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Elemental Stoichiometry (C, N, P) of Soil in the Wetland Critical Zone of Dongting Lake, China: Understanding Soil C, N and P Status at Greater Depth

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Abstract: Earth's critical zone is defined as a plant–soil–water system, which covers a wide area and has a large vertical thickness, but the soil elemental stoichiometry characteristics of the critical zone at different depths are still unclear. In this study, the spatial distribution of soil carbon (C), nitrogen (N) and phosphorus (P) in the critical zone of a typical wetland in Dongting Lake, China, and their ecological chemometric characteristics were analyzed. The results indicated that: (1) the average C, N and P contents were 18.05, 0.86 and 0.52 g/kg, respectively, with a decreasing trend from the surface to the deeper layers. The soil is relatively rich in C and P, while N is the main element limiting plant growth and development. (2) The mean values of soil C/N, N/P and C/P were 21.1, 1.7 and 35.4 respectively, with the C/N ratio and C/P ratio showing a trend of increasing and then decreasing in the vertical direction and reaching a maximum at a depth of 2–5 m below ground. (3) According to the correlation results, C, N and P in soils are coupled and influenced by each other ($p < 0.001$), and pH, infiltration coefficient and human activities are closely related to the spatial distribution of C, N and P. (4) Stable Redfield ratios (1:1.6:35.4) may exist in lake wetland soils, and future studies should be conducted for complete systems of the same type of wetlands. The results of the study will provide a theoretical basis for the sustainable development and scientific management of lake wetlands.

Keywords: soil; lake wetlands; carbon; nitrogen; phosphorus; ecological stoichiometry; Redfield ratio

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1. Introduction

Wetlands are a transition zone between terrestrial and aquatic ecosystems [1], with unique hydrological, soil, vegetation and biological characteristics, and are a sink for a variety of biogenic elements in the Earth's surface layer, where a variety of nutrients are continuously exchanged and energy is transferred between wetland soils and plants, driven by coupled physical, chemical, biological and geological processes [2,3]. Carbon (C), nitrogen (N) and phosphorus (P) are the most important nutrients in wetland ecosystems and the stoichiometric relationships between these elements influence the chemical and biological processes of ecosystems [4]. Soil C, N and P elements are coupled in the ecosystem cycle [5], C sequestration in the ecosystem is largely controlled by key elements such as N and P [6], plants that perform nitrogen fixation result in greater phosphatase activity [7] and P also promotes the functioning of biological photosynthesis and carbohydrate synthesis [8]. In recent years, human activities and climate change have threatened lake and coastal wetland ecosystems to varying degrees, resulting in wetland destruction, reduced ecosystem productivity and sharp declines in biodiversity, which in turn have led to a decrease in soil organic matter content and nutrient reductions [9,10]. Therefore, exploring the dynamic changes and synergistic mechanisms of C, N and P in wetland soils is of great scientific significance for gaining a deeper understanding of the biogeochemical cycles in wetland ecosystems in response to human activities and climate change.

Redfield's 1958 study [11] found that the atomic number ratios of C, N and P in marine plankton were similar to those of oceanic seawater (C:N:P(R_{CNP}) = 106:16:1); it also found that this ratio was regulated by environmental and biological interactions. The development of Redfield's theory led ecologists to look for similar patterns of constant stoichiometric ratios and interrelationships in terrestrial ecosystems [12], and gave rise to a new field of research—ecological chemometrics [13]. Ecological chemometrics is the study of the balance and coupling of multiple chemical elements (mainly N, P and C) in the ecological interactions of organisms [14]. In the field of ecological research, C, N and P ecological stoichiometry ratios are important indicators used to predict organic matter decomposition rates and determine nutrient limitation factors [15,16], which can effectively explain biological productivity, nutrient constraints and limitations, homeostatic regulation of ecosystems and evolution [17,18]. For example, Fan et al. [19] showed that stoichiometric characteristics are closely related to tree growth under nutrient limitations in subtropical forests; Zeng et al. [20] concluded that root N/P ratios can be used to indicate nutrient limitation in grassland species when using N/P ratios as indicators of plant nutrient limitation. While many previous results in ecological chemometrics have focused on the elemental ecochemistry of terrestrial and aquatic ecosystems, research on wetland ecosystems, which are in the transition zone between terrestrial and aquatic ecosystems, has been very limited and has mostly analyzed the elemental ecochemistry of plant tissues. The same emphasis has not been placed on the ecological chemistry of soil elements, which is important for revealing the availability of nutrients and the cycling and balancing mechanisms of elements such as carbon, nitrogen and phosphorus.

The Earth's critical zone is a complex system that controls soil development, water transport and elemental geochemical cycling processes, regulating the functions and properties of resources [21], and therefore critical zone research has become an increasingly active scientific frontier in the international geological community in recent years [22,23]. An integrated study of Earth's critical zones and wetland systems will enrich the scientific content of wetland critical zones. Liu et al. [24] integrated the mechanisms of various driving forces to give a conceptual model of wetland critical zones and an overall framework for the coupling of water, soil, rock and biology in wetland ecosystems, with spatial boundaries ranging from the vertical area of the wetland vegetation canopy (above) and the base of the aquifer (below). Such a framework suggests that the wetland critical zone covers a wider area and is vertically thicker than the surface wetland zone, the surface water-groundwater interaction zone, and the vertical infiltration zone, with more complex nutrient biogeochemical cycles, patterns and multiple influences, and where elemental transport, exchange, cycling and interaction responses are more active [25,26]. Therefore, by studying the changes in C, N and P contents in wetland critical zone soils and their ecological chemometric characteristics, we can more comprehensively reveal the drivers of nutrient transport and transformation in wetland ecosystems, and thus explore the biogeochemical cycling of nutrients and their coupling mechanisms.

Dongting Lake is the second-largest freshwater lake in China. It is a spillway-type lake that receives the Xiangjiang River, Zishui River, Yuanjiang River and Lishui River. As a result of sedimentation, the lake bed is constantly silted up and raised, forming large areas of lake wetlands. As the last natural barrier to reduce the input of watershed materials into the lake, lake wetlands not only intercept pollution entering the lake, but also play an important role in controlling eutrophication in the lake [27,28]. Therefore, this paper used wetland critical zone soils in the Dongting Lake area as the study object and selected representative sample sites with the aims of: (1) investigating the spatial and vertical distribution patterns of C, N and P contents and stoichiometric ratios in wetland soils; (2) revealing the C, N and P interrelationships in wetland soils and their ecological stoichiometric drivers; and (3) exploring whether stable Redfield ratios exist in lake wetlands.

2. Materials and Methods

2.1. Study Region

Dongting Lake, located in the middle reaches of the Yangtze River (about 28°300′ N–30°200′ N, 111°400′ E–113°100′ E), has a humid monsoon climate in central China, with abundant rainfall. Dongting Lake is a typical throughput lake, consisting of East Dongting Lake, South Dongting Lake and West Dongting Lake. The inland water system flowing into Dongting Lake includes Xiangjiang River, Zijang River, Yuanjiang and Lishui (Figure 1). The Dongting Lake basin covers an area of 2.63×10^5 km^2 with an average annual precipitation of 1.35×10^3 mm, of which the South Dongting Lake wetland covers 1.68×10^3 km^2, making it one of the largest freshwater wetlands in southern China [29,30], which preserves a rich and diverse wetland landscape ecosystem, natural scenery of the lake and human landscape resources, and is important in water storage. It plays an important role in water storage, flood regulation, climate control, soil and water conservation, water purification and biodiversity protection.

