SOIL PHYSICAL AND CHEMICAL PROPERTIES EFFECT THE SOIL MICROBIAL CARBON, NITROGEN, AND PHOSPHORUS STOICHIOMETRY IN A MANGROVE FOREST, SOUTH CHINA

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Abstract. Mangrove wetland ecosystem is a coastal ecological key area that combines ecological characteristics of land and marine environments. This study examined soil carbon (C), nitrogen (N), and phosphorus (P) and their stoichiometry in three dominant mangrove species (*Aegiceras corniculatum*, *Kandelia obovata*, and *Avicennia marina*) distributed in the Guangxi Beilun Estuary Nature Reserve, China. Results showed that soil organic carbon (SOC) was highest in *K. obovata*, whereas soil total nitrogen (TN) and phosphorus (TP) were highest in *A. corniculatum*. The C:N, C:P, and N:P ratios in *K. obovata* were greater than those in the others. The microbial biomass C (MBC), N (MBN), and P (MBP) concentrations varied in ranges of 33.45–249.44 mg kg^{-1}, 5.17–9.17 mg kg^{-1}, and 0.17–0.43 mg kg^{-1}, respectively. Similar to soil C, N, and P stoichiometry, *K. obovata* had the highest MBC, MBC:MBN, and MBC:MBP values, whereas the highest MBN and MBN:MBP were found in *A. marina*, and the highest MBP was found in mudflats. Overall, this study demonstrated that the soil stoichiometry and soil microbial biomass responded differently to different plant communities and these differences might be accounted for by variations in the environmental conditions of the three communities.
Keywords: *soil nutrients, ecological stoichiometry, microbial biomass, mangrove forest, estuarine wetland*

Introduction

Mangrove ecosystems are an essential ecotone between terrestrial and marine environments in tropical and subtropical coastlines (Pires et al., 2012). These intertidal forests contribute to coastline protection, reducing the effect of waves and tsunamis, producing detritus to sustain an extensive food web, and acting as nutrient filters between terrestrial and marine ecosystems (Luo et al., 2018). The stability of mangrove ecosystems is influenced by salinity; soil physical and chemical properties, such as nutrient content dynamics; and physiological tolerance (Tripathi et al., 2016).

Ecological stoichiometry focuses on the balance in multiple chemical substances (particularly carbon, C; nitrogen, N; phosphorus, P) in ecological interactions and processes of ecosystems (Elser et al., 2000; Meng et al., 2021). Soil C, N, and P stoichiometry in mangrove ecosystems is not only determined by primary production of the ecosystem and sedimentation processes but is also affected by environmental factors,

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such as species composition, soil properties, the tidal gradient, and water salinity (Liu et al., 2019; Meng et al., 2021). For example, the absorption of P by sediment particles in coastal waters results in low N:P ratios of mangrove soils under temperate inorganic sediment-rich coastal landforms (Harrison et al., 2005). In addition, C:N:P stoichiometric interactions can enhance or weaken the carbon-climate feedback, and reconciling site-specific mechanisms that regulate C:N:P stoichiometry in mangrove ecosystems is beneficial to improve capacity and predict carbon stocks in coastal wetlands (Rovai et al., 2018). Thus, exploring soil C, N, and P stoichiometry can help to improve our understanding of the potential impacts of nutrients on ecosystem processes under environmental change in mangrove forests.

