Resource stoichiometry, vegetation type and enzymatic activity control wetlands soil organic carbon in the Herbert River Catchment, North-east Queensland

Lu Yao[1*], Maria Fernanda Adame[1], and Chengrong Chen[1*]

[1]*Australian Rivers Institute and School of Environment and Science, Griffith University, Nathan, QLD 4111, Australia*

*Corresponding authors.

E-mail address: l.yao@griffith.edu.au (L. Yao); c.chen@griffith.edu.au (C. Chen)

Abstract

Wetlands are highly productive ecosystem with great potential to store carbon (C) and retain nitrogen (N) and phosphorus (P) in their soil. Changes in vegetation type and land use can affect organic matter inputs and soil properties. This work aimed to examine how these changes affected elemental stoichiometry and C-, N-, and P- associated enzyme activities and wetland soil organic C stock. We quantified organic C concentrations, and stoichiometric ratios of C, N, and P in total and microbial biomass pools, along with the activities and ratios of C-, N-, and P-associated enzymes for soils of natural coastal wetlands with different vegetation types, namely *Melaleuca* wetland (*Melaleuca spp*), mangrove forests (*Bruguiera spp),* and saline marsh (*Eleocharis spp*). We also compared these natural wetlands to an adjacent sugarcane plantation to understand the effects of vegetation types. Hypothesis-oriented path analysis was used to explore links between these variables and soil organic C stocks. Tidal forested soils (0-30 cm) had the highest organic C, N, and P contents and potential activities of C-, N-, P- acquiring enzymes, compared with other vegetation types. Mangroves soils had the highest total soil C:N and microbial biomass C:P ratios. Microbial biomass C:P ratios were significantly and positively related to total C:P, while microbial biomass N:P ratios were positively associated with total soil C:P and N:P ratios. Path analysis suggested that soil organic C stock was largely explained by total C:P ratio, microbial biomass N:P ratios, total P content, and the ratio of C- and P-associated enzymes. Different types of wetlands have different soil properties and enzymatic activities, implying their different capacity to store and process C and N. The resource quality and stoichiometry direct influence the organic C stock.

Keywords

Coastal wetlands, Biogeochemistry, Stoichiometry, Microbial enzymes, Carbon stock, *Melaleuca*.

1. Introduction

Wetlands can accumulate large amounts of carbon (C) and nitrogen (N) in their soil, thereby improving water quality and mitigating greenhouse gas emissions (Mitsch et al. 2001; Keddy et al. 2009). Degradation of wetlands in the Great Barrier Reef (GBR) catchment is a major issue (Adame et al. 2019), because large area of wetlands has historically been converted for agriculture, with the most significant losses in floodplain wetlands, including tidal forested wetlands of "tea tree swamps" dominated by *Melaleuca* spp. (Johnson et al. 2000). Adjacent to these wetlands, N fertilisers support agricultural activities and food production (Thorburn and Wilkinson 2013). However, some of this fertiliser can be exported to adjacent wetlands and eventually flow to coastal wetlands (Adame and Reef, 2020). Within these wetlands, N can be reduced through the process of denitrification, and accumulation in the soil profile (Adame et al. 2020). Due to the ability to store and process large amount of C and N, wetlands are now considered priority areas for conservation (Adame et al. 2014, 2019; Dixon et al. 2016).

Vegetation types can influence soil organic C stock by altering the form, quality and seasonality of organic matter inputs and nutrient availability (Schulze 2005). Plant aboveground detritus and fine roots provide major inputs of organic matter to soil (Kristensen et al. 2008), most of the inorganic nutrients in natural wetland soils comes from biological decomposition of plant residues (Manzoni et al. 2010). Variation in biomass production, lignin content, C:nitrogen (N) ratio, and phosphorus (P) availability directly affect soil microbial activity (Reddy and DeLaune 2008; Adame et al. 2013; Arnosti et al. 2013). The moist conditions of wetland soil influence organic matter turnover by microbes, whereas anaerobic environment created by water saturated conditions may decrease microbial metabolism and can result in long-term C stock. On the other hand, conversion to cultivation

62 land and agricultural management practises can lead to significantly reduction in soil organic
63 C (Nahlik and Fennessy 2016; Carnell et al. 2018; Pekkan et al., 2021). Thus, the covered
64 vegetation type is one of the major factors that influence the organic C stock in wetland soil
65 (Kristensen et al., 2008; Hayes et al., 2017).

66 In general, N and organic C accumulates in wetland soils because the rate of organic
67 matter input exceeds the rate of decomposition. The decomposition of organic matter is
68 mainly mediated by soil enzymes mainly excreted by micro-organisms and plants. From
69 these enzymes, β-1,4-glucosidase (BG) and chitinase play roles in the degradation of
70 cellulose and chitin, respectively. Acid phosphatases (AP) hydrolyse a variety of organic and
71 inorganic phosphomonoesters. The BG, chitinase, and AP are the key enzymes involved in
72 the C-, N-, and P-acquisition during decomposition processes (Sinsabaugh et al. 2008).
73 According to the principle of ecological stoichiometry, the microbial demand of C and
74 nutrients is determined both by the elemental ratio of microbial biomass and environmental
75 nutrients availability (Sterner and Elser 2002; Manzoni et al. 2008). Investment in enzyme
76 synthesis is assumed to reflect microbial nutrient demand (Follstad et al. 2014), therefore the
77 organic matter decomposition and nutrients cycling can be inferred from determination of
78 potential soil enzyme activities (Kuscu et al., 2008; Sinsabaugh and Follstad-Shah 2012;
79 Veres et al 2015). Moreover, soil enzyme activities are sensitive to the change of abiotic
80 factors, such as soil moisture content, pH, and electrical conductivity (EC). Hence, the
81 investigation of the interaction of soil enzyme activity, microbial biomass stoichiometry,
82 nutrients availability and soil properties can help improve the understanding of
83 biogeochemical cycles driven by microbial community under different vegetation types and
84 soil management in coastal wetlands.

85 A way to understand the interactions between C dynamics is the hypothesis-oriented

path analyses, which is a quantitative analysis method to identify the potential direct and indirect effects of soil variables on soil organic C dynamics, and has been widely used in multivariate interacting system (Grace and Kelley 2006; Luo et al. 2017). This analysis can help identify key parameters involved in soil, such as elemental stoichiometry, as microbial nutrients demand is determined by the ratios of microbial biomass in relation to ratios of resource (Sterner and Elser 2002), meanwhile, soil enzyme activities drive microbial nutrient acquisition from organic matter. Therefore, resource ratios may indirectly affect enzyme ratios through elemental ratios of biomass.

Here we examined stoichiometric ratios of C, N, and P in soil total and microbial biomass pools, and associated soil properties (inorganic N, plant available P, and the activities of C-, N-, and P-acquiring enzymes) in natural wetlands under different vegetation types (mangroves, saline marsh, and *Melaleuca* wetlands) in tropical Australia Northeast Queensland. We also compared soil properties to those of an adjacent sugarcane plantation, which was previously dominated by *Melaleuca* (Adame et al., 2019). We hypothesised that (1) the different vegetation types and associated management practices would affect soil organic C content, (2) nutrient availability and abiotic factors would affect soil enzyme activities, (3) C:N and C:P ratios in resource and edaphic factors would jointly lead to the change in soil organic C contents.

2. Materials and methods

2.1 Study area and field sampling

The study site was located at Insulator Creek (18° 53' S, 146° 15' E) within the catchments of the Herbert River in Northeast Queensland. Mean annual precipitation for the region is > 2000 mm, with most rainfall occurring between January and May (Australian

109 Bureau of Meteorology [BOM], 2018; 1907-2018). Mean monthly temperatures range from
110 22 to 34 ◦C (BOM 2018; 1907-2018). Sampling was conducted twice during two dry seasons,
111 August, 2016 and June, 2018. We sampled three types of wetlands: saline marsh, *Melaleuca*
112 wetlands, and a mangrove forest. The saline marsh was dominated by *Eleocharis* spp and the
113 mangroves by *Bruguiera gymnorhiza*. The *Melaleuca* wetlands was a tidal freshwater forest,
114 which was dominated by *Melaleuca quinquenervia*. *Melaleuca* spp. is a genus of the
115 Myrtaceae family native to eastern Australia and commonly located in many coastal wetland
116 environments, and they have high potential for C and N stock (Tran et al. 2013; Adame et al.
117 2019b). These wetlands in Australia are highly threatened by deforestation and increased
118 salinity (Salter et al. 2007; Adame et al. 2019b)

119 At each of sampling sites, 3–5 sampling plots (5×5 m each) were randomly selected in a
120 line transect which was perpendicular to the water edge. Each plot was separated at least by
121 20 m (Adame et al. 2015). At each plot, 3-5 surface soil core samples were collected using an
122 auger of approximately 7.5 cm in diameter and stored at 4 °C. In the laboratory, soil samples
123 were passed through 2 mm sieve to remove roots and other debris. A subsample of moist soil
124 was stored at 4 °C for analysis of pH, EC, labile C and nutrient, microbial biomass C and
125 nutrient, enzyme activity within two weeks of the sampling. Air-dried samples were finely
126 ground (<150 μm) for analyses of total C and nutrient contents. All results are expressed on
127 an oven-dry basis.

128 *2.2 Measurements of soil physical, chemical and biological properties*

129 Soil pH and electrical conductivity (EC) were measured using standard methods in a
130 solution of 1:5 soil to water by a combined glass electrode (Rayment and Lyons 2011). Soil
131 moisture was measured gravimetrically by oven drying the soil at 105 ºC for 24 h. Soil bulk
132 density was estimated by weighing intact soil cores. Soil inorganic N (NH_4^+-N and NO_3^--N)

of field-moist soils was extracted with 2 M KCl and determined with a Continuous Segmented Flow Analyzer (SEAL Auto Analyzer 3 HR, SEAL Analytical Limited, UK). Soil Olsen P concentrations were measured using the method described by Rayment and Lyons (2011).

