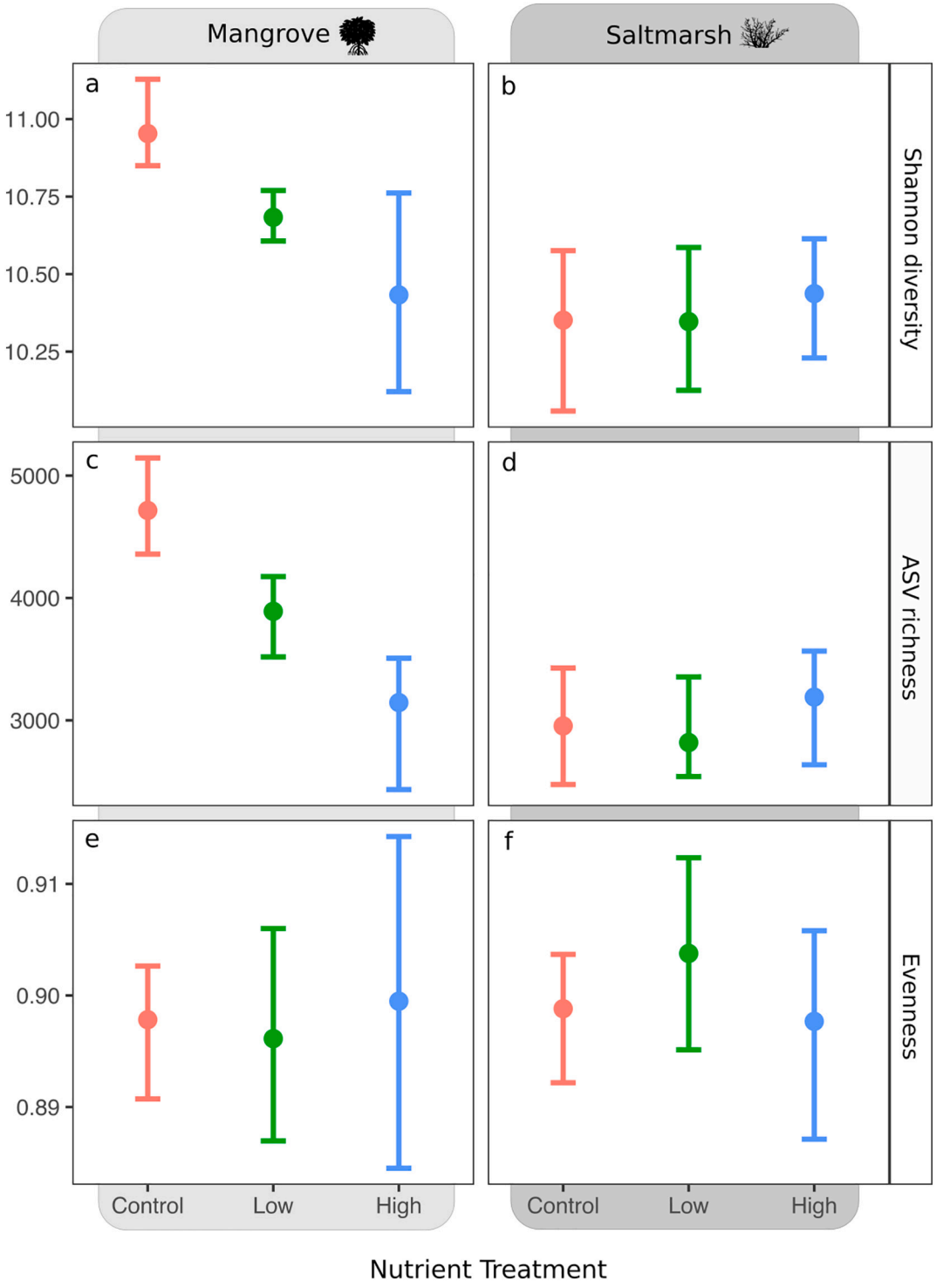

Fig. 5. Alpha diversity of the soil microbe community within mangrove (a,c,e) and saltmarsh plots (b,d,f) exposed to either control (red bars), low- (green bars) or high-nutrient treatments (blue bars). Results for Shannon diversity (a,b), ASV richness (c,d), and Pielou evenness (e,f) taken 56 days (T1) after the addition of nutrients. Values denote mean ± 95% confidence intervals. Values at 277 days (T4) available on Supplementary Materials (Fig. S7). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AIC selected against the inclusion of all other parameters – despite some of these showed significant effects in the fully-parameterised model: phosphate ($p = 0.011$), air temperature ($p = 0.021$), and CH_4 flux ($p = 0.042$). These results also highlight the differences between ecosystems: mangroves have higher C:N ratios (Fig. 3), CH_4 flux rates (Fig. 3) and nutrient concentrations (ammonium and phosphate; Fig. 2), whereas saltmarsh recorded warmer air temperatures (see Study site). We also noted that there was a clear separation between the first and last sampling for the saltmarsh communities, linked to higher ammonium concentrations at the first sampling (T1, Fig. 6).

4. Discussion

Coastal blue carbon ecosystems are under increasing pressure from

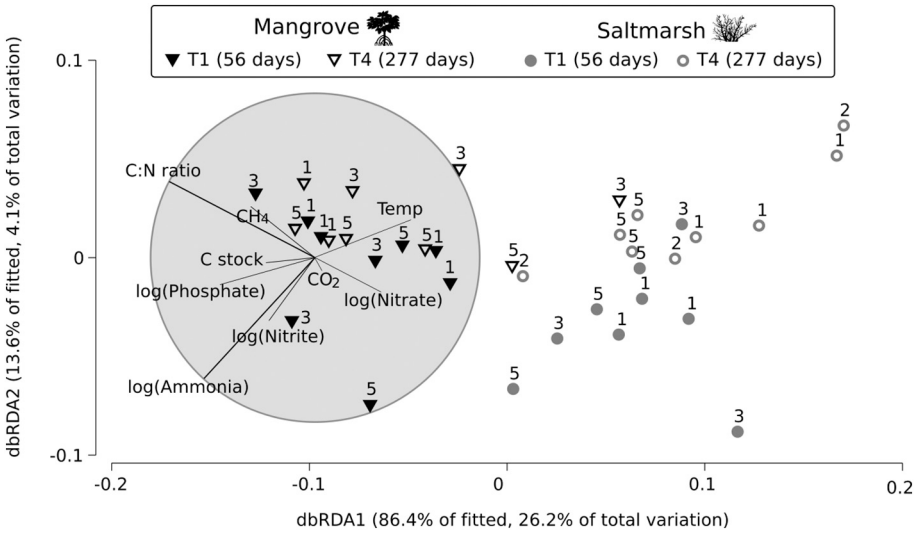