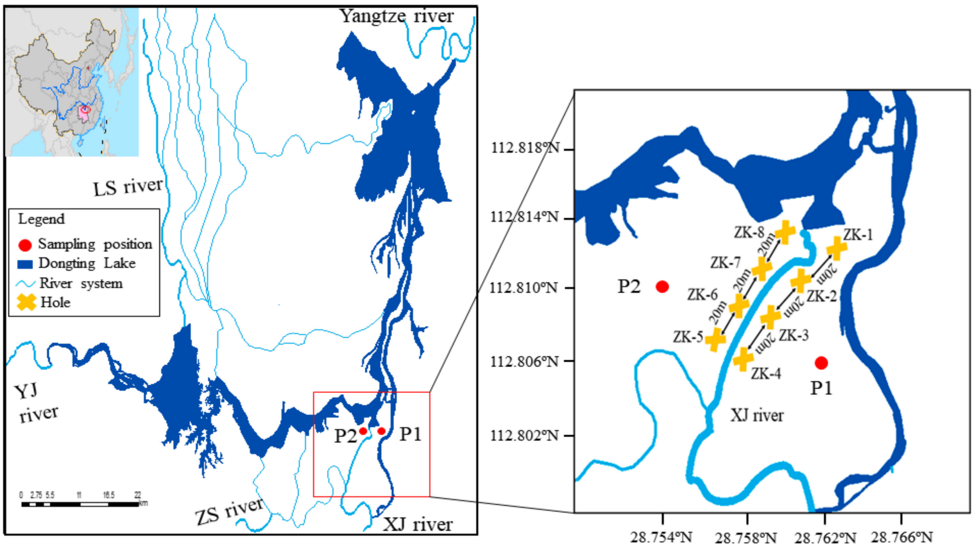


Figure 1. Soil sampling sites in the Lake Wetland Critical Zone of the Poyang Lake region, southern China; LS—Lishui; YJ—Yuanjiang; ZS—Zishui; XJ—Xiangjiang.

2.2. Soil Sampling and Measurements

2.2.1. Sample Collection

The most representative wetlands in the South Dongting Lake area were selected and two monitoring profiles (P1 and P2) were arranged perpendicular to the lakeshore in the critical zone of the wetland (Table 1), where P1 was a natural wetland with the dominant species being phragmites australis, suaeda glauca and typha orientails preslreed, with a large number of plant species and high coverage; P2 was a wetland that has undergone a small amount of reclamation, with the dominant species being *phragmites australis* and typha orientails preslreed, and human activities such as agricultural farming have resulted in fewer plant species and less coverage than P1, especially the destruction of vegetation along the lake shore. Each profile was provided with four monitoring holes, with a total of eight monitoring holes (ZK-1~ZK-8) (Figure 1), with a depth of 9 m–10 m and a diameter of 110 mm. The boreholes were drilled between 12 October and 14 October 2021 and soil samples were collected on site using a ring knife at a time when the soil moisture was suitable, the lake level was stable and the temperature was average for the study area, so that the lakeshore soil elements determinations were subject to interference caused by less lakeside sedimentation. Samples were taken at intervals of 1 m above the underground water level and 2 m below the water level, and 9–11 undisturbed soil samples were taken from each borehole in total. At the same time, in order to carry out comparative research, the paddy soil samples near the drilling hole were also collected (depth 0–1 m). The retrieved samples were packed in plastic bags, sealed and stored at 4 °C. Before testing, the samples were removed from the soil, dried naturally and then crushed and ground. Samples used to measure soil pH, water content (W) and permeability coefficient (K) were screened by a 20-mesh sieve; samples for measuring soil C, N and P content were screened by a 100-mesh sieve.

Table 1. Basic condition of study plots.

Section Number	Wetland Type	Latitude and Longitude	Plants Types	Soil Texture	Note
P1	Lake wetland	112°48′22″ E 28°45′29″ N	Phragmites australis, Suaeda glauca, Typha orientails Presl	Silty clay, Clay	Natural wetlands, less affected by human activities
P2	Lake wetland	112°48′36″ E 28°45′15″ N	Phragmites australis, Typha orientails Presl	Silty clay, Clay, Silt	Experienced wetland reclamation with minor anthropogenic impacts

2.2.2. Soil Sampling and Measurements

Soil pH was determined using the potentiometric method by first shaking air-dried soil (10 g, <2 mm) with 25 mL of deionized water for 1 min, leaving it for 30 min, then repeating the process and measuring it with a PHS-3C acidity meter. Soil water content was measured by the drying method, i.e., by weighing 15–30 g of soil sample into an aluminum box, then placing it in an oven at a constant temperature of 105 °C for 8 h and then removing it and weighing it. There are several methods to determine the permeability coefficient, and considering that the soil samples used in the test are mostly pulverized clay, the saturation permeability coefficient of the soil samples was determined by the variable head Darcy permeability test method [31], using a TST-55 permeameter. Soil total organic C content was measured by oxidative spectrophotometry with potassium dichromate [32]. Briefly, each soil sample of approximately 0.5 g was suspended in 5 mL of 0.8 M $K_2Cr_2O_7$ and homogenized with 7.5 mL of H_2SO_4. The homogenates were kept in a water bath at 135 °C for 30 min and absorbance was measured at 585 nm. Total N content was determined by Kjeldahl method using 1 g soil and 7.5 mL H_2SO_4 treatment [33]. Total soil P was determined by inductively coupled plasma emission spectrometry, i.e., 0.2 g of air-dried sample with 0.149 mm pore size was weighed, 1 mL of concentrated HNO_3, 1 mL of concentrated HCl and 2 mL of HF were added, and the determination was carried out by ICP-OES instrument after digestion.

2.3. Data Analyses

The variation of pH with depth was plotted using Excel 2010. Contour plots of soil moisture content of the profiles were plotted using Matlab 2020a. Tests for significant differences in C, N and P concentrations and stoichiometric ratios within groups were analyzed by the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA, 2008). One-way ANOVA (analysis of variance) was used for the significance of differences and Duncan's method was used for multiple comparisons with a t-test to determine whether the differences in the assays were significant. The C, N and P content data were processed and interpolated using the ordinary Kriging method to generate spatial distribution maps. Kriging spatial interpolation and mapping were performed using ArcGIS 10.3 (Esri Inc., Redlands, CA, USA, 2018). SPSS-based functions were fitted to the variation of C, N and P with soil depth in 81 soil samples and Pearson correlation analysis was performed to obtain the correlation between soil C, N, P, W and pH factors and R_{CN}, R_{CP} and R_{NP}. The R_{CN}, R_{CP} and R_{NP} were gridded, and finally the spatial variation of R_{CN}, R_{CP} and R_{NP} and the correlation heat map were plotted using the OriginPro 9.1 software package (OriginLab Inc., Northampton, MA, USA, 2018).

3. Results and Discussion

3.1. Physico-Chemical Properties of Soil

The critical zone of the Dongting Lake wetland is saline, with different perennial waterlogging and different soil physicochemical properties. The characteristic map of soil pH variation with depth is shown in Figure 2. The soil pH in the P1 profile of the critical zone of the wetland varies between 5.02 and 6.27, with the P1 profile being acidic, while the soil pH in the P2 profile varies between 5.86 and 7.64, with the P2 profile being neutral.