In addition to the effect of soil C, N, and P stoichiometry, soil microbes also determine the changes associated with the ecological process and function (Bai et al., 2021). Since soil microbial biomass has been proven to be highly correlated with environmental factors, such as soil pH (Rousk et al., 2009), moisture (Fierer et al., 2003), soil organic C (Xu et al., 2014), and biological properties (He et al., 2020), these soil microorganisms affect the changes in ecological processes, such as organic matter decomposition, nutrient cycling, and mineralization (Yang et al., 2010; Rawat et al., 2021). In addition, the variation in soil microbial biomass can indicate the soil fertility and stability (Angst et al., 2018). For example, soil microbial biomass carbon (MBC) is used to predict alterations in soil carbon stocks (Luo et al., 2020; Srivastava et al., 2020). Meanwhile, soil microbial biomass serves as a crucial buffer for soil nitrogen and nitrogen immobilization in fall seasons when nitrogen availability is higher, while releasing nitrogen in early spring when the nitrogen availability is low (Zak et al., 2003). Though extensive research has been carried out on the ecology, structure, and function of the mangrove ecosystem, very limited research is available concerning an array of soil microbial biomass that is a sensitive indicator of environmental change. Therefore, the primary objectives of this study were as follows: to (a) analyze soil and microbial stoichiometry with different mangrove species; (b) explore which environmental variables are most strongly correlated with soil and microbial stoichiometry. This study will provide a basis for the differential restoration of coastal ecosystems in the future.

Materials and Methods

Study area

The Beilun Estuary, Fangchenggang City, Guangxi Zhuang Autonomous Region, P.R. China is located at 21°31′00″–21°37′30″N and 108°00′30″–108°16′30″E with a 150 km coastline, including a land area of 53 km^2 and mangrove forest area of 12.74 km^2 (*Figure 1*). It is dominated by a subtropical climate, and the mean annual temperature, mean annual precipitation, and evaporation are 22.3 °C, 2220.5 mm, and 1400 mm, respectively (Zhou et al., 2020). The rainy season is mainly from June to August. This ecosystem consists of two typical ecological niches (i.e., mudflat and mangrove forest). *Aegiceras corniculatum*, *Kandelia obovata*, and *Avicennia marina* are the three dominant mangrove species in the Beilun estuarine wetland (Zhou et al., 2020).

Sampling

In this study, we chose four sites, each of which was from mudflat to mangrove forests, to collect surface (0–20 cm) soils in May of 2020 (*Table 1*). For each sample, three

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subsamples were mixed to compose one sample. The soil samples were homogenized and sieved through a mesh size of 2 mm to remove plant debris and roots and then transported to the lab immediately for further analyses. Soil samples were stored at 4 °C to determine the physicochemical characteristics.

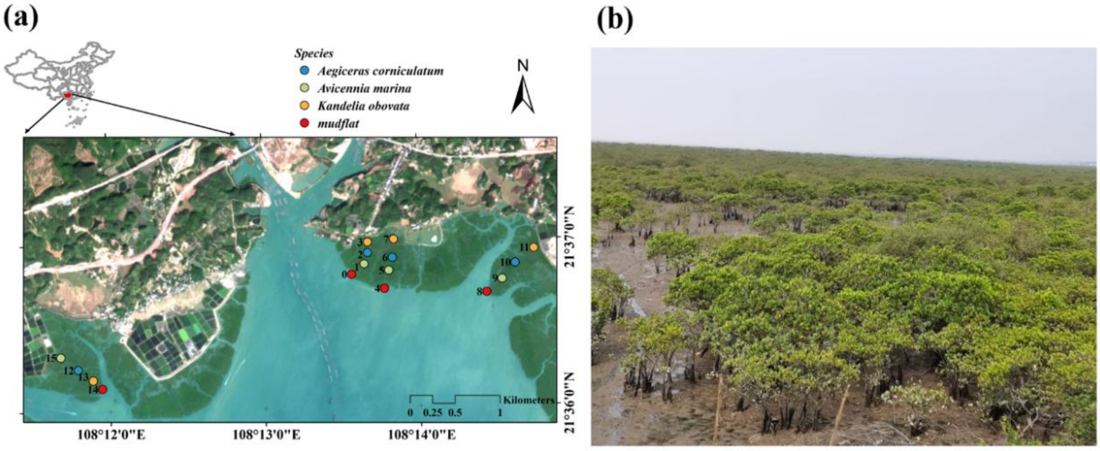

Figure 1. *(a) Map of soil samples collecting locations in mangrove forest ecosystems; (b) sampling environment in the study*