Fig. 6. Distance-based redundancy analysis for the beta diversity of the soil microbe community within mangrove (triangles) and saltmarsh plots (circles). Numbers represent the 'Block' number (1–5). While the best-fitting model following AIC only included two variables (i.e., C:N ratio and ammonia), we here present the results of the fully parametrised model. See Fig. 1 for details and a map of the 'Blocks' position.

land-derived eutrophication in most developed coastlines worldwide. Here, we examined the impact of a nutrient pulse on the carbon sink capacity of an Australian temperate saltmarsh and mangrove ecosystem. We found that a single environmentally-relevant load of fertiliser (~25 g N m^{-2} yr^{-1} and ~2 g P m^{-2} yr^{-1}) had a significant, but short-lived effect on superficial soil carbon stocks. Over the first two months, we evidenced a decoupling between the soil carbon inputs and outputs, in which the nutrient load was insufficient to boost carbon inputs, yet enough to reduce soil carbon stocks by ~23% and slightly accelerate litter decay rates. During this time, mangroves also experienced a ~33.3% reduction in microbe ASV richness and a shift in community structure linked to elevated ammonium concentrations. Before the end of the 277-day experiment, the effect of the nutrient pulse had dissipated with surface soil carbon- and nitrogen-cycling returning to background control levels. Although these results highlight the resilience of blue carbon ecosystems, coastal eutrophication should be regarded as a critical environmental issue that at high levels or frequency could limit their capacity to serve as carbon sinks (e.g., Deegan et al., 2012; Macreadie et al., 2012; Bulseco et al., 2019).