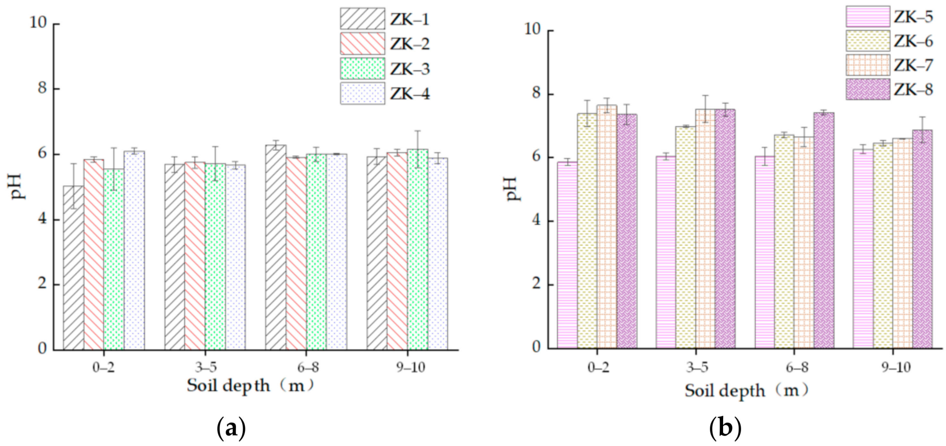


Figure 2. Characteristics of pH variation with depth in wetland critical zone soils ((**a**) belongs to section P1, (**b**) belongs to section P2, ZK–1~ZK–8 respectively represent eight monitoring holes).

The water content characteristics of the wetland critical zone soil samples obtained by the drying method were plotted in Figure 3. The average water content of the P1 profile in the wetland critical zone was 38.8% and that of the P2 profile was 36.6%. The water content of the P1 profile was slightly higher than that of the P2 profile, which was mainly due to the fact that the P2 profile had undergone wetland reclamation and had less vegetation cover compared to the P1 profile (Table 1). The relatively exposed P2 profile, therefore, receives direct solar radiation, with high evaporation from the surface and a consequent reduction in water content. However, according to the results of the one-way ANOVA, the overall variability in water content between profiles was not significant, which may be related to the fact that the lake wetlands were under flooding conditions for a long time.

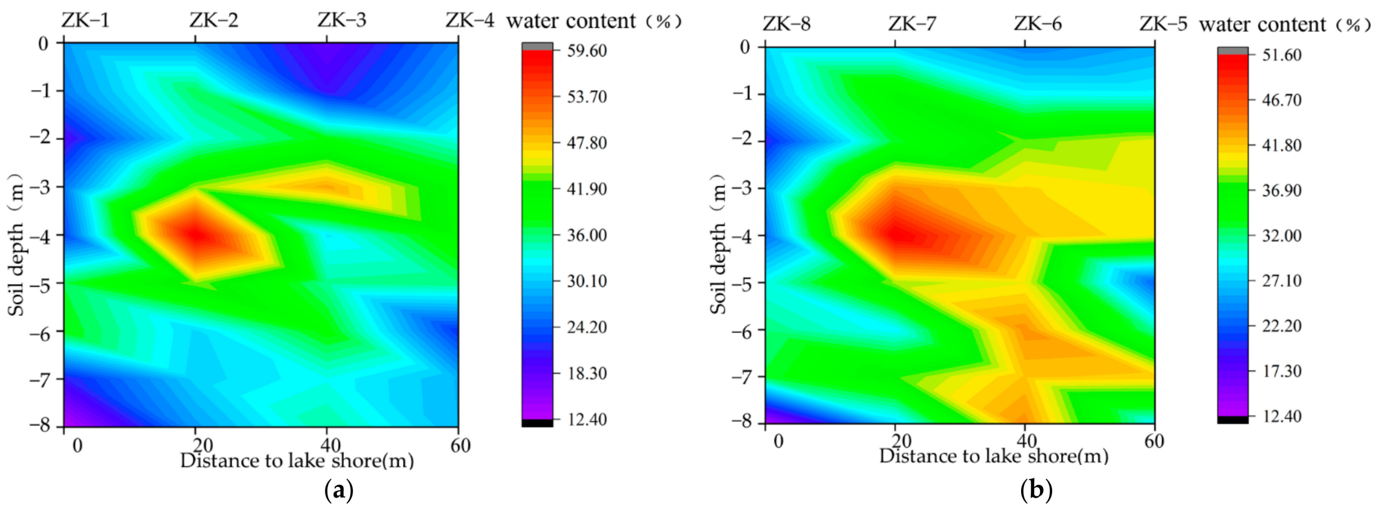
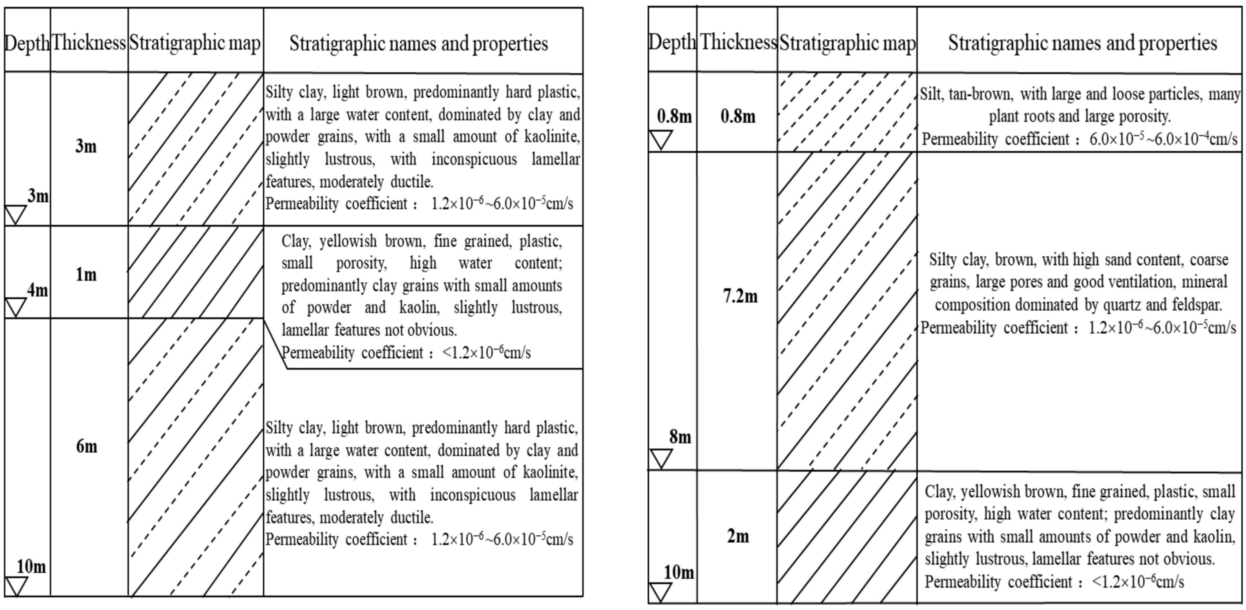


Figure 3. Characterization of soil water content changes in the wetland critical zone ((**a**) belongs to section P1, (**b**) belongs to section P2, ZK−1~ZK−8 respectively represent eight monitoring holes).

The stratigraphic column of the wetland critical zone is shown in Figure 4. The P1 profile of the wetland critical zone is dominated by clay layers, while the P2 profile is dominated by sand and clayey sand layers. The clay layer is less porous and less permeable than the sand layer, but has a higher sorption capacity [34]. As the permeability coefficient (K) of soils is an important indicator of soil texture [35], the correlation between K values and C, N and P in each soil layer was explored in this study to investigate how soil texture affects the C, N and P content of soils in the wetland critical zone and their ecological stoichiometric ratios. Overall, the soils in the study area are generally acidic, with high water content, and the soil textures are mostly silt clay layers.