Table 1. *Coordinates and dominant species of sampling sites*

Sampling site	Longitude	Latitude	Dominant species
0	108.226044	21.613317	Mudflat
1	108.2274	21.614351	*A. marina*
2	108.227793	21.615475	*A. corniculatum*
3	108.227816	21.61652	Mudflat
4	108.229551	21.611845	Mudflat
5	108.230035	21.613669	*A. marina*
6	108.230447	21.61495	*A. corniculatum*
7	108.23061	21.616781	*K. obovata*
8	108.240517	21.61133	*K. obovata*
9	108.242201	21.612635	*A. marina*
10	108.243625	21.614211	*A. corniculatum*
11	108.24566	21.615661	*K. obovata*
12	108.19651	21.60421	*A. corniculatum*
13	108.198069	21.60313	*K. obovata*
14	108.199072	21.602287	Mudflat

Soil physical and chemical properties

For the determination of the soil moisture content, soil samples were oven dried at 105 °C for 24 h. The pH was determined in a 1:2.5 (soil:water) slurry using a Delta 320 pH meter (Model Delta 320, Mettler Toledo, Switzerland). The salinity of soil was quantified using the dregs-drying math method. Soil organic carbon (SOC) concentrations were measured by the $K_2Cr_2O_7$–H_2SO_4 oxidation method (Zhang et al., 2009). TN concentrations were measured using a Carlo Erba CNS Analyzer (Carlo Erba, Milan, Italy) (Gallaher et al., 1976). TP concentrations were measured via perchloric-acid

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digestion followed by ammonium-molybdate colorimetry (Wang et al., 2006). The units (g kg^{-1}) of SOC, TN, and TP concentrations were transformed to mol kg^{-1} to calculate the C:N, C:P, N:P ratios of each soil sample as the molar ratios (atomic ratio).

Soil microbial biomass C, N, and P

Soil MBC, MBN, and MBP concentrations were analyzed by the chloroform fumigation-extraction method after 65 h of incubation (Brookes et al., 1982; Wu et al., 1990). The soil was split into two parts, and one half was placed in a desiccator with chloroform. The desiccator was evacuated until the $CHCl_3$ had boiled for 2 min, and samples were fumigated for 24 h at room temperature. For MBC and MBN analysis (multi N/C 2100, Analytik Jena), the samples were extracted with 0.5 M K_2SO_4 with a soil:solution ratio of 1:5. For MBP analysis (FIALAB, MLE Dresden), a Bray-1 solution (0.03 M NH_4Fe, 0.025 M HCl) with a soil:solution ratio of 1:10 was used. MBC:MBN:MBP ratios were calculated from molar MBC, MBN, and MBP concentrations.

Statistical analysis

One-way analysis of variance and pair-wise comparison tests (Tukey’s HSD) were used to compare the concentrations of essential elements in the different mangrove species. Relationships between nutritional elements and microbial biomass were determined by correlation analysis. Multiple comparisons of the means were performed using Tukey’s test at the 0.05 significance level. All statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

The variable importance in the projection (VIP) with the PLSR model was conducted to reflect the importance of soil physical and chemical properties (soil moisture, salinity, pH; soil C, N, and P concentrations; and C:N, C:P, and N:P) for the soil microbial biomass C, N, and P concentrations and stoichiometry. Larger VIP values (≥ 1) represent the most relevant parameter for explaining the dependent variable (Zhou et al., 2017). The VIP values of these properties were calculated with SIMCA-P+13.0 (Umetrics AB, Sweden).

Results

Soil physical properties

Results showed a significant difference in soil properties, such as soil moisture, pH, and salinity, between mudflats and the three mangrove species (*Figure 2*). The average soil moisture contents of mudflats (23.64%) and *A. marina* (23.52%) were significantly lower than those in *A. corniculatum* (29.08%) and *K. obovata* (30.76%). The highest pH (4.97) and lowest salinity (0.99%) were in mudflats, whereas there was no significant difference among the three mangrove species in pH and salinity.