Soil microbial biomass C, N, and P were measured using the chloroform fumigation-extraction method, with conversion factors of 2.64, 2.22, and 0.40, respectively (Brookes et al. 1982: 1985; Vance et al. 1987). The activities of β-1,4-glucosidase (BG), chitinase (CHIN), and acid phosphatase (AP) were measured using ρ-nitrophenyl spectrophotometric methods (Eivazi and Tabatabai 1988; Sinsabaugh and Linkins 1990; Tabatabai and Bremner 1969). The Sigma codes for the BG, CHIN, and AP substrates used were N7006, N9376 and P4744, respectively. Soil total and microbial biomass C:N, C:P, and N:P ratios were calculated as molar ratios (atomic ratio). Stoichiometric ratios of BG:CHIN, BG:AP, and CHIN:AP were calculated as the ratio of potential activity of each enzyme.

Total C and total N content were analysed using a Leco TruMac TCN Determinator (LECO Corporation, USA). For the organic C concentration analysis, 1 g of air-dried sample was placed into ceramic boat fitted with nickel boat liner to which 5 to 10 ml of a 5-6% sulphurous acid was added to remove any carbonates from the soils. Once reaction ceased, the samples were placed on a hotplate to remove water and excess sulphurous acid (Balduff, 2007). The organic C content of the samples was then determined using the Leco TruMac TCN Determinator. Soil total P concentration was measured via inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perlin Elemer, USA) after the samples were digested in nitric acid following a modified microwave-digestion protocol based on 3051A of the USEPA (1988). In brief, 0.2 g fine-ground soil samples were added with 10 ml of concentrated nitric acid, the mixture were digesting at 200 °C for 40 mins in a microwave

digester (TITAN MPSTM, Perkin Elmer). After cooling, the digested solution was diluted with 10 ml deionised water, the supernatants were then analysed by ICP-OES.

2.3 Statistical analysis

Data were tested for normality with a Shapiro-Wilk tests, and were natural logarithm or square-root transformed when required to achieve a normal distribution. For Olsen P and microbial biomass C, Box-Cox transformation was applied. The differences in soil properties between different vegetation types for each sampling time were analysed with repeated measures analysis of variance (ANOVA) using a generalized linear model. Tukey's Honestly Significant Difference (HSD) method was used to determine whether these effects were significantly different ($p < 0.05$); homogeneity of variance and normality for all soil properties data were assessed using the Leven's test ($p > 0.05$). Repeated measures ANOVA and Tukey's HSD analyses were performed with SPSS version 25.0 (IBM SPSS Inc., Chicago, USA). Pearson correlations were used to determine relationships among soil physical, chemical, and biological parameters and stochiometric ratios of C, N, and P in different pools. Principal component analysis (PCA) was applied to the correlation matrix among the variables and distinguish the effects of vegetation types on soil. PCA analyses were performed with R version 3.5.2 (R Core Team 2018).

Path analysis was conducted using partial least squares path modelling (PLS-PM). The calculation of the model was performed using the package 'plspm' in R version 3.5.2 (ver. 0.4.9, Sanchez et al., 2013). A non-parametric bootstrapping (1000 resamples) was used to estimate the precision of the parameter estimates. Non-significant relationships ($p > 0.05$) were excluded from the results. The path model was formulated using composite variables while five latent variables were included to assess the direct and indirect effects on soil organic C stock. The environmental factors were categorized into five latent variables: 'soil

abiotic properties', 'biotic properties', 'soil microbial biomass ratios', 'soil enzyme ratios', and 'soil resource ratios'. The latent variables were explained by measured variables.

All measurements of soil properties including pH, EC, moisture content, concentrations of NH_4^+–N, NO_3^-–N, Olsen P, total P, and total N were considered as indicators for the latent variable 'abiotic properties'. The 'biotic properties' were reflected by microbial biomass C, N, and P, the activities of β-1,4-glucosidase, chitinase, and acid phosphatase. We estimated total C:P ratio, N:P ratio, and inorganic N to Olsen P ratio as indicators for 'resource ratios'. The 'microbial biomass ratios' was reflected by microbial biomass C:N, microbial biomass C:P, microbial biomass N:P. The stoichiometric ratios of three enzyme activity were considered as potential indicators for the latent variable 'enzyme ratios'. The correlations between each latent variable and the measured variables were indicated by the loadings, with values > 0.7 indicating that the variability in the observed variables was significantly captured by their latent variables (Urakawa et al. 2016).

3. Results

3.1 Wetland soil edaphic variables and biological properties

The effect of vegetation type on soil bulk density, pH, EC, moisture, total organic C and nutrient concentrations, available nutrient concentrations, and enzyme activities were significant ($p < 0.05$; Table 1). Sampling time (2016 or 2018) exerted significant influence over soil biotic factors associated with C and P biogeochemical cycles, namely concentrations of total P, microbial biomass C, and β-1,4-glucosidase activity ($p < 0.05$). Vegetation type and sampling time had an interactive influence on soil Olsen P, soil microbial biomass C, N, and P, and the potential activities of C-, N-, and P- acquiring enzymes ($p < 0.05$; Table 1).

Melaleuca wetlands had the highest average soil organic C stock, with a value of 13.2 ± 7.4 kg m^{-2} to a depth of 30 cm, compared to 5.3 ± 1.1, 6.9 ± 4.6, and 5.1 ± 0.2 kg m^{-2} in mangroves, marsh, and sugarcane, respectively ($p < 0.05$; Table 2). Soil bulk density was lowest under mangroves and *Melaleuca* wetlands, highest under sugarcane and intermediate under marsh ($p < 0.05$). Soil pH was significantly lower under *Melaleuca* wetlands than in the other three soils, and soil moisture was significantly lower under sugarcane ($p < 0.001$). Soil EC was lower under *Melaleuca* plants and sugarcane than under mangroves and Marsh ($p < 0.001$) (Table 2). Concentrations of total organic C, N, and P were highest in *Melaleuca* wetlands soil ($p < 0.05$) (Table 2). Concentrations of NH_4^+-N were highest in the marsh soil, lowest in the mangrove soil and intermediate in *Melaleuca* wetlands soils and sugarcane soils ($p < 0.001$). Concentrations of NO_3^--N were significantly highest in *Melaleuca* wetlands soils, lowest in the marsh soil and intermediate in mangrove and sugarcane soils ($p < 0.05$). Concentrations of Olsen P were the highest in mangroves and marsh soils, lowest in the sugarcane soil and intermediate in the *Melaleuca* wetlands soils ($p < 0.05$).

There were no significant differences in the concentrations of microbial biomass C, N and P among the four vegetation types (Table 2). The activity of β-1,4-glucosidase was significantly higher in *Melaleuca* wetlands soils, lower in the marsh soil and intermediate in mangroves and sugarcane soils ($p < 0.05$). The activity of chitinase was higher in the *Melaleuca* wetlands soils, lower in mangrove soil and intermediate in marsh and sugarcane soils ($p < 0.05$). Acid phosphatase activity was higher in *Melaleuca* wetlands and sugarcane soil and lower in mangroves and marsh soils ($p < 0.05$) (Table 2). Overall, *Melaleuca* wetlands soils had the highest organic C, N, and P content, as well as the highest potential activities of C-, N-, P- acquiring enzymes ($p < 0.05$).

3.2 Wetland soil C, N, and P stoichiometric characteristics under different vegetation types

228 Vegetation type and sampling time had complex effects on the stoichiometric ratios of
229 C-, N-, and P-related variables (Table 3). Soil total C:N, N:P, microbial biomass C:N, and
230 BG:CHIN ratios were significantly influenced by sampling time and the interaction of
231 vegetation type and sampling time ($p < 0.05$). Soil C:P ratio was significantly influenced by
232 sampling time ($p < 0.05$). Soil microbial biomass C:P ratio was influenced by vegetation
233 types and sampling time ($p < 0.05$). The ratio of soil inorganic N to Olsen P was significantly
234 influenced by vegetation type ($p < 0.05$). BG:AP ratio was significantly affected by
235 vegetation type, sampling time and their interaction ($p < 0.05$) (Table 3).

236 The ratio of total C:N was highest in the mangroves, lowest in the marsh and
237 intermediate in *Melaleuca* wetlands and sugarcane soils ($p < 0.05$) (Table 4). There was no
238 significant difference in soil total C:P and N:P ratios among the four vegetation types. The
239 ratio of inorganic N to Olsen P was the highest in the sugarcane soil, lowest in the mangrove
240 soil and intermediate in marsh and *Melaleuca* wetlands soils ($p < 0.05$). There were no
241 differences in microbial biomass C:N and N:P ratios among the four vegetation types. The
242 ratio of microbial biomass C:P was highest in mangrove and marsh soils, lowest in sugarcane
243 soil and intermediate in *Melaleuca* wetlands soils ($p < 0.05$). There was no difference in the
244 stoichiometric ratio of β-1,4-glucosidase to chitinase among vegetation types. The ratios of
245 β-1,4-glucosidase to acid phosphatase and chitinase to acid phosphatase were both highest in
246 the mangrove soil, lowest in marsh and sugarcane soils and intermediate in the tidal
247 freshwater forests soil ($p < 0.05$).