(**a**) (**b**)

Figure 4. Histogram of soil stratigraphy in the wetland critical zone ((**a**) belongs to section P1, (**b**) belongs to section P2).

3.2. Spatial Changes of C, N, P Contents in Soils

The C, N and P contents of the soil in the critical zone of the Dongting Lake wetland varied from 1.8–37.2, 0.08–1.89 and 0.15–1.03 g/kg, with mean values of 18.05, 0.86 and 0.52 g/kg, respectively (Table 2), all of which were lower than the Chinese soils and those in the vicinity of the study area (Table 3). This is due to the fact that the Dongting Lake wetland is in a subtropical region characterized by high precipitation and high temperature, which intensifies soil erosion and loss of C, N and P [36]; on the other hand, the vegetation along the lake shore has an underdeveloped root system, which leads to the fact that soil C, N and P elements are not easily accumulated, while the application of N and P organic fertilizers in agricultural fields not only directly and significantly increases the effective soil N and P nutrients, but also its own biomass humification decomposition can indirectly supply nutrients to the soil [37]. However, in comparison, soil C, N and P contents in the critical zone of lake wetlands were higher than those in grassland and shrub soils (Table 3), probably due to the fact that lake wetlands are mostly in a flooded state and the anaerobic environment can reduce nitrogen loss and inhibit organic matter decomposition, thus facilitating soil C, N and P storage [38].

The C, N and P profiles of soils in the critical zone of the Dongting Lake wetland were obtained using Kriging (Normal Kriging) as shown in Figure 5. The overall decreasing trend of C, N and P elements in soils from the surface to the deeper layers in the critical zone of the wetland is due to the high return of plant residues to the soil in the lake wetland and the abundance of organic matter in the surface soil, which decreases as the soil layer deepens and there is less and less root distribution and apoplastic material for microbial decomposition. Coefficients of variation in critical zone soils were 44.81%, 36.24% and 32.63% (Table 2), with elemental spatial variability of P < N < C (Tables 2 and 4). This is mainly because the dominant species in the lake wetland is *phragmites australis*, which has high productivity and complete return of litter to the soil, with a large soil carbon sequestration effect [44]; on the other hand, due to the large diurnal temperature difference in the study area, low temperatures inhibit the ability of plants in the critical zone to sequester N and P [45]. Compared to soil P content, the C and N content of soils in the critical zone of the Dongting Lake wetland was significantly higher in the topsoil than in all other layers (Figure 5), mainly because vegetation, apoplankton and humus contributed most to soil C and N content, and these mainly acted in the topsoil and weakened with deeper soil depth [46]. The small range of soil P variability in the critical zone of the Dongting Lake wetlands is consistent with previous results, as soil P is mainly derived from rock weathering, a long and relatively stable process that is less susceptible to biotic and abiotic conditions, and therefore has less spatial variability [47].

Table 2. Statistical characteristics of soil C, N and P in the Dongting Lake wetland critical zone.

	C	N	P	R_{CN}	R_{NP}	R_{CP}
Minimum	3.23 g/kg	0.08 g/kg	0.15 g/kg	11.4	0.6	9.6
Maximum	37.20 g/kg	1.89 g/kg	1.03 g/kg	49.1	2.5	86.2
Mean	18.05 g/kg	0.86 g/kg	0.52 g/kg	21.1	1.7	35.4
CV	44.81%	36.24%	32.63%	38.5%	21.2%	42.8%

Table 3. Comparison of the average values of C, N, P and their ecostoichiometric ratio in the Dongting Lake wetland critical zone with other regions.

Type	C (g/kg)	N (g/kg)	P (g/kg)	R_{CN}	R_{NP}	R_{CP}	R_{CNP}	Reference
Global soil				14.3	186.0	13.1		Cleveland and Liptzin 2007 [39]
Chinese soil	24.6	1.9	0.8	11.9	5.2	61	60:5:1	Tian et al., 2010 [40]
Chinese wetland				18.2	13.6	245.2	245:13.6:1	Zhang et al., 2016 [41]
Grassland	3.9	0.4	0.5	9.3	0.8	7.2		Jiao et al., 2013 [42]
Shrub	5.6	0.7	0.3	8.5	2.1	18.1		Guo et al., 2020 [43]
Lake wetland critical zone	18.1	0.9	0.5	21.1	1.7	35.4	1:1.6:21	This study
Paddy soil	37.9	2.4	1.4	15.8	1.7	27.1		This study

Table 4. Concentrations of C, N and P (unit; g/kg) and their stoichiometric ratios in different profiles.

		C	N	P	R_{CN}	R_{NP}	R_{CP}
P1	Range	5.81–37.2	0.21–1.89	0.19–1.03	14.9–49.1	1.1–2.3	24.4–86.2
	SE	1212	50.9	30.7	1.5	0.04	2.3
	Mean	20.3 ^{a}	0.9 ^{a}	0.52 ^{a}	24.1 ^{a}	1.7 ^{a}	40.8 ^{a}
P2	Range	3.23–32.0	0.08–1.56	0.15–0.74	11.4–26.7	0.6–2.5	9.6–67.2
	SE	1361	52.2	23.9	0.7	0.07	2.2
	Mean	15.8 ^{b}	0.82 ^{a}	0.52 ^{a}	18.2 ^{b}	1.6 ^{a}	30 ^{b}

Different letters represent the significant difference of the mean values at each soil horizon ($p < 0.05$), whereas the same letters indicate insignificant difference.

Most of the plants in the Dongting Lake wetlands are hydrophilic herbaceous plants (Table 1), which absorb CO_2 from the atmosphere, absorb solar energy through photosynthesis and contribute significant amounts of C and other nutrients to the soil ecosystem through apoplastic litter [48], so the C, N and P content of the soil is closely linked to plants. The mean values of C, N and P in our study were higher in the P1 profile than in the P2 profile, and the variability in C content was significant (Table 4), due to the relatively low and less diverse vegetation cover and fragile environment in the P2 profile (Table 1).

There was a decreasing trend of C and N content in profile P1 from the monitoring holes along the lake to those away from the lake shore, while the opposite trend was shown in profile P2 (Figure 5), which was due to the large vegetation cover of the soil near the lake shore in the P1 profile, while the vegetation of the soil near the lake shore was destroyed after the wetland reclamation in the P2 profile. In addition to plant growth, anthropogenic influences also lead to differences in elemental content [49]. Soil C and N content in profile P1 are highest in the surface layer of the soil and gradually decreases with increasing soil depth, while soil C and N content in profile P2 tend to increase and then decrease with increasing depth and reaches a maximum at 3–5 m below ground (Figure 5). This is mainly due to agricultural tillage measures in profile 2, which disrupted the natural structure of the soil layer and caused a change in the spatial distribution of elements (Table 1). From the lake shore to the area away from the lake shore, the trend of change in soil P content in profiles P1 and P2 is not obvious, which is due to the relatively fixed source of soil P, which is mainly related to the soil type and soil-forming parent material; vertically, the soil P content in profiles P1 and P2 generally show a decreasing trend, but the decrease is smaller compared to the soil C and N content. This is because the wetland soil type is mainly silty clay (Table 1), which has a weak fixation of phosphorus and releases accumulated phosphorus more easily, even at low levels of P accumulation, and still has a high potential for phosphorus leaching. Phosphorus leaching causes soil P levels to be lower than soil C and N levels, and therefore the decline is smaller [50].