Soil C, N, and P concentrations and stoichiometry

The C, N, and P concentrations and stoichiometry of soil exhibited marked difference, as in *Figure 3*. The concentrations of SOC ranged from 5.05 to 12.63 g kg^{-1}, with an average of 9.95 g kg^{-1}. The TN and TP concentrations varied from 0.38 to 0.60 g kg^{-1} and 0.08 to 0.18 g kg^{-1}, with mean values of 0.49 and 0.14 g kg^{-1}, respectively. The SOC concentration was higher in the *K. obovata* plant community than in the other plant communities, whereas the N and P concentrations in *A. corniculatum* plant communities

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were both higher than those in the other plant communities. The C:N, C:P, and N:P ratios exhibited the ranges of 21.63 to 26.31, 138.82 to 258.72, and 6.21 to 9.66, respectively. The C:N, C:P, and N:P ratios in *K. obovata* plant communities were greater than those in the other plant communities.

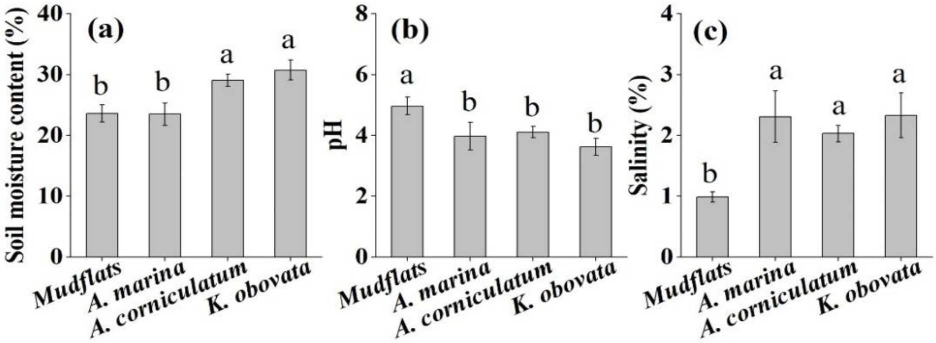

Figure 2. *Characteristics of soil moisture content (a), pH (b), and salinity (c) content for four zones. Values are means ± SE. Different letters indicate significant difference among treatments at 0.05 significance level*

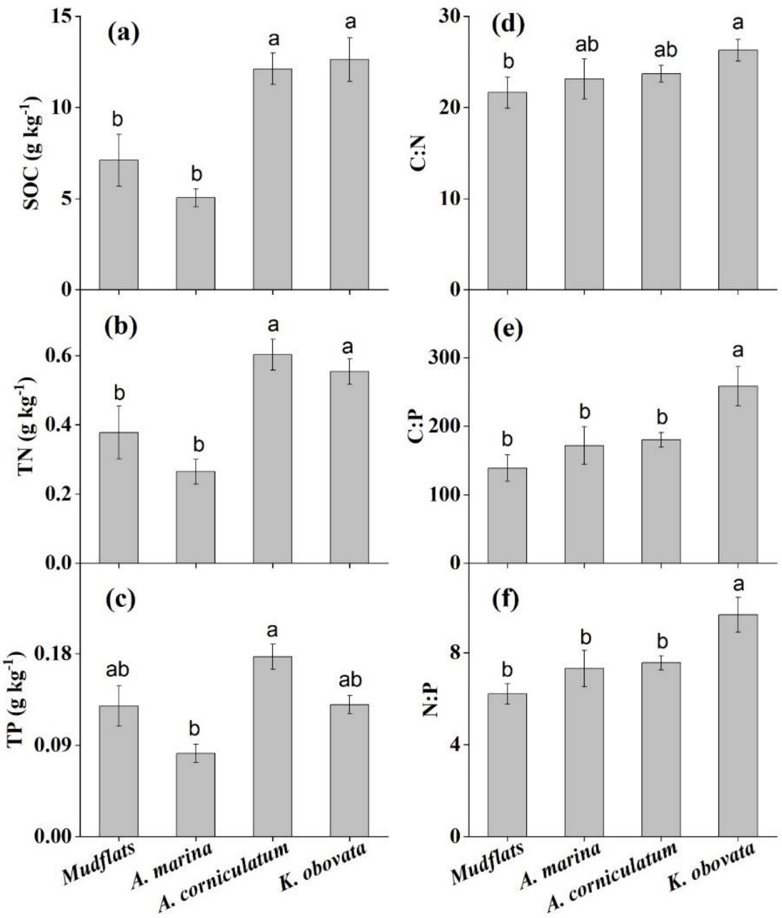

Figure 3. *Concentrations of SOC (a), TN (b) and TP (c), ratios of C:N (d), C:P (e) and N:P (f) for four zones. Values are means ± SE. Different letters indicate significant difference among treatments at 0.05 significance level*