248 *3.3 Correlations among soil variables*

249 Soil bulk density was negatively ($p < 0.05$) associated to soil moisture content and
250 concentrations of organic C and total N, while positively ($p < 0.01$) related to pH. Soil pH
251 was positively ($p < 0.05$) related to EC and the concentration of Olsen P, and negatively ($p <$

252 0.01) related to concentrations of organic C, total N, total P, and the activities of β-1,4-
253 glucosidase, chitinase, and acid phosphatase. Soil EC was positively ($p < 0.001$) related to
254 moisture and concentration of Olsen P, while negatively ($p < 0.01$) related to soil NO_3^--N
255 concentration and the activities of β-1,4-glucosidase, chitinase, and acid phosphatase. Soil
256 moisture was positively ($p < 0.01$) corelated with concentration of total P and Olsen P, while
257 negatively ($p < 0.01$) related to the NO_3^--N concentration (Fig. 1).

258 Overall, there were significant ($p < 0.05$) correlations among soil total organic C, N, and
259 P concentrations, as well as microbial biomass C, N, and P and C-, N-, P-associated enzyme
260 (β-1,4-glucosidase, chitinase, and acid phosphatase, respectively) activities (Figs. 2, 3 and
261 S2). Additionally, the organic C concentration was positively ($p < 0.05$) associated with
262 microbial biomass C, the activities of β-1,4-glucosidase, chitinase, and acid phosphatase.
263 Total N concentration was positively ($p < 0.05$) related to microbial biomass C and N, and
264 the activities of β-1,4-glucosidase, chitinase, and acid phosphatase. Total P concentration was
265 positively ($p < 0.05$) related to microbial biomass C and P, and the activities of β-1,4-
266 glucosidase, chitinase, and acid phosphatase (Fig. 1). There were no significant relationships
267 among the NH_4^+-N concentration and other soil parameters. Soil NO_3^--N concentration was
268 positively ($p < 0.01$) related to β-1,4-glucosidase and acid phosphatase activity, while
269 negatively ($p < 0.01$) related to Olsen P concentration. Olsen P concentration was negatively
270 ($p < 0.05$) related to β-1,4-glucosidase activity. Microbial biomass C was positively ($p < 0.05$)
271 related to β-1,4-glucosidase, chitinase, and acid phosphatase activities, microbial biomass N
272 was positively ($p < 0.05$) correlated with chitinase and acid phosphatase activities, and
273 microbial biomass P was positively ($p < 0.01$) related with acid phosphatase activity (Fig. 1).

274 Soil microbial biomass C concentrations were negatively ($p < 0.01$) related to the ratio
275 of chitinase and acid phosphatase. Microbial biomass N concentrations were negatively ($p <$

0.05) correlated with β-1,4-glucosidase to acid phosphatase ratios, and chitinase to acid phosphatase ratios. Microbial biomass P concentrations were negatively related to total C:P and N:P ratios, biomass N:P ratios and chitinase to acid phosphatase ratios (Fig. S2). Microbial biomass C:N ratios were positively ($p < 0.05$) related to the ratio of β-1,4-glucosidase and chitinase activity (Figs. 2b and S2). Microbial biomass C:P ratios were significantly ($p < 0.001$) and positively related to total C:P ratios at each sampling time separately, however, the relationship was not statistically significant when analysed with combined data (Fig. 2b). Microbial biomass N:P ratios were positively ($p < 0.001$) related to total C:P and N:P ratios (Figs. S2 and 3C).

3.4 PCA and path analysis

The PCA divided the four sampling sites into three groups (mangrove and marsh as one group; *Melaleuca* wetlands as the second group; sugarcane as the third group). The groups differ mainly from each other along the 2nd axis, i.e., in moisture and EC (also drained vs. sites flooded with brackish/saltwater and nitrate fertilization). The average loadings on component 1 are rather similar between the groups. The *Melaleuca* wetlands group especially is highly variable, possibly suggesting several subtypes (Fig. 3; Table S1).

The loadings scores from the path analysis suggested that soil total P concentration, acid phosphatase activity, the ratio of microbial biomass N to P, the ratio of β-1,4-glucosidase to acid phosphatase, and the ratios of total C and N to P were representative indicators of 'abiotic properties', 'biotic properties', 'microbial biomass ratios', 'enzyme ratios' and 'resources ratios', respectively (Table 5). The goodness of fit, which indicates the average prediction of the entire model, was 0.48 (Fig. 4). Soil organic C stock was significantly and directly affected by 'abiotic properties', 'resource ratios' and 'biotic properties. The 'enzyme ratios' did not have significant direct effects on soil organic C stocks (Fig. 4). The latent

300 variable ‘microbial biomass ratios’ were significantly and directly affected by ‘resource
301 ratios’, while ‘enzyme ratios’ were negatively affected by ‘biotic properties’ (Fig. 4).

302 The relationship between scores of ‘abiotic properties’ and organic C stock (0-30 cm),
303 which are the value of latent variables of each site derived from path analysis, are shown in
304 Fig. 5a. The broad latent variable ‘abiotic properties’ indeed had a significant effect on
305 organic C stock (Fig. 4), but significant correlations were only evident for *Melaleuca*
306 wetlands (R^2 = 0.80, $p < 0.001$) and sugarcane plantation (R^2 = 0.83, $p < 0.001$) soils.
307 Additionally, scores of ‘resource ratios’ and organic C stock were significantly correlated
308 across the four sampling sites (Fig. 5b; R^2 = 0.15, $p < 0.05$), therefore the entire effect of
309 ‘resource ratios’ and organic C stock was significant across the four sampling sites.

310 **4. Discussion**

311 *4.1 Vegetation type and wetland organic C stock - why tidal Melaleuca wetlands have higher*
312 *organic C stock?*

313 Organic C stock of natural coastal wetlands were higher than the sugarcane plantation,
314 with highest values in the tidal freshwater forest, suggesting that *Melaleuca* wetlands in the
315 Insulator Creek of Northeast Queensland store large amount of soil C, averaging 132 ± 74 tC
316 ha^{-1} to a depth of 30 cm. Adame et al. (2019) found that the surface soil accumulation rates in
317 the *Melaleuca* wetlands of tropical Australia were 0.6 ± 0.2 Mg C ha^{-1} y^{-1}. Due to the
318 development of accommodation space (the vertical and lateral space available for fine
319 sediments to accumulate and be colonized by wetland vegetation) relative to sea–level
320 change (Schuerch et all., 2018; Rogers et al., 2019), *Melaleuca* wetlands are highly
321 heterogeneous. Therefore, the soil organic carbon stock is spatially variable for *Melaleuca*
322 wetlands. Meanwhile, Mangrove and marsh wetlands stored soil C of 53 ± 11 and 69 ± 46 tC
323 ha^{-1}, respectively. The values are comparable to other coastal carbon rich wetlands. For

example, soil C standing stocks of tidal freshwater wetlands in Atlantic coastal rivers ranged from 181 to 1259 Mg C ha-1. Saltmarshes have ecosystem C stocks ranging from 100 to 800 Mg C ha-1 (Chmura et al., 2003).

Soil under sugarcane plantation stored 51 ± 2 tC ha^{-1}, where agricultural practices has led to C stock reductions in the region, similar to other parts of Australia (Luo et al. 2010). Agricultural activities, such as harvesting and fertiliser application, have a significant influence on C and nutrient dynamics (Braunack et al., 2006a,b). Compared with the sugarcane cultivation field within the Queensland sugarcane growing areas, the mean value (0.076 dS m^{-1}) of soil EC in the present study was slightly higher than the range of 0.040–0.070 dS m^{-1} reported by Page et al. (2013), and within the range of 0.030–0.260 reported by Braunack et al. (2006a). Excessive N fertiliser, combined with increased soil compaction and soil structural degradation from traffic during crop management operations (Hamza and Anderson, 2003; Armour et al., 2013), contributed largely to the separation of sugarcane land use from natural wetlands in the present study.

Soil organic C stock could increase either by higher plant residue input or slower decomposition rates. Tree has more structural C compared with herbs and shrubs (Ma et al. 2018). It was reported that *Melaleuca* trees produce woody tissues with a high amount of recalcitrant organic materials and have a high rate of litter fall but low decomposition rates, largely due to the high content of essential oils in the leaves (Bolton and Greenway 1997). Therefore, soil organic compounds are stable for long periods. We found the soil pH in *Melaleuca* wetlands were significantly lower than the other three sampling sites (Table 2). Meanwhile, the positively relationship between soil pH and bulk density in this study was probably due to the presence of soil organic matter. Previous study also observed the significant correlation between soil bulk density and pH (Jia et al., 2005, Table 4), while

348 Rokosch et al. (2009) reported non-significant relationship between soil bulk density and pH.
349 Changes in bulk density in the soil profiles reflect soil organic matter development, with the
350 density of the soil decreasing as soil organic matter increases (Ballantine and Schneider,
351 2009). Additionally, increased pH generally accelerates the leaching of dissolved organic C
352 and thus result in lower organic C levels in the surface soil (Grybos et al., 2009). Research
353 has shown that *Melaleuca* plants can also influence the metal mobility, reactivity and
354 availability in soil through the alteration of soil pH and redox potential, therefore causing
355 substantial changes to biogeochemical cycling in wetland soils (Johnston et al. 2003). Above
356 all, our results can further support the suggestion that the potential for tidal freshwater forest
357 wetlands to store C and N were probably underestimated (Tran and Dargusch 2016; Adame
358 et al. 2019).