4.1. Nutrient effects

The nutrient addition pulse had variable effects on the carbon production and turnover components measured in this study. First, we found that aboveground plant cover was unaffected by the nutrient pulse, which contrasts to several studies reporting an increase of carbon inputs with nutrient loads (Vivanco et al., 2015, Morris et al., 2002, Logan, 2018). The lack of change in plant cover could be due to several non-exclusive factors: (i) the relatively low nutrient concentration of the pulse (most studies >70 g N m^{-2} yr^{-1}; Morris and Bradley, 1999, Vivanco et al., 2015, Simpson et al., 2020); (ii) a lack of nitrogen or phosphorous-limitation in these ecosystems; and/or (iii) a slow response to nutrient uptake. Additionally, it is important to note that a lack of change in plant cover does not necessarily mean a lack of change in plant productivity. For example, saltmarsh productivity could have increased through the growth of taller canopies, denser or thicker leaves, or more leaves per shoot, despite the unchanged plant cover. Unfortunately, our results do not fully exclude an effect of nutrients on plant carbon input and further studies are needed to clarify this finding.

Second, we found that elevated nutrients led to significant changes in surface carbon stocks, soil C:N ratios, and a slight increase in litter decomposition rate. However, these effects on remineralisation and decomposition were limited to the first months after the nutrient release. Approximately two months into the experiment, superficial soil carbon stocks had been reduced by 23% in the high-nutrient treatment compared to controls for both ecosystem types. Although carbon efflux (CO_2 and CH_4) remained unaffected, the loss of superficial soil carbon stock suggests carbon-cycling and remineralisation occurred at some point before our first sampling (T1 = 56 days). A similar nutrient effect on the early stages of litter decay has been shown for saltmarsh (Mueller et al., 2018; Koceja et al., 2020), mangroves (Simpson et al., 2020), and eutrophic freshwater ecosystems (Seelen et al., 2019), suggesting exogenous nutrient sources facilitate microbial metabolism of fresh litter. It is possible that the early carbon-cycling and loss of carbon stocks mainly affected the fresh organic carbon deposits from the surface (top 5 cm), which are generally more labile and potentially more susceptible to remineralisation. After three months (T2), carbon stocks from nutrient-enriched plots were similar to controls, suggesting that the fertiliser effect had mostly disappeared and the effects of the nutrient treatment on remineralisation and decomposition had become negligible. Given the lack of nutrient monitoring between T1(two months) and T4 (nine months), we can only speculate that the fertiliser pellets released most of the nutrient load before T2 (three months).

In response to the nutrient pulse, we also detected an early microbial response on the top 1 cm of mangrove soil, further suggesting that isolated nutrient pulses have a significant but short-term effect on superficial coastal vegetated soil. We found a decrease in mangrove microbial alpha diversity two months after the nutrient release (T1), likely due to the enhancement of nitrogen-cycling favouring the oxidation of complex forms of organic matter into more bioavailable forms (Bulseco et al., 2019). In both saltmarsh and mangrove soils, the high-nutrient plots had higher relative abundances of the ammonium-oxidising archaea (AOA) *Nitrosopumilus* and Nitrososphaeraceae ASVs and the ammonium-oxidising bacteria (AOB) Nitrosococcaceae in comparison to control plots. These taxonomic groups have been previously found in enriched saltmarsh soils after fertilisation treatments (Peng et al., 2013); though the presence of this functional group of archaea in control soils also suggests that the diversity AOAs may respond differently to changes in soil conditions (Peng et al., 2013). The strong correlation between mangrove microbial communities and ammonium concentrations further supports the impact of nutrient addition on the microbe diversity and structure. While we do not have data on the nitrogen-cycling genes used by the soil microbiome, the higher relative abundance of *Nitrosopumilus* sp. and Nitrososphaeraceae, as well as putative sulphate-

oxidising, nitrate-reducing Desulfobulbaceae suggests both ammonium-oxidising and nitrate-reducing pathways, respectively, are important in cycling these nutrient inputs (Reyes et al., 2017; Bulseco et al., 2020; Murphy et al., 2020).

4.2. Ecosystem effects

Mangroves and saltmarshes from Towra Point greatly differed in their carbon cycling and remineralisation. In particular, mangrove soils had higher C:N ratios, CH_4 emissions, microbial diversity, and greater initial decomposition of rooibos tea than saltmarsh soils. Many of these differences are likely driven by the specific soil properties (i.e., anoxic conditions and coarser size fraction in mangrove soil) and tidal influence (i.e., greater waterlogged soils in mangrove than saltmash) that characterises each ecosystem. For example, the frequent tidal flushing in mangrove soils likely enhanced the early leaching (passive loss of soluble cell contents) and greater initial mass loss of the labile green tea (Mueller et al., 2018; Seelen et al., 2019). Instead, the drier saltmarsh soil could have delayed the decomposition of the recalcitrant rooibos tea (Hemminga et al., 1988). While we did not measure soil oxygen concentration, we previously showed that oxygen only penetrates to 0.8 mm in mangrove and to more than 5–6 mm in saltmarsh soils from Towra Point (Brodersen et al., 2019). This difference in oxygen availability in the top 1 cm of soil, and the observed water-logged conditions, was likely an important variable in shaping the prokaryotic community of the mangrove soil microbiome.