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Microbial biomass C, N, and P stoichiometry

The microbial biomass concentration (MBC, MBN, MBP) and their stoichiometry (MBC:MBN, MBC:MBP, MBN:NBP ratios) in the studied soil samples are illustrated in *Figure 4*. The MBC, MBN, and MBP varied from 33.45 to 249.44 mg kg^{-1}, 5.17 to 9.17 mg kg^{-1}, and 0.17 to 0.43 mg kg^{-1}, with averages of 150.33 mg kg^{-1}, 6.35 mg kg^{-1}, and 0.26 mg kg^{-1}, respectively. The highest MBC, at a significance level, was observed in *K. obovata* plant communities, followed by that in *A. corniculatum*, mudflats, and *A. marina*. In contrast to that for MBC, significantly higher MBN was shown in *A. marina* than in the other soils. The highest MBP was observed in mudflats. Similar to that with MBC:MBN, a markedly higher MBC:MBP ratio was found in *K. obovata*, whereas a higher MBN:MBP ratio was shown in *A. marina*.

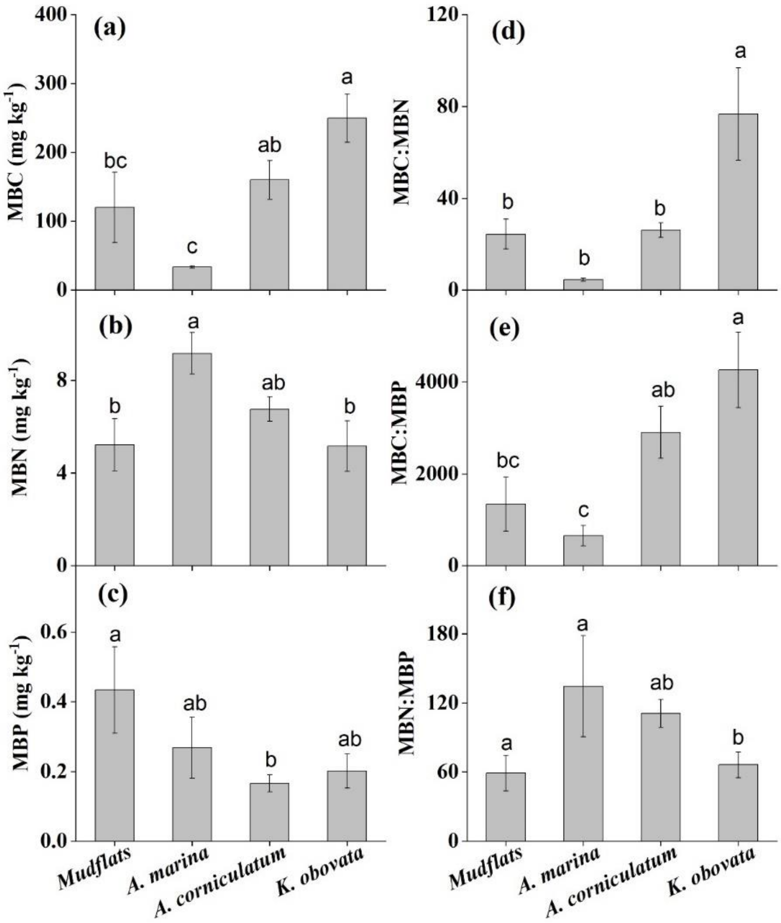

Figure 4. *Characteristics of soil microbial biomass (a-c) and ratios (d-f) for four zones. Values are means ± SE. Different letters indicate significant difference among treatments at 0.05 significance level. MBC, microbial biomass C. MBN, microbial biomass N. MBP, microbial biomass P*