359 *4.2 Effects of nutrient availability and soil enzyme activities on soil organic C stock*

360 The interplay between nutrient availability and decomposition rate of organic matters
361 are crucial in understanding the soil organic C stock. Soil nutrient dynamics can be affected
362 by vegetation type in addition to belowground biological and geochemical processes, thereby
363 influence soil organic C stock (Viscarra Rossel and Bui 2016; Weiss et al. 2016). It has been
364 reported that soil with a high amount of organic C may probably result in higher microbial
365 metabolism and increasing microbial activities (Jiang et al. 2019). Moreover, P availability in
366 tropical soils tends to increase organic C concentration (Chen et al. 2015), and organic matter
367 accumulation was positively associated with organic P levels (Hou et al. 2014; Fonte et al.
368 2014). Additionally, the close association of soil total P concentration with soil organic C
369 content in our study was expected, as the soil in Australia is derived from old landscapes and
370 considered to be P deficient (Bui and Henderson 2013).

371 Soil enzyme activities are also strongly affected the C stock in our study. The marked

increase in acid phosphatase activity and soil organic C in *Melaleuca* wetlands indicated an association between P biogeochemical cycle and organic C concentration. It has been reported that soil enzyme activities directly indicate soil microbials' metabolic requirements and available nutrients, thereby regulating the processing of C in the soil system (Cenini et al., 2016). Vegetation type plays an important role in determining soil enzyme activity, thereby result in variations in C stock (Cui et al. 2018; Luo et al. 2018; Jiang et al. 2019). As we discussed before, *Melaleuca* plants have a higher rate of litter fall (Bolton and Greenway 1997). Johnston (2003) also reported that *Melaleuca* plants can influence metal mobility, reactivity and availability in soil through the alteration of soil pH and redox potential, thereby causing substantial changes to biogeochemical cycling in wetland soils. Sinsabaugh et al. (2009) further suggested that soil enzyme activities are strongly related to soil pH. It noteworthy that β-1,4-glucosidase and chitinases activity was the highest in the *Melaleuca* wetlands. On the other hand, the distribution of organic layers in coastal wetland soil profiles is spatially variable. Australian tidal marshes may have sub-surface horizons compared to lower elevation wetlands (Adame et al., 2019). Meanwhile, Senga et al. (2015) reported that the activity of phosphatases from plant roots may lead to high AP activity in the surface layers. Those were likely reasons for the large variation of the potential AP activity observed in marsh in the present study (Table 2). Thus, increased enzyme activity is proportionally lead to improved nutrient availability for plants and microorganism utilization, and eventually increase the amount of organic matter input into soils.

4.3 The combined effects of C, N, and P ratios and edaphic factors on wetland organic C stock

Soil microorganisms do not simply respond to resource stoichiometry and microbial biomass stoichiometry but can adjust their metabolic activities through regulation of available energy and substrate (Austin et al. 2014). Enzyme activities link decomposition processes

with microbial community, and the production of enzymes involved in C-, N-, and P-acquisition are stoichiometrically constrained across ecosystems (Sinsabaugh et al. 2009). This was supported by the strong correlations among soil enzymes across three different wetland vegetation types. Additionally, stoichiometric ratios of the three enzymes, especially the chitinase to acid phosphatase ratio, were significantly correlated with microbial biomass C, N, and P in our study. The microbial biomass N to P ratio was strongly associated with the resource stoichiometry, consistent with ecological stoichiometry theory. This result, along with the significant relationships among the activities of three enzymes and total C, N, and P concentrations, further suggest that enzyme expression was a product of microbial demand for energy and nutrient, and was largely driven by resource stoichiometry.

The path analysis revealed that C and nutrient ratios of resource and soil abiotic properties had direct influence on organic C stock and dynamics. Organic C stock differs among vegetation types, largely due to different litter inputs, enzyme activities, and composition of soil microbes that contribute to decomposition processes (Fierer et al. 2009, Ouyang and Lee 2020). Soil enzyme activities ratios reflect the biogeochemical equilibirium between microbial growth and stoichiometry of resource and microbial biomass (Sinsabaugh et al. 2009). Therefore, our result suggests that soil stoichiometric ratios of resource C and nutrients directly govern the organic C stock, while environmental conditions modify microbial activities, and hence, may reflect in C and nutrient biogeochemical dynamics. However, the goodness of fit of this model was 0.49, indicating half of the direct and indirect effect of variables on the organic C stock were not explained by this model. Besides, the microbial biomass ratios, grouped as a latent variable, is overall a weak factor on other groups. This is probably due to that soil microbial biomass only accounted for approximately 1–5 % of soil organic matter (Jenkinson 1990). Moreover, to maintain biomass growth, soil microbes regulate the production and release of extracellular enzymes in response to

environmental resource availability (Sinsabaugh et al., 2012), thereby influencing environmental substrate abundances, but the correspondence is not a direct mechanistic link. Overall, enzyme activities were the dominant factors discriminating between soil properties under different vegetation types in our study (Table 2). Meanwhile, previous study suggested that soil enzyme activities should be considered in the context of interactions of microorganisms and vegetation (Schimel and Weintraub 2003; Hoyos-Santillan et al. 2018), thus, the model structure might change with improved dataset on microbial community composition and litter input derived by vegetation.

It is acknowledged that there is some limitation in this study in understanding temporal variation and seasonal dynamics of wetland soil properties due to lack of more frequent sampling (only in 2016 and 2018). The result from this study is case specific. More frequent sampling and deep soil C measurement should be required in the relevant future field study.

4.4 Implications for future management of the coastal wetlands

Repeated soil measurement is used to estimate changes in organic C stock over time and under climate change, in the present study, vegetation types presented a significant influence on organic C stock, which highlights the potentially important role of vegetation cover on the coastal ecosystem. Mangroves and marsh plants respond more robustly to environmental changes than *Melaleuca* plants which require freshwater conditions, implying a high priority for conservation management when experiencing sea-level rise. The results of this study have improved our understanding of the processes that govern the C cycling in tidal freshwater forest wetlands (Lovelock and Duarte 2019), and further supporting the incorporation of tidal freshwater forest wetlands for conservation and rehabilitation as a complementary strategy to minimise N inputs to waterways and estimate the C releases caused by deforestation and degradation (Adame et al. 2019). In addition, results of this research will facilitate a more

446 comprehensive understanding of soil biogeochemistry of a gradient from landward to
447 seaward in the GBR, therefore provide sound management for wetland ecosystems in
448 response to global change, such as sea level rise or nutrient leaching from land use.

449 **5. Conclusions**

450 Our results have demonstrated that the surface organic C stock and nutrient dynamics
451 varied among different vegetation types. *Melaleuca* wetlands in the Insulator Creek of
452 Northeast Queensland store large amount of soil C. Compared with that in mangroves, marsh
453 wetlands and sugarcane plantation, the highest organic C stock in tidal freshwater forest
454 wetlands was probably due to the high organic material inputs and high nutrient content
455 available for plant utilization. Our results have confirmed that resource stoichiometry
456 governed the organic C stock, while soil enzyme activities modified by environmental
457 conditions also contributed to the shift in nutrient dynamics and organic C stock. These
458 results have improved our understanding of the biogeochemical processes that govern the C
459 cycling in tidal freshwater forest wetlands, which has significant implications on improving
460 planning and management of the coastal wetland ecosystems.

461 **Acknowledgements**

462 We acknowledge the Traditional Owners of the land in which the field study was
463 conducted. We thank for Orpheus M. Butler and Xiaoguang Ouyang for assistance in proof
464 reading and suggested improvements to the manuscript. Lu Yao is supported by a Griffith
465 University (GU) and University of Chinese Academy of Sciences (UCAS) under a joint GU-
466 UCAS agreement.

467 **References**

468 Adame MF and Reef R (2020) Potential pollution sources from agricultural activities on
469 tropical forested floodplain wetlands revealed by soil eDNA. Forests 11:892

Adame MF, Reef R, Wong VNL, Balcombe SR, Turschwell MP, Kavehei E, Rodríguez DC, Kelleway JJ, Masque P, Ronan M (2019) Carbon and nitrogen sequestration of melaleuca floodplain wetlands in tropical Australia. Ecosystems:1–13

Adame MF, Hermoso V, Perhans K, Lovelock CE, Herrera-Silveira JA (2014) Selecting cost-effective areas for restoration of ecosystem services. Conserv Biol 29:493–502

Adame MF, Santini NS, Tovilla C, Vázquez-Lule A, Castro L, Guevara M (2015) Carbon stocks and soil sequestration rates of tropical riverine wetlands. Biogeosciences 12:3805–3818

Adame MF, Kauffman JB, Medina I, Gamboa JN, Torres O, Caamal JP, Reza M, Herrera-Silveira JA (2013) Carbon stocks of tropical coastal wetlands within the Karstic landscape of the Mexican Caribbean. PLoS One 8:e56569

Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen AD, Stromberger M, Wallenstein M, Weintraub MN (2013) Extracellular enzymes in terrestrial, freshwater, and marine environments: Perspectives on system variability and common research needs. Biogeochemistry 117:5–21

Armour JD, Nelson PN, Daniells JW, Rasiah V, Inman-Bamber NG (2013) Nitrogen leaching from the root zone of sugarcane and bananas in the humid tropics of Australia. Agric Ecosyst Environ 180:68–78.