The ecosystems differences were also reflected in the overall prokaryotic microbial community structure. The difference in the microbe communities between the two ecosystems was partly driven by the higher relative abundances of obligate anaerobic taxonomic groups in the mangrove soils. For example, there were higher relative abundances of putative aerobes in the saltmarsh surface soils, such as Kiloniellaceae, Geminicoccaceae, and *Halogranum*, in addition to the ammonium oxidiser Nitrososphaeraceae. In contrast, the mangrove soils had enriched relative abundances of Syntrophobacteraceae, a family that includes syntrophic bacteria involved in methane cycling and sulphate reduction (Kuever et al., 2014). There were also enriched relative abundances of putative ammonium oxidiser *Nitrosopumilus* sp., and nitrate-reducers and sulphate-reducers within the Desulfobacterales (Kearns et al., 2016; Murphy et al., 2020).

Additionally, the soil C:N ratio and ammonium concentrations were the environmental parameters that explained most of the variability in beta diversity, driven by higher concentrations in the mangrove soils. The presence of the bacterial groups, along with the high ammonium levels recorded in the mangrove soils, suggest the dissimilatory nitrate reduction to ammonium pathway (DNRA) was favoured in the system, with most of the bioavailable nitrogen being recycled and stored as ammonium (Fernandes et al., 2012; Murphy et al., 2020). Although several studies have reported a significant N_2O boost from mangrove enrichment (Muñoz-Hincapié et al., 2002; Kreuzwieser et al., 2003), here the DNRA pathway probably limited an early release of N_2O via a denitrification pathway.

4.3. Conclusion

Overall, our results highlight the significant, but short-lived impact that a nutrient pulse has on the surface soil carbon stocks of temperate mangrove and saltmarsh ecosystems. Fortunately, both coastal wetlands returned to control conditions after 100 days, proving the resilience of these blue carbon ecosystems to short-term nutrient pulses and the robustness of their carbon- and nitrogen-cycling. Under the experimental context of this research, we did not find concluding evidence that an environmentally-relevant eutrophication load ($\sim$25 g N m^{-2} yr^{-1} and $\sim$2 g P m^{-2} yr^{-1}) impacted the long-term carbon sink capacity of these systems. Instead, given their resilience and strategic location between land and sea, our results highlight the critical role coastal wetlands play as nutrient sinks (either by assimilation to plant biomass or conversion to gaseous products) potentially intercepting and buffering the impact of land-derived eutrophication on highly vulnerable marine habitats (seagrass meadows and coral reefs; Koop et al., 2001, Burkholder et al., 2007). Management plans in coastal areas with delicate marine systems and high risk of terrestrial runoff (i.e., Queensland coast, Australia; Kroon et al., 2012) should prioritise the protection of fringing coastal wetlands which can intercept and retain high amounts of land-derived nitrogen (Valiela and Cole, 2002; Zhao et al., 2019). Despite the sort-lived or limited net effects evidenced here and in other studies (e.g., Keuskamp et al., 2015b; Simpson et al., 2020), land-derived eutrophication should still be considered an environmental issue of critical concern as consecutive pulses, chronic exposure or acute eutrophication loads have been found to dampen the long-term capacity of coastal wetlands to serve as carbon sinks (Wigand et al., 2009; Deegan et al., 2012; Macreadie et al., 2012; Bulseco et al., 2019).

CRediT authorship contribution statement

M.M. Palacios: Conceptualization, Methodology, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **S.M. Trevathan-Tackett:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **M. E. Malerba:** Formal analysis, Writing – review & editing. **P.I. Macreadie:** Conceptualization, Methodology, Writing – review & editing, 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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Appendix A. Supplementary data

Supplementary data to this article (Fig. S1-S9; Tables S1-S2) can be found online at https://doi.org/10.1016/j.marpolbul.2021.112024.

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