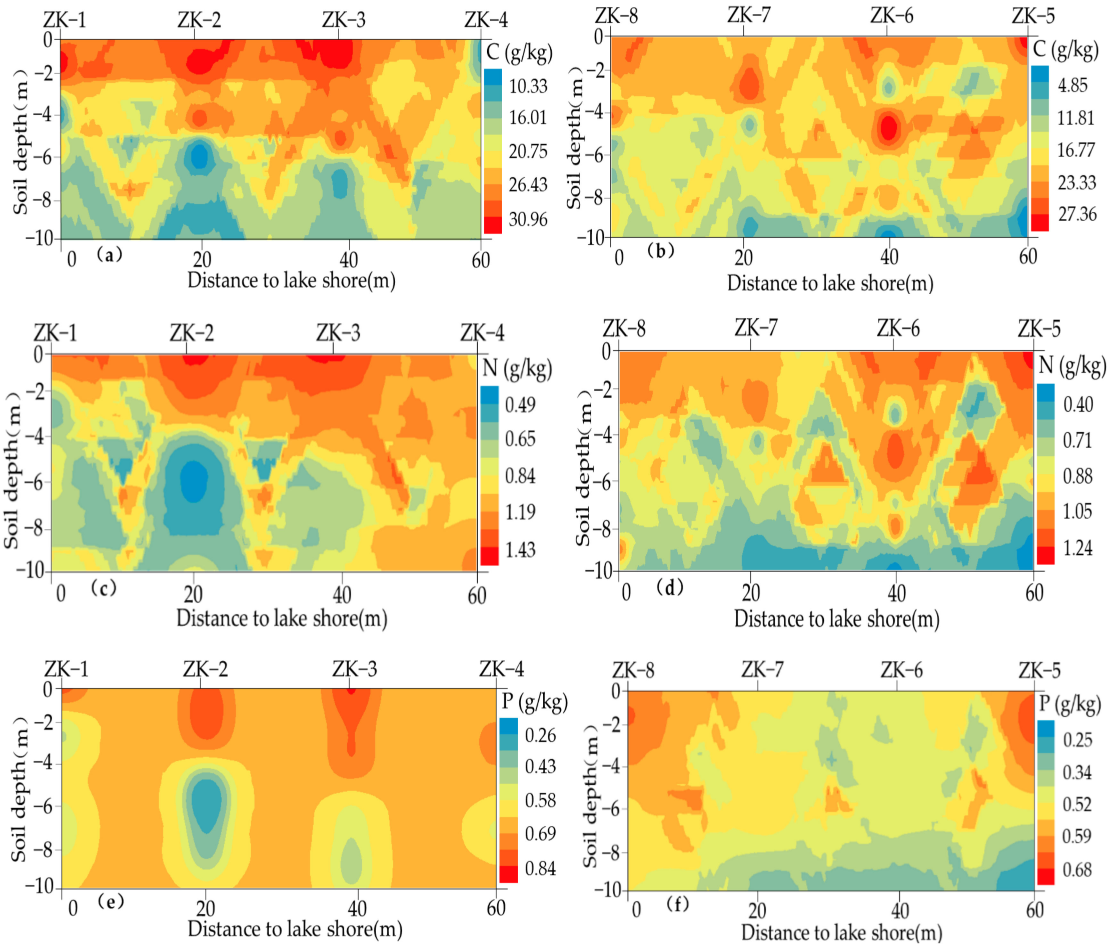


Figure 5. Spatial distributions of soil C, N and P contents in the lake wetland critical zone ((**a**,**c**,**e**) belong to section P1, (**b**,**d**,**f**) belong to section P2, ZK−1~ZK−8 respectively represent eight monitoring holes).

To gain a deeper understanding of the information on the behavior of nutrients in critical zone soils, we averaged the carbon, nitrogen and phosphorus content of each layer in the profiled soils and fitted the relationship between soil nutrient content and depth using 11 common functions, including linear, quadratic, cubic, composite, growth, logarithmic, S, exponential, inverse, power and logistic functions (Table 5). The results show that the variation of N and P content with depth in the critical zone soils can be well fitted with cubic power functions and that there is a significant correlation ($p < 0.05$). The relationship between C content and depth, which is significantly different in the critical zone soils, is most consistent with the type of growth function in the P1 profile, with a highly significant correlation ($p < 0.001$), while the relationship between C content and depth in the P2 profile is consistent with N and P, and can be well fitted with cubic function with a significant relationship ($p < 0.05$).

Table 5. The correlation between soil carbon, nitrogen and phosphorus content and soil depth in the wetland critical zone.

Nutrients	Profile	Regression Equation	Correlation Coefficient	*p*-Value
C	P1	$y = e^{-0.079x+3.337}$	0.903 ***	0.0001
	P2	$y = 20.945 - 1.470x + 0.274x^2 - 0.025x^3$	0.813 *	0.0287
N	P1	$y = 1.351 - 0.229x + 0.025x^2 - 0.001x^3$	0.846 **	0.0057
	P2	$y = 1.347 - 0.291x + 0.059x^2 - 0.004x^3$	0.917 **	0.0040
P	P1	$y = 0.770 - 0.064x - 0.003x^2 + 0.001x^3$	0.917 **	0.0012
	P2	$y = 0.759 - 0.135x + 0.028x^2 - 0.002x^3$	0.847 *	0.0176

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

3.3. Ecological Stoichiometry Characteristics of Soil

The C, N and P stoichiometric ratios in the soils of the critical zone of the Dongting Lake wetland ranged from 11.4–49.1, 0.6–2.5 and 9.6–86.2, with mean values of 21.1, 1.7 and 35.4, respectively (Table 2). Soil R_{CN} is a key parameter to characterize the rate of soil organic carbon mineralization, when soil $R_{CN} < 25$, microbial activity is relatively high and soil mineralization rate is fast, when soil C decomposition rate is faster than accumulation rate, resulting in an increase in effective N content in the soil, while organic matter decomposition is not limited by N. The opposite is true when soil $R_{CN} > 25$ [20,51]. The R_{CN} in most of the soils in this study area is higher than 25 and greater than the other regional soil averages, so overall the degree of soil organic matter humification in the key zone of the Dongting Lake wetland is low, the mineralization of organic matter is slow and organic matter decomposition is limited by elemental N. Bengtsson et al. [52] concluded that the R_{CN} of soil is inversely proportional to the rate of C decomposition, and the higher the R_{CN}, the slower the organic matter mineralization and the more conducive to the maintenance of soil fertility. R_{CN} in wetland soils in this study area was significantly higher than the Chinese soil average (Table 3), predicting larger soil C stocks in the critical zone of the Dongting Lake wetland, which is consistent with the results of Liu et al. [38]. R_{NP} is often used as a diagnostic indicator of N saturation, which can be used to determine the threshold of nutrient limitation and can indirectly predict the level of soil supply and limitation of plant nutrients [53]. The lower R_{NP} in the wetland soils of this study area, 3.5 smaller than the mean Chinese soil R_{NP} (Table 3), suggests that N is a key factor limiting plant growth and development in the critical zone of the Dongting Lake wetland. Soil R_{CP} is an important index to reflect the availability of P. The lower the R_{CP} value, the higher the activity of soil P, which is beneficial to the release of P during the decomposition of organic matter. [54]. The R_{CP} values of the wetlands in this study area were lower than the Chinese soil average (Table 3), indicating a net mineralization of organic P from soil microbial objects in the study area of the wetland critical zone and a high activity of soil P. Overall, R_{NP} and R_{CP} were relatively low and R_{CN} was relatively high in the Dongting Lake wetland critical zone, indicating that the soils of the Dongting Lake wetland were relatively enriched in C and P nutrients and relatively deficient in N nutrients (Figure 6). Recent studies have also demonstrated the prevalence of N limitation in wetland ecosystems [55,56]. This is because denitrifying bacteria and ammonia-oxidizing archaea are widespread in flooded wetlands and the reaction rates of denitrification and anaerobic ammonia oxidation increase with increasing flooding, leading to an increase in the concentration of ammonium ions and a decrease in nitrate in the soil [57]. Although nitrification is present in anaerobic wetland soils, the rate of nitrification is very small, even almost zero in deep soils of subtropical estuarine wetland [58], resulting in most of the available N being available only in the form of ammonium and a lack of nitrate, thus causing the phenomenon of N limitation in wetlands.