Austin AT, Vivanco L, González-Arzac A, Pérez LI (2014) There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. New Phytol. 204:307–314

Balduff DM (2007) Pedogenesis, inventory, and utilization of subaqueous soils in Chincoteague Bay, Maryland (PhD dissertation). University of Maryland

Ballantine K and Schneider R (2009) Fifty-five years of soil development in restored freshwater depressional wetlands. Ecol Appl 19(6):1467–1480

495 Bolton KG, Greenway M (1997) A feasibility study of Melaleuca trees for use in constructed
496 wetlands in subtropical Australia. Water Sci and Technol 35:247–254
497 Braunack MV, McGarry D, Venture SYDJ (2006) Traffic control and tillage strategies for
498 harvesting and planting of sugarcane (*Saccharum officinarum*) in Australia. Soil Tillage
499 Res 89:86–102.
500 Braunack MV, Arvidsson J, Håkansson I (2006) Effect of harvest traffic position on soil
501 conditions and sugarcane (*Saccharum officinarum*) response to environmental
502 conditions in Queensland, Australia. Soil Tillage Res 89:103–121.
503 Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass
504 phosphorus in soil. Soil Biol Biochem 14:319–329
505 Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the
506 release of soil nitrogen: A rapid direct extraction method to measure microbial biomass
507 nitrogen in soil. Soil Biol Biochem 17:837–842
508 Bui EN, Henderson BL (2013) C:N:P stoichiometry in Australian soils with respect to
509 vegetation and environmental factors. Plant Soil 373:553–568
510 Carnell PE, Windecker SM, Brenker M, Baldock J, Masque P, Brunt K, Macreadie PI (2018)
511 Carbon stocks, sequestration, and emissions of wetlands in south eastern Australia.
512 Global Change Biol 24:4173–4184.
513 Cenini VL, Fornara DA, McMullan G, Ternan N, Carolan R, Crawley MJ, Clement JC
514 Lavorel S (2016) Linkages between extracellular enzyme activities and the carbon and
515 nitrogen content of grassland soils. Soil Biol Biochem 96:198–206
516 Chen CR, Hou EQ, Condron LM, Bacon G, Esfandbod M, Olley J, Turner BL (2015) Soil
517 phosphorus fractionation and nutrient dynamics along the Cooloola coastal dune
518 chronosequence, southern Queensland, Australia. Geoderma 257:4–13
519 Cui Y, Fang L, Guo X, Wang X, Zhang Y, Li P, Zhang X (2018) Ecoenzymatic

stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. Soil Biol Biochem 116:11–21

Davidson, NC (2014) How much wetland has the world lost? Long-term and recent trends in global wetland area. Mar Freshw Res 65:934–941

Dixon MJ, Loh J, Davidson NC, Beltrame C, Freeman R, Walpole M (2016) Tracking global change in ecosystem area: The Wetland Extent Trends index. Biol Conserv 193:27–35.

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20:601–606

Fierer N, Grandy AS, Six J, Paul EA (2009) Searching for unifying principles in soil ecology. Soil Biol Biochem 41:2249–2256

Follstad JJ, Brian S, Kevin HH (2014) Extracellular enzyme kinetics scale with resource availability. Biogeochemistry 287–304

Fonte SJ, Nesper M, Hegglin D, Velásquez JE, Ramirez B, Rao IM, Bernasconi SM, Bünemann EK, Frossard E, Oberson A (2014) Pasture degradation impacts soil phosphorus storage via changes to aggregate-associated soil organic matter in highly weathered tropical soils. Soil Biol Biochem 68:150–157

Grace JB, Keeley JE (2006) A structural equation model analysis of postfire plant diversity in California shrublands. Ecol Appl 16:503–14

Grybos M, Davranche M, Gruau G, Petitjean P and Pédrot M (2009) Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. Geoderma 154(1–2):13–19

Hamza MA and Anderson WK (2003) Responses of soil properties and grain yields to deep ripping and gypsum application in a compacted loamy sand soil contrasted with a sandy clay loam soil in Western Australia. Australian Journal of Agricultural Research, 54:273–282.

Hayes MA, Jesse A, Hawke B, Baldock J, Tabet B, Lockington D, Lovelock CE (2017) Dynamics of sediment carbon stocks across intertidal wetland habitats of Moreton Bay, Australia. Global Change Biol, 23:4222–4234

Hou EQ, Chen CR, Wen, DZ, Liu X (2014) Relationships of phosphorus fractions to organic carbon content in surface soils in mature subtropical forests, Dinghushan, China. Soil Res 52:55–63

Hoyos-Santillan J, Lomax BH, Turner BL, Sjögersten S (2018) Nutrient limitation or home field advantage: Does microbial community adaptation overcome nutrient limitation of litter decomposition in a tropical peatland? J Ecol 106:1558–1569

Hu Y, Wang L, Tang Y, Li Y, Chen J, Xi X, Zhang Y, Fu X, Wu J, Sun Y (2014) Variability in soil microbial community and activity between coastal and riparian wetlands in the Yangtze River estuary - Potential impacts on carbon sequestration. Soil Biol Biochem 70:221–228

Jenkinson DS (1990) The turnover of organic carbon and nitrogen in soil. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 329(1255):361–368

Jia GM, Cao J, Wang C and Wang G (2005) Microbial biomass and nutrients in soil at the different stages of secondary forest succession in Ziwulin, northwest China. For Ecol Manag 217(1):117–125

Jiang Y, Lei Y, Qin W, Korpelainen H, Li C (2019) Revealing microbial processes and nutrient limitation in soil through ecoenzymatic stoichiometry and glomalin-related soil proteins in a retreating glacier forefield. Geoderma 338:313–324

Johnson AKL, Ebert SP, Murray AE (2000) Land cover change and its environmental significance in the Herbert River catchment, north-east Queensland. Australian Geographer, 31(1):75–86

570 Johnston SGA (2003) Alteration of groundwater and sediment geochemistry in a sulfidic
571 back swamp due to Melaleuca quinquenervia encroachment. Soil Res 1343–1367
572 Johnston SGA, Slavich PG, Hirst P (2003) Alteration of groundwater and sediment
573 geochemistry in a sulfidic back swamp due to *Melaleuca quinquenervia* encroachment.
574 Aust. J Soil Res 41:1343–1367
575 Keddy PA, Fraser LH, Solomeshch AI, Junk WJ, Campbell DR, Arroyo MTK, Alho CJR
576 (2009) Wet and Wonderful: The World's Largest Wetlands Are Conservation Priorities.
577 Bio Science 59:39–51
578 Kristensen E, Bouillon S, Dittmar T, Marchand C (2008) Organic carbon dynamics in
579 mangrove ecosystems: a review. Aquat Bot 89: 201–19
580 Kuscu KSI, Cetin M, Yigit N, Savaci G, Sevik H (2008) Relationship between enzyme
581 activity (urease-catalase) and nutrient element in soil use. Pol J Environ Stud 27: 2107–
582 2112
583 Lovelock CE, Duarte CM (2019) Dimensions of blue carbon and emerging perspectives. Biol
584 Lett 15:20180781
585 Luo L, Wu R, Gu JD, Zhang J, Deng S, Zhang Y, Wang L, He Y (2018) Influence of
586 mangrove roots on microbial abundance and ecoenzyme activity in sediments of a
587 subtropical coastal mangrove ecosystem. Int Biodeterior Biodegrad 132:10–17
588 Luo ZK, Wang EL, Sun OJ (2010). Soil carbon change and its responses to agricultural
589 practices in Australian agro-ecosystems: a review and synthesis. Geoderma, 155:3-4,
590 211–223.
591 Luo ZK, Feng WT, Luo YQ, Baldock J, Wang EL (2017). Soil organic carbon dynamics
592 jointly controlled by climate, carbon inputs, soil properties and soil carbon fractions.
593 Global Chang Biol, 23: 4430–4439.
594 Ma SH, He F, Tian D, Zou DT, Yan ZB, Yang YL, Zhou TC, Huang KY, Shen HH, Fang JY

595 (2018) Variations and determinants of carbon content in plants: a global synthesis.
596 Biogeosciences 15: 693–702
597 Manzoni S, Jackson RB, Trofymow JA, Porporato A (2008) The global stoichiometry of
598 litter nitrogen mineralization. Science 321:684–686
599 Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on
600 carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol Monogr 80:89–
601 106
602 Mitsch WJ, Day JW, Wendell GJ, Groffman PM, Hey DL, Randall GW, Wang NM (2001)
603 Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River Basin:
604 strategies to counter a persistent ecological problem. BioScience 51:373–388
605 Nahlik AM, Fennessy MS (2016) Carbon storage in US wetlands. Nat Commun 7:13835
606 Ouyang XG, Lee SY (2020) Improved estimates on global carbon stock and carbon pools in
607 tidal wetlands. Nat Commun 11:317
608 Pekkan OI, Kurkcuoglu MAS, Cabuk SN, Aksoy T, Yilmazel B, Kucukpehlivan T, Dabanli
609 A, Cabuk A, Cetin M (2021) Assessing the effects of wind farms on soil organic carbon.
610 Environ Sci Pollut Res 1–18
611 R Core Team (2015) R: A language and environment for statistical computing. R Foundation
612 for Statistical Computing, Vienna, Austria
613 Page KL, Bell M, Dalal RC (2014) Changes in total soil organic carbon stocks and carbon
614 fractions in sugarcane systems as affected by tillage and trash management in
615 Queensland, Australia. Soil Res 51:608–614
616 Rayment GE, Lyons DJ (2011) Soil Chemical Methods: Australasia. vol. 3. CSIRO
617 publishing
618 Reddy KR, DeLaune RD (2008) Biogeochemistry of wetlands: science and applications,
619 CRC press