Correlations among soil and microbial biomass stoichiometry, and environment

Soil moisture was significantly positively associated with salinity, concentrations of SOC, TN, MBC, MBN, and ratios of C:N, C:P, N:P, and MBC:MBP, but was negatively associated with MBP concentrations. Soil pH was negatively associated with SOC and

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ratios of C:N, C:P, N:P, and MBC:MBN but was positively associated with MBP. Soil salinity was positively related to ratios of N:P and MBN:MBP and negatively related to MBP (*Figure 5*).

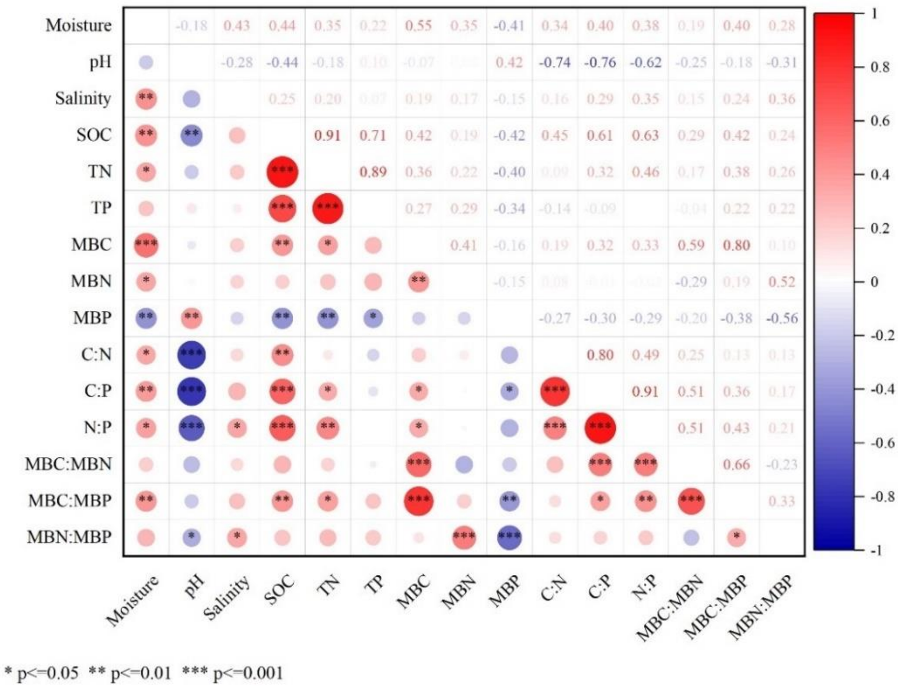

Figure 5. *Pearson's correlations between soil physical, chemical, and biological properties. The color of circle corresponds to the direction of correlations. Positive correlations are shown in blue, while negative correlations in red. '*' indicates significant correlation at P< 0.05; **P< 0.01; ***P< 0.001. MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P*

Overall, there were significant correlations among SOC, TN, TP concentrations. Additionally, soil properties were significant correlate with microbial properties. SOC and TN were positively associated with MBC and MBC:MBP but were negatively associated with MBP. TP was negatively associated with MBP. MBC was positively associated with MBP and ratios of C:P, N:P. MBP was negatively associated with ratios of C:P (*Figure 5*).

The weight plots indicated the variables with the highest weights in the optimal model (*Figure 6a*). In the soil and microbial biomass stoichiometry model, the first component was dominated by the soil moisture, with positive PLSR weights. The second component was dominated by pH, with negative PLSR weights (*Figure 6a*). A more convenient expression of the relative importance of the soil physical chemistry factors could be obtained from their VIP values and regression coefficients (*Figure 6b*). For microbial biomass models, the soil moisture was the key variable. All considered variables were related to soil microbial biomass, but those with a VIP < 1 were considered of minor importance for prediction purposes. Therefore, the discussion is limited to variables with a VIP > 1.