In this study, although the soil R_{CN}, R_{CP} and R_{NP} ratios in the critical zone of the Dongting Lake wetland fluctuated with profile changes (Figure 6, Table 4), the overall variation was small, which is consistent with the dynamic equilibrium theory of ecological chemistry [59]. The spatial distribution and variation patterns of global soil C, N and P concentrations and their stoichiometric ratios have not been clearly concluded, but decreasing soil stoichiometric ratios from surface to deeper layers are common [60,61], and Tian et al. [40] showed that soil 0–10 cm R_{CN}, R_{CP} and R_{NP} ratios were significantly higher than other soil levels. However, our study showed that, in wetland critical zone soils, R_{CN} and R_{CP} showed a trend of increasing and then decreasing in the vertical direction, reaching a maximum at 2–5 m below ground level, while R_{NP} showed an insignificant trend (Figure 6). The reasons for this difference are as follows: (1) Tian et al. used data from the second soil census in China, which were standardized to eliminate soil spatial heterogeneity. (2) Tian et al. used soils from different land-use types, whereas the current study only focused on wetlands. (3) Previous studies have mostly focused on the 0–1 m soil layer, while the segmental layer standard used in this study is relatively coarse and deeper in profile. The variation in C, N and P stoichiometric ratios from lake to shore in this study may be related to soil enzymes. Soil enzymes, as active components of the soil, are involved in the decomposition and synthesis of organic matter and the release of nutrients such as C and N. They directly or indirectly influence a range of biochemical reactions in the soil and are often used as indicators of the intensity of the material decomposition cycle in wetlands [62]. It has been shown that soil enzyme activity is affected by changes in distance from the lake shore, increasing elevation gradients and succession of plant communities to land [63], and therefore C, N and P stoichiometric ratios may change accordingly. For example, the R_{CN} and R_{CP} of the topsoil in this study are usually at a high level (Figure 6), which may be related to the β-glucosidase enzyme that directly affects organic carbon decomposition, and a large number of studies have shown that plant litter increases the activity of β-glucosidase [64,65]; urease and phosphatase directly determine soil R_{CN} and soil phosphorus activity respectively [66], and Li et al. [67] recently showed that both alkaline phosphatase and urease activities trend downwards as wetlands reverse their succession from plant communities to light panel sites, which may also be the main reason for the significant differences in R_{CN} and R_{CP} between profiles (Table 4).

3.4. Influencing Factors of Soil C, N and P Content and Ecological Stoichiometric Ratio

Correlations between elemental content and elemental stoichiometric ratios in soils of the Dongting Lake wetland critical zone are shown in Figure 7 (where autocorrelations exist, no correlation analysis was performed). In this study, although the correlation coefficient between R_{NP} and C content was low, the high level of significance of the correlation ($p < 0.01$) implies that soil C content in the wetland critical zone may be the most significant factor affecting soil C, N and P stoichiometric ratios (compared to N and P), due to the fact that C storage is to some extent closely related to the availability of N and P [68]. An abundance of N and P means a relative excess of C; and a scarcity of N and P means a relative deficit of C. Combined with the lack of N in this study, it is clear that the factor limiting effective C cycling in lake wetlands may be N (Figure 6). Most plants grow by taking up N and P mainly from the soil and C from the atmosphere for photosynthesis, so there is generally a significant positive correlation between C, N and P in the soil (with the exception of symbiotic nitrogen-fixing plants) [39]. Our study confirms this phenomenon (Figures 8 and 9), with highly significant two-by-two correlations ($p < 0.001$) between C, N and P contents in soils of the critical zone of the Dongting Lake wetland, indicating that the cycles of C, N and P elements in the critical zone of the wetland are coupled and interact with each other.

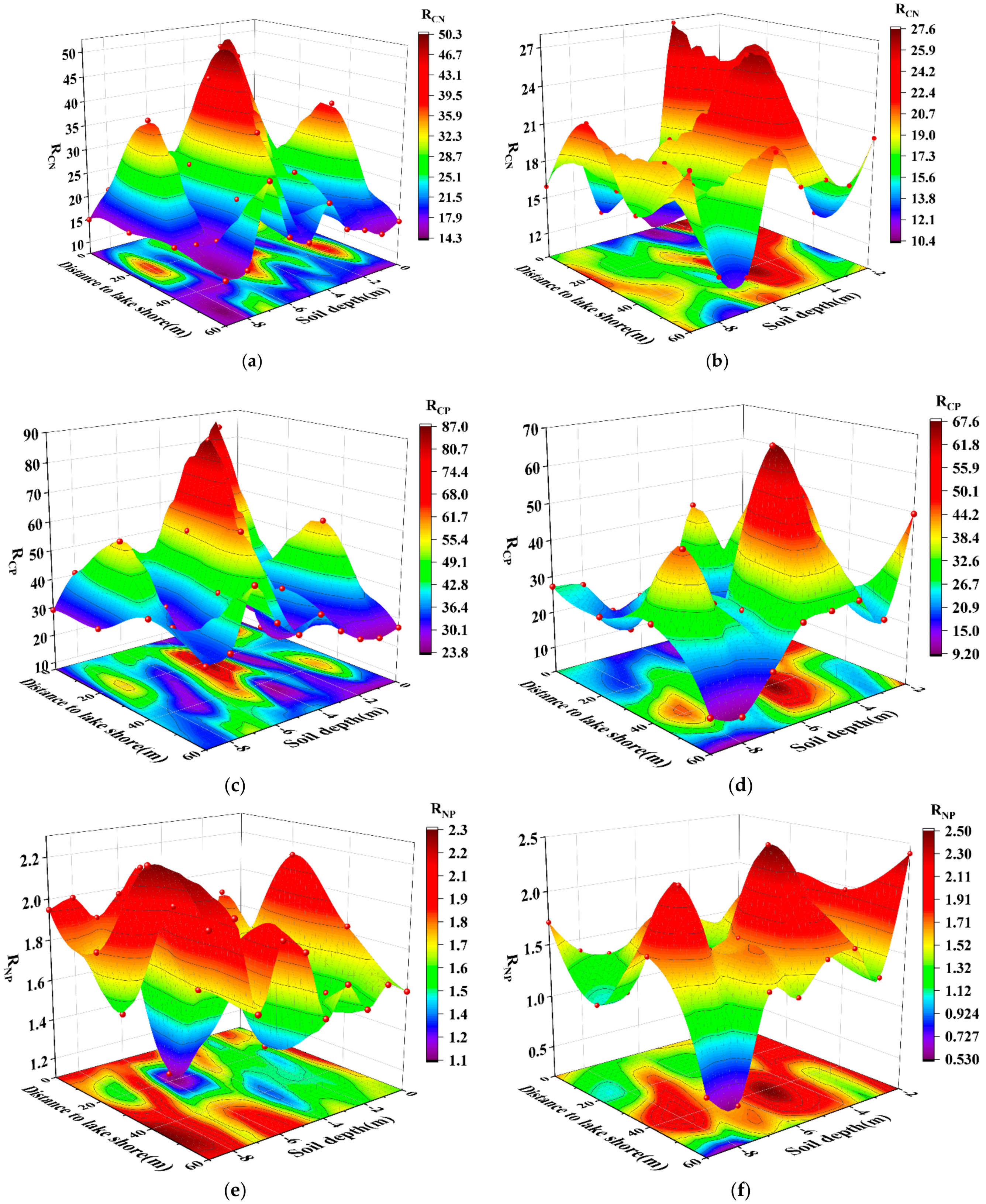


Figure 6. Spatial distribution map of soil C, N, P ecological stoichiometric ratios ((**a**,**c**,**e**) belong to profile P1, (**b**,**d**,**f**) belongs to profile P2).