620 Rokosch AE, Bouchard V, Fennessy S and Dick R (2009) The use of soil parameters as
621 indicators of quality in forested depressional wetlands. Wetlands 29(2):666–677
622 Salter J, Morris K, Bailey PCE, Boon PI (2007) Interactive effects of salinity and water depth
623 on the growth of *Melaleuca ericifolia* Sm. (Swamp paperbark) seedlings. Aquat Bot
624 86:213–222
625 Sanchez G (2013) PLS Path Modeling with R. Trowchez Editions. Berkeley, USA
626 Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial
627 carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–
628 563
629 Schulze E-D, Beck E, Müller-Hohenstein K. 2005. Plant Ecology. Springer: Dordrecht.
630 Sinsabaugh RL, Linkins AE (1990) Enzymic and chemical analysis of particulate organic
631 matter from a boreal river. Freshw Biol 23:301–309
632 Sinsabaugh RL, Follstad-Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory.
633 Annu Rev Ecol Evol Syst 43:313–343
634 Sinsabaugh RL, Hill BH, Follstad-Shah JJ (2009) Ecoenzymatic stoichiometry of microbial
635 organic nutrient acquisition in soil and sediment. Nature 462:795–798Sinsabaugh RL,
636 Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack
637 D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS,
638 Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH
639 (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264
640 Sterner RW, Elser JJ (2002) Ecological Stoichiometry: The Biology of Elements from
641 Molecules to the Biosphere. Princeton University Press
642 Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil
643 phosphatase activity. Soil Biol Biochem 1:301–307
644 Thorburn PJ, Wilkinson SN (2013) Conceptual frameworks for estimating the water quality

benefits of improved agricultural management practices in large catchments. Agr Ecosyst Environ 180:192–209

Tran DB, Dargusch P, Herbohn J, Moss P (2013) Interventions to better manage the carbon stocks in Australian *Melaleuca* forests. Land Use Pol 35: 417–420

Tran DB, Dargusch P (2016) Melaleuca forests in Australia have globally significant carbon stocks. For Ecol Manage 375:230–237

Urakawa R, Ohte N, Shibata H, Isobe K, Tateno R, Oda T, Hishi T, Fukushima K, Inagaki Y, Hirai K, Oyanagi N (2016) Factors contributing to soil nitrogen mineralization and nitrification rates of forest soils in the Japanese archipelago. For Ecol Manage 361:382–396.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

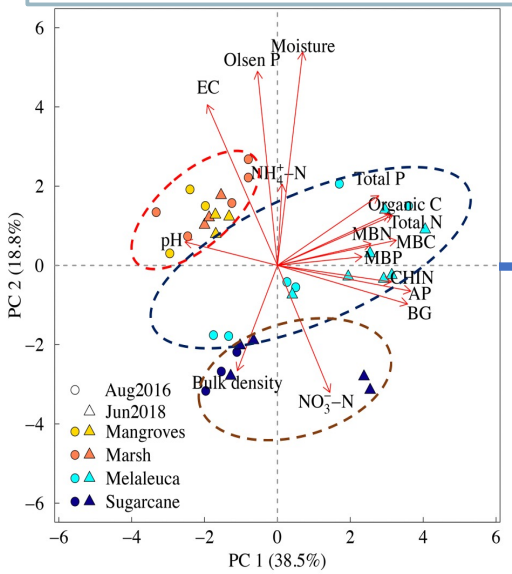
Veres Z, Kotroczó Z, Fekete I, Tóth JA, Lajtha K, Townsend K, Tóthmérész B (2015) Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability. Appl Soil Ecol, 92:18–23

Viscarra Rossel RA, Bui EN (2016) A new detailed map of total phosphorus stocks in Australian soil. Sci Total Environ 542:1040–1049

Weiss C, Weiss J, Boy J, Iskandar I, Mikutta R, Guggenberger G (2016) Soil organic carbon stocks in estuarine and marine mangrove ecosystems are driven by nutrient colimitation of P and N. Ecol Evol 6:5043–5056

Organic carbon stock in coastal wetlands are largely underestimated. ? The determinants

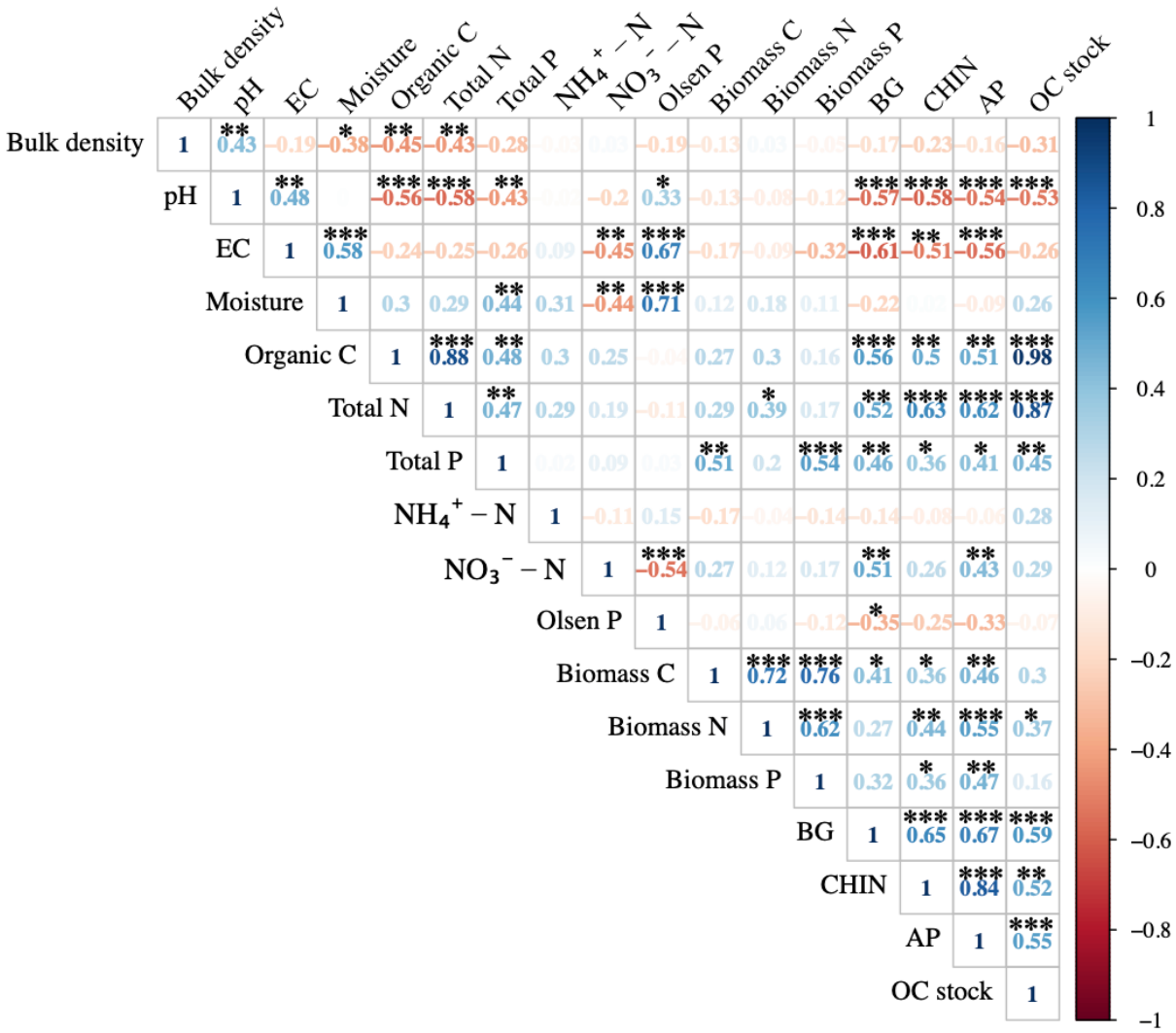
PCA biplot of the four vegetation types based on soil properties.



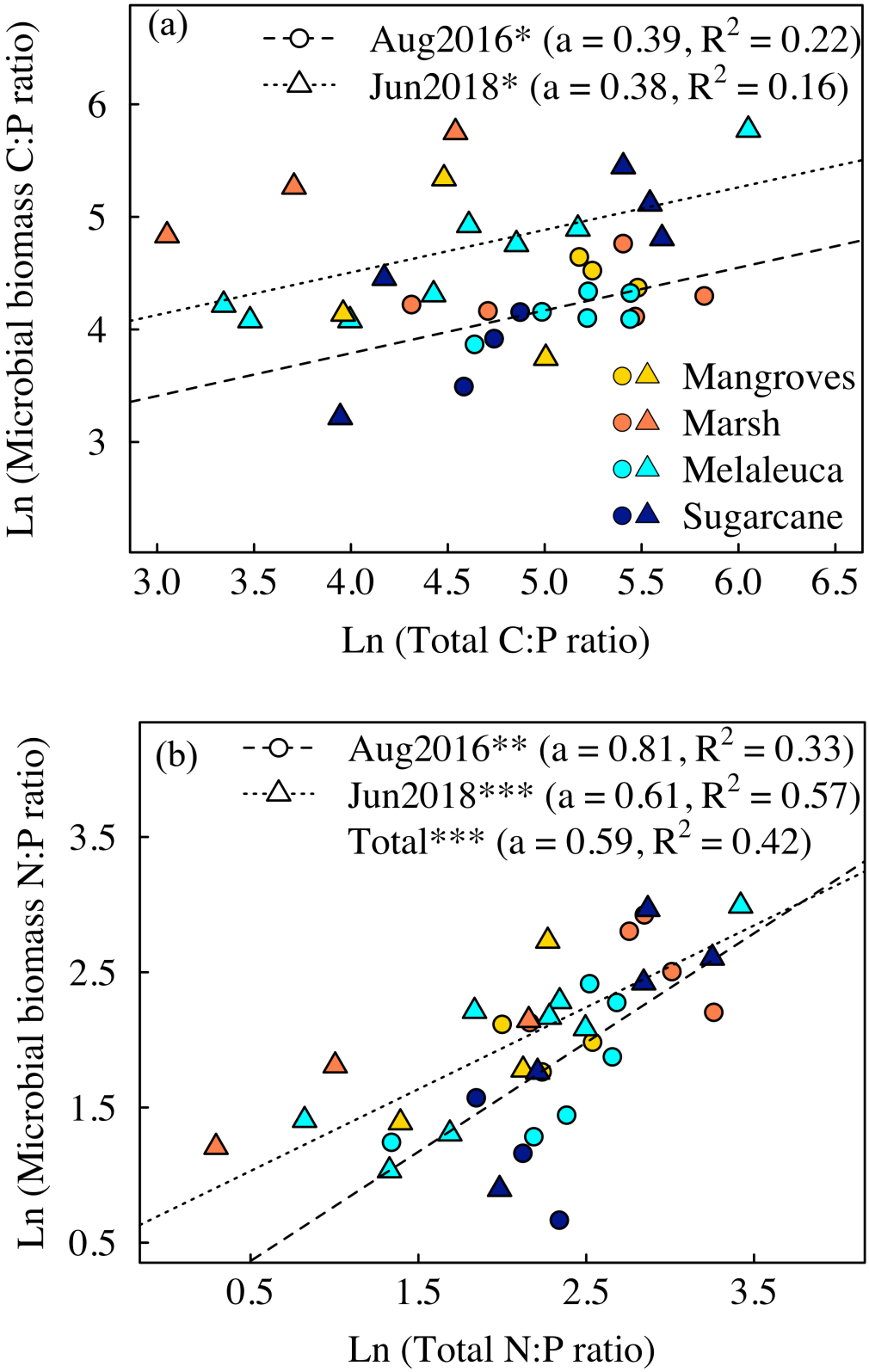
- Abiotic properties
- Biotic properties
- Resource ratios
- Biomass ratios
- Enzyme ratios