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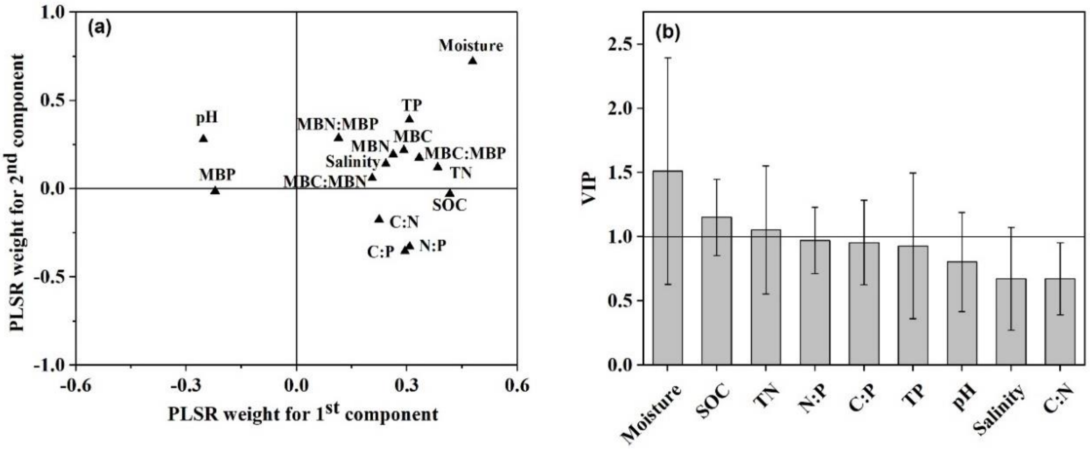

Figure 6. *(a) Weight plots of the first and second PLS components for soil microbial biomass and stoichiometry; (b) Variable importance for the projection (bars) and regression coefficients (lines) of each predictor of soil microbial biomass. The straight solid line indicates a threshold above which predictors are considered to be important for interpretation*

Discussion

All of soil SOC (9.23 g kg^{-1}), TN (0.45 g kg^{-1}), and TP (0.13 g kg^{-1}) in this study area were lower than the mean values reported previously for China (19.33 g kg^{-1}, 1.04 g kg^{-1}, and 0.75 g kg^{-1}, respectively) (Tian et al., 2010), Chongming island (15.94 g kg^{-1}, 0.74 g kg^{-1}, and 0.74 g kg^{-1}, respectively) (Yun et al., 2009), and Minjiang estuary wetland (17.02 g kg^{-1}, 1.02 g kg^{-1}, and 0.24 g kg^{-1}, respectively) (Wang et al., 2012). The difference might be related to various comprehensive factors, such as the source of elements, decomposition process of organic matter, root exudates, and tidal and human disturbances. The N:P ratio (7.69) was approximately equal to that reported in China (Zhou et al., 2020), whereas the ratios of C:N (23.7) and C:P (187.35) were approximately two times higher than those in China (13 and 105, respectively). This could be caused by the activity of soil microorganisms and the oxidation of organic carbon being restrained, as well as the accumulation of SOC in wetlands (Yang et al., 2013; Wang et al., 2014). In addition, mangrove forests are the most important ecosystems for organic carbon sequestration and storage, largely owing to the high SOC levels produced by burial rates, which are higher on average than those in other terrestrial ecosystems (McLeod et al., 2011; Breithaupt et al., 2019).

Soil microbial biomass serves as a pool of nutrients in the soils, which plays an important role in ecosystem sustainability and is a sensitive indicator under the changing environmental conditions. In the present study, the mean concentrations of MBC (33.45-249.44 mg kg^{-1}), MBN (5.17–9.17 mg kg^{-1}), and MBP (0.17–0.43 mg kg^{-1}) in the three mangrove species were lower than those on South Andaman Islands, India (141-489 mg kg^{-1}, 14–38 mg kg^{-1}, and 3.3–15.4 mg kg^{-1}, respectively) (Dinesh and Chaudhuri, 2013). The reason for this might be that land-use changes in mangrove forests in India have altered the soil microbial biomass and microbial community over the long-term. In addition, plants provide the energy to the soil system in the form of litter and root exudates, which eventually are turned into soil microbial biomass (Ohtonen et al., 1999). Several studies suggested that the shifts in stoichiometry of microbial biomass C:N, C:P, and N:P are predictable across a range of biological scales (Sistla et al., 2015; Yao et al.,