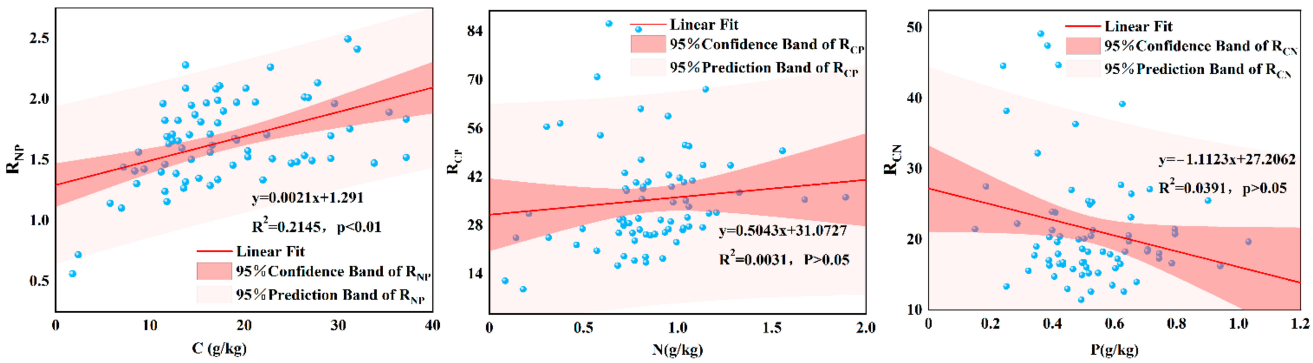


Figure 7. Correlation between soil C, N and P contents and their stoichiometry (if there is autocorrelation, no correlation analysis will be conducted).

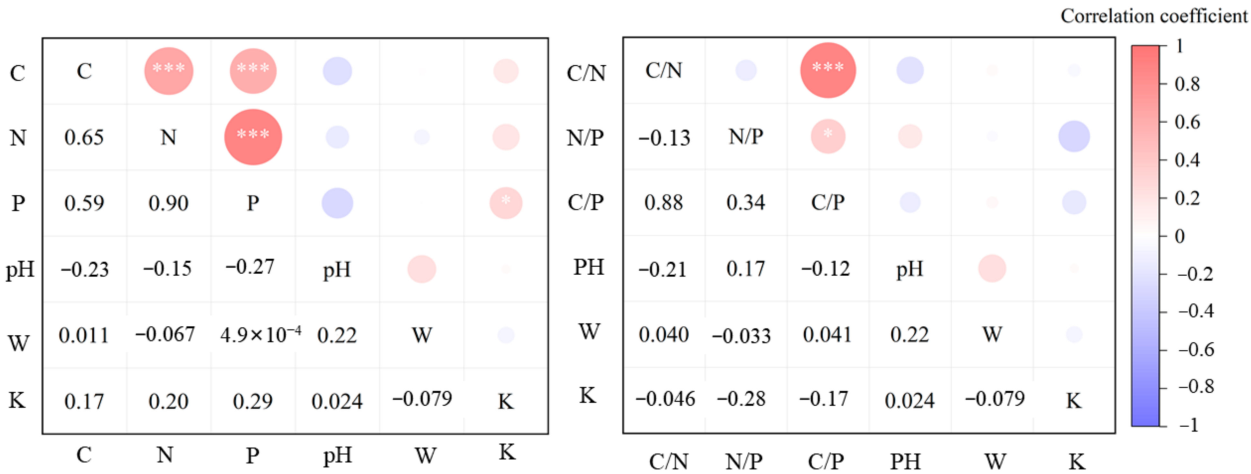


Figure 8. Correlation coefficients of C, N and P contents and their stoichiometry with environmental factors. (The numbers in the figure indicate the correlation coefficient between each sample and the color of the circles indicates the size of the correlation. The larger the correlation, the darker the color; * $p < 0.05$and *** $p < 0.001$).

Soil nutrients are synergistically influenced by soil physicochemical properties and environmental factors, and the correlation between soil element concentration and its stoichiometric ratio and soil physicochemical properties in the critical zone of Dongting Lake wetlands is shown in Figure 8. The C, N and P concentrations of the soils in this study were all negatively correlated with pH, a finding that is more common in wetland soils [69,70], as pH affects soil microbial activity and indirectly affects the efficiency of fixation, decomposition and accumulation of chemical elements in the soil. Both R_{CN} and R_{CP} in the soils of this study were negatively correlated with pH and R_{NP} was positively correlated with pH because a decrease in pH slows the rate of degradation of soil organic matter and increases the rate of accumulation, reflecting the accumulation of soil C. Microbial respiration will consume large amounts of P if in a flooded state and will also limit denitrification [71]. The correlation between soil elemental and stoichiometric ratios and water content in this study was weak (|R| < 0.1), which may be related to the sampling time. The sampling time in this study was October, which was mostly the rainy season and the lake wetland itself was in a water-saturated state, resulting in little variation in the water content of the soil layers. However, from the above discussion, it is clear that the flooding condition of the soil may be the main reason why the ecological stoichiometry of the wetland soil is significantly different from that of other soils. In this study, the anaerobic environment formed by soil flooding led to the inhibition of soil C mineralization and the phenomenon of N limitation, thus resulting in a condition of higher R_{CN} and lower R_{NP} in wetland soils, a trait also reflected by the weaker correlation in Figure 5. As the infiltration coefficient affects the transport and accumulation of elements in the soil ecological process, the higher the infiltration coefficient, the better the interception and storage of water and the accumulation of elements, resulting in higher soil C, N and P contents [72]. The significant positive correlation between soil infiltration coefficient and P content ($p < 0.05$), as well as the negative correlation between R_{NP} and R_{CP} in soil and infiltration coefficient in this study, suggest that the effect of infiltration on element accumulation in lake wetland soils is significantly present in P elements.