Management implications ⊕ Organic carbon stock

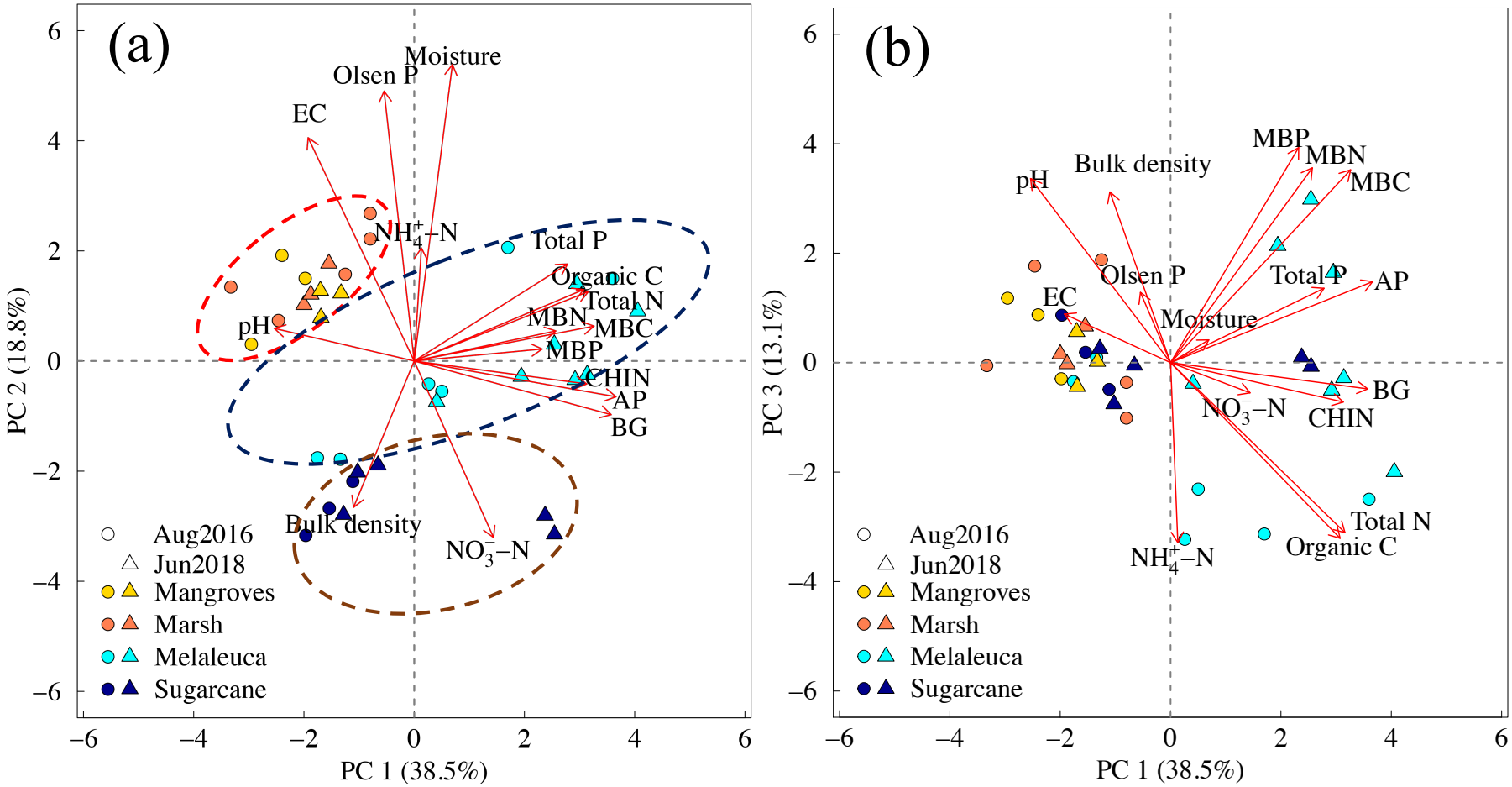
665



666 **Fig. 1** Pearson's correlations between soil physical, chemical and biological properties (n =
667 18 for all properties). The colour of circles corresponds to the direction of correlations.
668 Positive correlations are shown in blue, while negative correlations in red. '*' indicates
669 significant correlation at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. EC = electrical conductivity; C
670 = carbon; N = nitrogen; P = phosphorus; Biomass C = microbial biomass C; Biomass N =
671 microbial biomass N; Biomass P = microbial biomass P; BG = β-1,4-glucosidase activity;
672 CHIN = chitinase activity; AP = acid phosphatase activity.

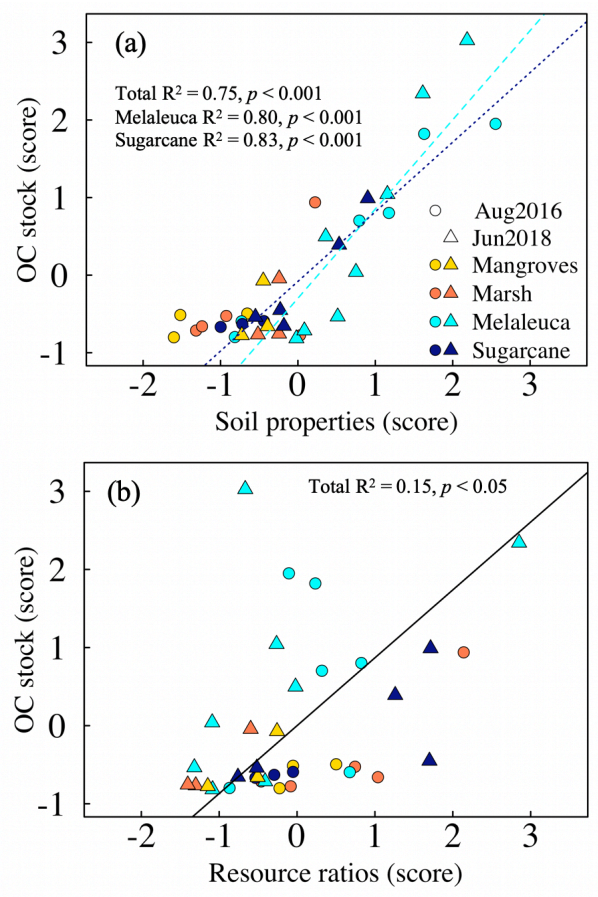


673 **Fig. 2** The relationships between microbial biomass C:P ratio and total C:P ratio (a),
674 microbial biomass N:P and total N:P ratio (b). Dash line represent the fitted linear regression
675 curve. The slope of each regression curve is parameterised by a in the legend.
676



677 **Fig. 3** Principal component analysis (PCA) scores of the (a) PC1× PC2 biplot, and (b) PC1× PC3 biplot of soil physical, chemical and biological
678 properties. EC = electrical conductivity; C = carbon; N = nitrogen; P = phosphorus; MBC = microbial biomass C; MBN = microbial biomass N;
679 MBP = microbial biomass P; BG = β-1,4-glucosidase activity; CHIN = chitinase activity; AP = acid phosphatase activity. Soil samples were
680 obtained in August in 2016 and June in 2018.

681 **Fig. 4** Path analysis results on the direct and indirect effects of 5 latent variables on soil organic C stock. Numbers show the path coefficients.
682 Dashed path indicates the effect is insignificant. See Fig. 4 for the indicators for the five latent variables. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



683 **Fig. 5** Relationships between soil properties and organic C stock (a) and resource ratios and
684 organic C stock (b). Score is the value if the latent variables belonging to each site derived
685 from path analysis model. Line represent the fitted linear regression curve between the scores
686 and measured organic C stock. Soils from the tidal freshwater forest wetlands and sugarcane
687 sites are indicated in cyan and dark-blue dash line (a), soil samples in total are indicated in
688 dark solid line (b). Total indicates samples collected across the four sampling sites.
689

690 **Table 1** Results of repeated measures ANOVA for testing the effects of the vegetation type,
691 sampling time and their interactions effect on the soil (0-30 cm) parameters.