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2019). Globally, the average soil microbial biomass C:N ratio is 7.6, estimated by Xu et al. (2013), and 8.6, estimated by Cleveland and Daniel (2007). This study suggested that the microbial biomass C:N is consistently higher (except for *A. marina*, with a microbial biomass C:N of 4.57) than these global average values. This is probably due to the presence of more organic matter and the low N availability to soil microbes (Rawat et al., 2021). Compared to the results of a study by Dinesh and Chaudhuri (2013), the soil microbial C:P was higher in this study. This could be due to poor soil P availability in the soil (Rawat et al., 2021). Meanwhile, Xu et al. (2013) reported that the soil microbial biomass N:P ratio was more constrained than soil N:P ratios. Cleveland and Daniel (2007) suggested that the soil microbial biomass N:P ratio (6.9) could be an indicator of ecosystem nutrient limitations, and a relatively higher microbial biomass N:P ratio indicates P limitation. Higher biomass N:P ratios were found in the present study, indicating that the mangrove forest might be more likely to be P-limited.

Furthermore, MBC and MBN concentrations were significantly positively correlated with SOC and TN concentrations ($p < 0.01$). The reasons for this might be that soil C and N could promote soil microbial activity, and their availability to the soil microorganisms is possibly increased through root exudates. Soil C availability is one of the vital ecological driving factors for microbial community dynamics and has a crucial effect on the microbial community structure under nutrient-limited conditions. For example, de Vries et al. (2012) indicated that the SOC can provide more readily available C and energy for soil microbial communities. Soil N can also affect the soil microbial composition and MBN by directly supplying N for soil microbial metabolism (Huang et al., 2014). Results in this study are consistent with previous results, suggesting that MBC and MBN concentrations are closely related to changes in the soil microorganism concentrations (Huang et al., 2015). In addition, the positive relationship between soil moisture and microbial biomass C confirms the findings of Curtin et al. (2012) and Rawat et al. (2021). SOC decomposition could be associated with physical protection, thee inhibited diffusion of catabolites, and interactivity with the surface of soil organic matter (Zhao et al., 2020).

In wetlands, larger numbers of microorganisms might require more oxygen to decompose SOC, and when soil moisture exceeds the optimal level, increased water availability can suppress microbial activity. Water-logged soil with limited oxygen availability has been shown to reduce the SOC decomposition rate (Qu et al., 2021). However, the negative relationship between soil moisture and microbial biomass P in our study is not in accordance with the results of the study by Rawat et al. (2021). A possible reason for this might be that frequent drying and rewetting increases the volatility of microbial biomass P (Bagheri et al., 2020). Soil pH is the one of the factors affecting nutrient cycling in soil and microbial biomass in the present study. Low pH might also lead to the higher SOC concentration owing to slow decomposition processes under low pH conditions, and this result agrees with the finding of Liu et al. (2018). Salinity was positively and strongly correlated with N:P and MBN:MBP, which might be due to a shift in the microbial community composition leading to variance in soil nutrients and altering the stoichiometry of microbial biomass, as reported by Hu et al. (2019). However, this finding was contrary to that of Hartzell and Jordan (2012), who proposed that spatiotemporal changes in the dynamics of N and P could shift nutrient availability in the estuaries owing to the mixing of freshwater and seawater.

Conclusions

Our results confirmed that the soil and microbial biomass stoichiometry is influenced by plant communities and soil properties in the mangrove forest. However, these results are based on a preliminary field investigation, and further controlled incubation experiments are needed to confirm the mechanisms by which plant communities and soil properties influence soil stoichiometry characteristics.

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Conflict of Interests. The authors declare that they have no competing interests.

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