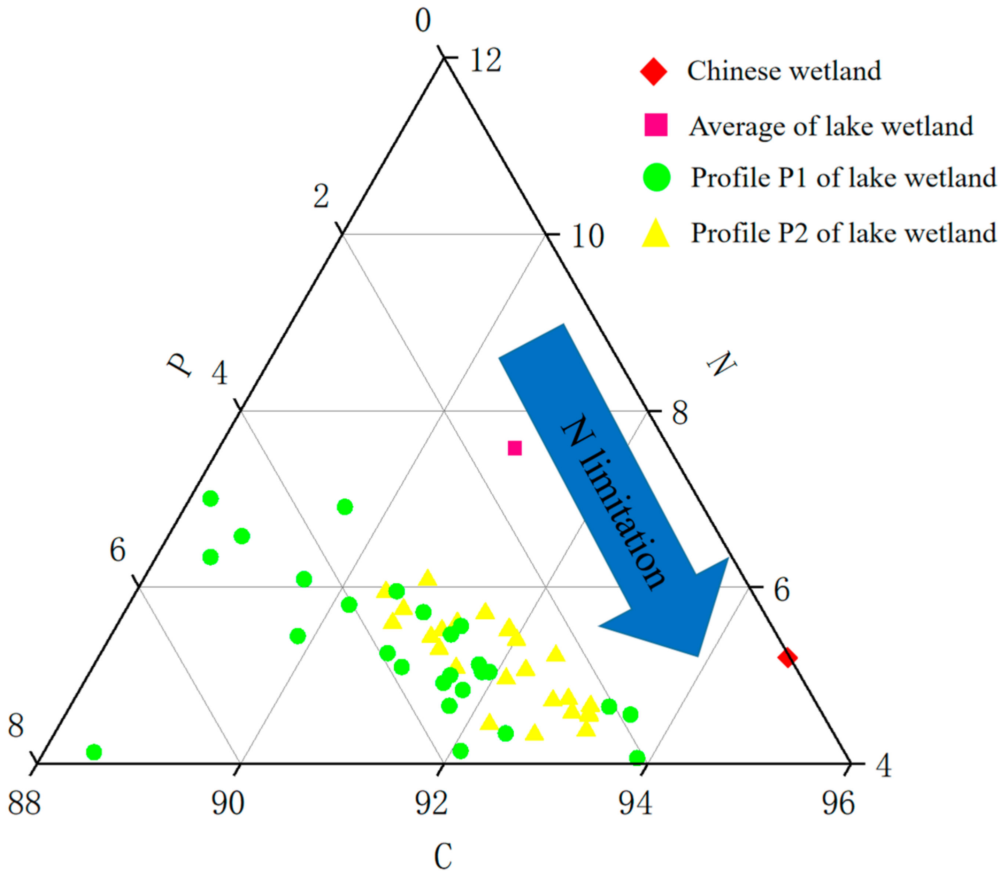


Figure 9. C:N:P ternary diagram (based on original C:N:P quality data). The direction of the colored arrow indicates that the intensity of N-limit increases.

Disturbances from human activities also have a profound impact on the storage and cycling of soil C, N and P elements, disrupting the original hydrothermal and environmental conditions of the wetland, affecting not only soil respiration and plant photosynthesis, but also altering the yield and decomposition rate of apoplastic matter [73,74]. The additional rice field soil sample used in this study is an artificial wetland, where periodic drainage, fertilizer application and harvesting during the growth of its crop can affect the input of soil elements and the humification process of organic matter. Tillage disrupts the structure of soil surface aggregates, increases soil permeability, accelerates microbial respiration and decomposition rates, promotes SOC mineralization, reduces soil SOC content, and decreases R_{CN} and R_{CP} (Table 3) [75]. In addition, fertilizer application will increase the organic matter content and SOC density of the soil in the cultivated layer of farmland and improve soil enzyme activity, but too much N and P fertilizer application will easily lead to eutrophication of surrounding water bodies due to N and P loss [76].

3.5. Is There a Stable Redfield Ratio in the Critical Zone of the Lake Wetlands?

Ecological stoichiometry theory is based on the law of conservation of mass and the law of constant proportions, leading to a long-term balance of energy and elements in the context of interactions between ecosystems [77]. This framework suggests that there is a strong link between nutrient demand, use and cycling, which is the significance of the Redfield ratio [11]. At the global scale, previous work has confirmed the existence of similar Redfield ratios in terrestrial ecosystems, such as plants or forests, and grassland soils. Although much has been achieved, there is a lack of research on wetlands.

In this study, the coefficients of variation of R_{CN}, R_{CP} and R_{NP} were found to be at a high level in the Dongting Lake wetland ecosystem, ranging from 21.2% to 42.8% (Tables 2 and 5, Figure 9), which is because wetlands in ecological staggered zones are often vulnerable to the combined effects of environmental factors such as climate change and hydrological fluctuations and human activities [78], allowing changes in the content and distribution of C, N and P elements in the soil. Nevertheless, statistical analysis showed that R_{CNP} in most wetland soils in China was within the range of 12.5 to 27.5, with a mean value of approximately 245:13.6:1 (Table 3, Figure 9), accounting for approximately 90% of all samples [69]. The mean R_{CNP} in this study was 1:1.6:35.4, with 85% of the samples also falling within the range of 12.5 to 27.5 (Figure 9). The significant two-by-two correlation between C, N and P content in the soil (Figure 8) and the small spatial variation in R_{CN}, R_{CP} and R_{NP} (Figure 6) suggest that a stable ratio of R_{CNP}, the Redfield ratio, may exist in the soil of the critical zone of the lake wetland. This phenomenon deviates from the research of Zhang et al. (2017) [79]. One reason for this is that Zhang et al. (2017) chose a depth of approximately 40 cm for each profile, and the fluctuation range of the profile depth was controlled within 10 cm, whereas the critical zone in this study area is a completely dynamic system with a larger scale and less human disturbance, which is more stable. On the other hand, as there are various types of wetlands, including marshes, peatlands, lakes, rivers, beaches, etc., the present study area belongs to lake wetlands, while Zhang et al.'s study area belongs to peatlands. The differences in hydrological fluctuations, plant composition and soil biology between different wetlands are strong and far less stable than in forest and grassland soil environments [80]. To ensure environmental homogeneity, future studies should be conducted for complete systems of wetlands of the same type.

4. Conclusions

The mean C, N and P contents of the soils in the critical zone of the Dongting Lake wetland were 18.05, 0.86 and 0.52 g/kg respectively, all of which were lower than the mean of the Chinese soils, and the elemental contents showed a decreasing trend from the surface to the deeper layers of the soil, and their relationship with the depth of the soil could be well fitted with a cubic function, where the C and N contents of the surface soils were significantly higher than all other layers, and the greatest variation in C content was found in the soils of the critical zone. However, the range of variation in soil P was not significant. The mean values of R_{CN}, R_{NP} and R_{CP} in the critical zone soils of the lake wetland were 21.1, 1.7 and 35.4 respectively. The ecological stoichiometric ratios fluctuated with changes in the profile, but the overall changes were not significant, with R_{CN} and R_{CP} showing a trend of increasing and then decreasing in the vertical direction, reaching a maximum at 2–5 m below ground level, and R_{NP} showing an insignificant trend. In general, the soils of the Dongting Lake wetlands are relatively rich in C and P nutrients, while relatively deficient in N. N is also the main element limiting plant growth and development and effective C cycling in the critical zone of the Dongting Lake wetlands, which may be caused by the unique anaerobic environment of the flooded wetlands.

There were highly significant two-by-two correlations between C, N and P contents in the soils of the critical zone of the Dongting Lake wetland. pH, infiltration coefficient and human activities were all closely related to C, N and P contents and their stoichiometric ratios, with soil C content being the most important factor affecting soil C, N and P stoichiometric ratios. A stable ratio of R_{CNP}, the Redfield ratio, may exist in wetland critical zone soils and future studies should be conducted for complete systems of the same type of wetland. The present data and analyses provide valuable insights into the stoichiometric patterns of C, N and P in wetland soils and critical zones globally.

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