Soil parameters	Vegetation type $F_{(3,15)}$ value	Time $F_{(1,15)}$ value	Vegetation type × Time $F_{(3.15)}$ value
Bulk density	**6.15****	0.05	0.22
pH	**19.44*****	3.38	1.81
EC	**106.31*****	0.08	1.92
Moisture	**17.95*****	0.37	0.35
Organic C stock	**8.59****	0.12	0.05
Organic C	**10.74*****	0.01	0.11
Total N	**5.41****	1.07	1.06
Total P	**6.40****	**10.61****	0.59
NH_4^+-N	**9.14*****	1.26	1.53
NO_3^--N	**5.44****	0.17	2.05
Olsen P	**56.27*****	4.16	**6.00****
MBC	0.96	**6.36***	3.23
MBN	0.94	0.64	**14.20*****
MBP	2.22	0.15	**3.30***
BG	**47.80*****	**30.97*****	**3.66***
CHIN	**27.21*****	2.29	**7.28****
AP	**27.10*****	3.91	**5.85****

692 The four vegetation types were (1) Mangroves, (2) Marsh, (3) Melaleuca and (4) Sugarcane.
693 The time represents the sampling time (1) Aug-2016 and (2) Jun-2018. The number in bold
694 illustrates significant differences at $p < 0.05$. EC = electrical conductivity; Organic C = soil
695 total organic carbon; Total N = soil total nitrogen; Total P = soil total phosphorus; MBC =
696 microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass
697 phosphorus; BG = β-1,4-glucosidase activity; CHIN = chitinase activity; AP = acid
698 phosphatase activity. Significance levels: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.
699

700

701 **Table 2** Physical, chemical and biological parameters of soils (0-30 cm) under four
702 vegetation types (mean ± sd, over two sampling times).

Soil parameters	Vegetation type			
	Mangroves n = 6	Marsh n = 6	Melaleuca n = 12	Sugarcane n = 6
Organic C stock (0-30 cm) (kg m^{-2})	5.3 ± 1.1B	6.9 ± 4.6B	13.2 ± 7.4A	5.1 ± 0.2B
pH (water)	5.4 ± 0.9A	5.3 ± 0.3A	4.1 ± 0.4B	4.8 ± 0.3A
EC (μS cm^{-1})	2033 ± 621A	1613 ± 334A	239 ± 104B	75.6 ± 48.1A
Moisture (%)	49.2 ± 4.0A	54.1 ± 12.8A	35.9 ± 20.5A	14.1 ± 1.8B
Bulk density (g cm^{-3})	1.1 ± 0.2B	1.2 ± 0.2AB	1.1 ± 0.2B	1.3 ± 0.2A
Organic C (mg g^{-1})	16.3 ± 5.2B	19.2 ± 14.2B	43.8 ± 29.4A	13.4 ± 2.8B
Total N (mg g^{-1})	0.93 ± 0.38B	2.2 ± 1.4AB	3.1 ± 2.1A	1.2 ± 0.4AB
Total P (mg g^{-1})	0.21 ± 0.06B	0.27 ± 0.13AB	0.66 ± 0.49A	0.30 ± 0.02AB
NH_4^+-N (mg kg^{-1})	4.4 ± 0.3C	8.5 ± 1.6A	8.2 ± 3.4AB	4.6 ± 0.6BC
NO_3^--N (mg kg^{-1})	3.1 ± 1.0AB	1.0 ± 1.8B	5.3 ± 3.3A	4.9 ± 1.1AB
Olsen P (mg kg^{-1})	16.8 ± 1.0A	17.1 ± 1.1A	14.0 ± 0.8B	8.4 ± 0.5C
MBC (mg kg^{-1})	331 ± 61.1A	335 ± 230A	269 ± 202A	328 ± 142A
MBN (mg kg^{-1})	29.7 ± 2.9A	66.7 ± 43.7A	24.7 ± 12.2A	24.3 ± 7.6A
MBP (mg kg^{-1})	9.5 ± 2.1A	10.6 ± 5.0A	10.8 ± 7.5A	16.9 ± 2.2A
BG (mg kg^{-1} dwt h^{-1})	14.8 ± 11.1B	4.1 ± 1.9C	69.4 ± 27.2A	9.1 ± 0.5B
CHIN (mg kg^{-1} dwt h^{-1})	10.7 ± 2.4BC	11.2 ±14.1C	26.1 ± 8.2A	12.2 ± 5.5B
AP (mg kg^{-1} dwt h^{-1})	132 ± 37B	347 ± 337B	474 ± 196A	243 ± 21B

703 Means within a row followed by the same letter indicate insignificant difference at $p < 0.05$
704 within vegetation type effect by Tukey's HSD analysis. EC = electrical conductivity; Organic
705 C = soil total organic carbon; Total N = soil total nitrogen; Total P = soil total phosphorus;
706 MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial
707 biomass phosphorus; BG = β-1,4-glucosidase activity; CHIN = chitinase activity; AP = acid
708 phosphatase activity.

709 **Table 3** Results of repeated measures ANOVA for testing the effects of the vegetation type,
710 sampling time and their interactions effect on the soil (0-30 cm) stoichiometry properties.

Soil parameters	Vegetation type $F_{(3,15)}$ value	Time $F_{(1,15)}$ value	Vegetation type × Time $F_{(3.15)}$ value
Total C:N ratio	3.16	**14.44****	**5.84****
Total C:P ratio	1.21	**11.99****	1.16
Total N:P ratio	0.27	**7.87***	**5.73****
Inorganic N:Olsen P	**16.06*****	1.02	2.16
Microbial biomass C:N ratio	0.55	**10.51****	**14.44*****
Microbial biomass C:P ratio	**3.49***	**5.79***	1.21
Microbial biomass N:P ratio	1.38	0.04	**3.69***
BG:CHIN	0.47	**16.42****	**4.89***
BG:AP	**7.62****	**5.37***	**7.50****
CHIN:AP	**7.09****	3.48	0.52

711 Significance levels: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

712

713
714 **Table 4** Stoichiometry of carbon (C), nitrogen (N) and phosphorus (P) (molar ratio) in
715 wetland soils under four vegetation types (mean ± sd, over across two sampling times).

Soil parameters	Vegetation type			
	Mangroves (n = 6)	Marsh (n = 6)	Melaleuca (n = 12)	Sugarcane (n = 6)
Total C:N ratio	21.1 ± 2.6A	11.0 ± 3.5C	17.9 ± 4.5AB	14.0 ± 1.4B
Total C:P ratio	202 ± 33A	197 ± 106A	180 ± 49.5A	114 ± 17A
Total N:P ratio	9.8 ± 2.7A	17.6 ± 6.4A	10.8 ± 4.0A	8.4 ± 2.0A
Inorganic N:Olsen P	0.45 ± 0.09C	0.56 ± 0.21BC	0.96 ± 0.26B	1.10 ± 0.01A
Microbial biomass C:N ratio	13.1 ± 2.5A	6.2 ± 1.7A	12.0 ± 5.3A	19.8 ± 12.9A
Microbial biomass C:P ratio	91.7 ± 12.4A	76.8± 22.9A	64.0 ± 10.8AB	49.0 ± 15.5B
Microbial biomass N:P ratio	7.1 ± 1.2A	13.0 ± 4.5A	6.5 ± 3.2A	3.3 ± 1.4A
BG:CHIN	1.3 ± 0.9A	1.3 ± 1.2A	2.8 ± 0.8A	1.0 ± 0.5A
BG:AP	0.10 ± 0.05A	0.04 ± 0.04B	0.16 ± 0.08A	0.04 ± 0.01B
CHIN:AP	0.08 ± 0.03A	0.03 ± 0.01B	0.06 ± 0.04AB	0.05 ± 0.02B

716 Means within a row followed by the same letter indicate insignificant difference at $p < 0.05$
717 within vegetation type effect by Tukey's HSD analysis. BG:CHIN = the ratio of BG to CHIN;
718 BG:AP = the ratio of BG to AP; CHIN:AP = the ratio of CHIN to AP.
719

720

721 **Table 5**. Correlation coefficient (loading score) between each latent variable and its blocks of
722 observed variables

Latent variable	Observed variable	Loading score	Latent variable	Observed variable	Loading score
Abiotic properties			Microbial biomass ratios		
	pH	0.75		MBCN	0.18
	EC	0.43		MBCP	0.81
	Moisture	0.26		MBNP	0.95
	NH_4^+-N	0.30	Enzymatic ratios		
	NO_3^--N	0.35		BG:CHIN	0.48
	Olsen P	0.12		BG:AP	0.97
	Total P	0.91		CHIN:AP	0.46
	Total N	0.67	Resource ratios		
Biotic properties				Total C:P	0.96
	MBC	0.88		Total N:P	0.91
	MBN	0.80		IN:Olsen P	0.41
	MBP	0.60			
	BG	0.87			
	CHIN	0.79			
	AP	0.97			

723 MBC = microbial biomass C; MBN = microbial biomass N; MBP = microbial biomass P; BG
724 = β-1,4-glucosidase activity; CHIN = chitinase activity; AP = acid phosphatase activity;
725 MBCN = the ratio of MBC to MBN; MBCP = the ratio of MBC to MBP; MBNP = the ratio
726 of MBN to MBP; BG:CHIN = the ratio of BG to CHIN; BG:AP = the ratio of BG to AP;
727 CHIN:AP = the ratio of CHIN to AP; IN:Olsen P = the ratio of inorganic N to Olsen P.

728
729