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Microorganisms Directly Affected Sediment Carbon–Nitrogen Coupling in Two Constructed Wetlands

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Abstract: Clarifying the carbon–nitrogen coupling pattern in wetlands is crucial for understanding the driving mechanism of wetland carbon sequestration. However, the impacts of plants and environmental factors on the coupling of carbon–nitrogen in wetland sediments are still unclear. Sediment samples from plant (*Typha angustifolia* and *Phragmites australis*)-covered habitats and bare land were collected in two constructed wetlands in northern China. The contents of different forms of carbon and nitrogen in sediments and plants, and the sediment microbial community were detected. It was found that the sediment carbon to nitrogen (C/N) ratios did not differ significantly in the bare sites of different wetlands, but did in the plant-covered sites, which highlighted the different role of plants in shifting the carbon–nitrogen coupling in different constructed wetlands. The effects of plants on the sediment carbon–nitrogen coupling differed in two constructed wetlands, so the structural equation model was used and found that sediment microorganisms directly affected sediment C/N ratios, while water and sediment physicochemical properties indirectly affected sediment C/N ratios by altering sediment microbial functions. Multiple linear regression models showed that water pH, sediment moisture content, water dissolved oxygen, and water depth had a greater influence on the carbon metabolism potential of the sediment microbial community, while sediment moisture content had the greatest impact on the sediment microbial nitrogen metabolism potential. The study indicates that variations in environmental conditions could alter the influence of plants on the carbon and nitrogen cycles of wetland sediments. Water environmental factors mainly affect microbial carbon metabolism functions, while soil physicochemical factors, especially water content, affect microbial carbon and nitrogen metabolism functions.

Keywords: carbon–nitrogen coupling; sediment carbon to nitrogen ratio; constructed wetlands; sediment microorganisms; wetland plants

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1. Introduction

Wetlands represent a significant ecosystem that is instrumental in regulating climate change, conserving biodiversity and purifying water [1–3]. Despite covering only 5–8% of the global land, wetlands store 29–45% of soil carbon found on land [4], thus making them key areas for climate change mitigation [5]. The degradation and loss of natural wetlands has become an indisputable fact, while the constructed wetlands area is currently expanding worldwide [6,7]. By simulating natural wetlands, constructed wetlands are designed to degrade harmful substances and control water pollution. [8,9]. In constructed wetlands, carbon and nitrogen exist with different forms and are coupled within the atmosphere–plant–sediment system through sediment microbial action, plant photosynthesis and respiration, and the inputs and decomposition of carbon and nitrogen from litters [10–12]. Clarifying the pattern of carbon–nitrogen coupling in constructed wetlands will aid in understanding the carbon sink mechanism in wetlands.

Carbon and nitrogen cycles are tightly coupled due to competition of plants and soil organisms for nitrogen [13], and the variations of carbon–nitrogen coupling are mainly reflected by the changes in the carbon to nitrogen ratio [14]. Carbon in wetland sediments is mainly derived from above- and below-ground plant biomass, as well as root exudates [15,16]. The main sources of nitrogen in wetland sediments are plant deposition, algae, and exogenous pollutants [17,18]. Therefore, plants play an important role in wetlands [19] in terms of both the carbon and nitrogen cycle. Plant carbon inputs increase the sediment organic carbon content [20,21], while nitrogen is the primary nutrient limiting plant growth [22,23], and nitrogen availability affects plant carbon inputs to sediments by limiting net primary production [15,24]. The input of plant litter can stimulate microbial activity, thereby increasing the content of available nitrogen in sediments [21,25,26]. In addition, plants obtain nitrogen while distributing carbon underground to build roots and support microbial communities [27,28], and sediment microbes can convert inert nitrogen in sediments into reactive nitrogen that can be utilized by plants [23]. Changes in soil nitrogen affect plant and microbial growth [29], with plants further transporting carbon to the soil in the form of litter and root exudates [15]. When the environmental conditions change, plants will regulate the nitrogen concentration in the root system according to the nutrient demand so that the carbon and nitrogen ratio of each organ will be stabilized [30]. In short, plants are a significant factor in regulating the carbon and nitrogen cycle in wetland sediments, and other environmental factors might impact the effect of plant.

Some studies have indicated that the vegetation composition and plant carbon inputs of wetlands are direct factors affecting sediment carbon distribution [20,31], while climate and sediment properties (water content, pH, and bioavailable carbon) are the main factors affecting sediment nitrogen distribution [32]. However, it has also been shown that changes in vegetation can affect the composition of microbial communities relevant to nitrogen metabolism in the sediments and thus affecting the changes in sediment nitrogen [33], and that the physicochemical properties of sediment (particularly the water content) can affect microbial heterotrophic respiration and thus affect the sediment carbon fluxes [34,35]. In summary, vegetation and sediment physicochemical properties influence sediment carbon and nitrogen cycling through the regulation of microbial communities [36]. Furthermore, previous studies have shown that the water environment affects wetland sediment carbon; for instance, the duration of flooding limits microbial carbon metabolism and thus affects sediment carbon [37]. It has also been shown that the groundwater level is the main factor influencing carbon sinks of wetland sediments considering factors such as climate, vegetation, soil, and hydrology [38]. Plants, sediment physicochemical properties, sediment microorganisms, and water environment were the main factors affecting the sediment carbon–nitrogen coupling in wetland, and their effects on wetland sediments might vary between different wetlands.

Therefore, exploring the driving factors of the carbon–nitrogen coupling pattern in different wetland sediments and revealing the general rules involved are crucial for understanding the carbon–nitrogen coupling mechanism in wetlands. In this study, we chose two constructed wetlands (the Luohe Constructed Wetland and Xinxue River Constructed Wetland) in northern China and collected samples from plant (*Typha angustifolia* and *Phragmites australis*)-covered plots and bare land plots in each wetland. By detecting the contents of different forms of carbon and nitrogen in sediments and plants and detecting sediment microorganisms with high-throughput sequencing, this study aimed to test the following hypotheses: (1) the plant carbon–nitrogen coupling was not affected significantly by site differences but by the plant species; (2) plants and environmental factors would affect the sediment carbon–nitrogen coupling by influencing microorganisms.

2. Materials and Methods

2.1. Area Selection and Sample Collection

The study areas were Luohe Constructed Wetland in Jinan and Xinxue River Constructed Wetland in Jining, both located in Shandong Province, northern China. Luohe Constructed Wetland was completed in 2017 and the reclaimed water treated by the sewage treatment plant is discharged into the constructed wetland [39]. Xinxue River Constructed Wetland was established in 2007 and water from the Xinxue River enters the constructed wetland through a diversion channel [40]. Both constructed wetlands were used for micro-polluted water treatment. Field sampling proceeded in the summer of 2021 in the two constructed wetlands. A total of 10 *P. australis*-covered plots, 10 *T. angustifolia*-covered plots, and 5 bare land plots were selected at equal intervals from upstream to downstream in the two constructed wetlands. The size of each sampling plot was 1 × 1 m. Well-grown plants were selected and dug out from the plant-covered plots, and the sediment closely attached to the plant roots was collected and stored at −80 °C for the detection of microorganisms. According to the five-point sampling method, 3~5 plants were selected and the above-ground parts in the plant-covered plots were dug out. Then, a soil drill was used to collect the surface sediment of plant roots, and the sediment was stored in a sealed bag at 4 °C. Collected plant and sediment samples were taken to the laboratory for the determination of carbon and nitrogen content. In the bare land plots, one sediment sample for microbial analysis and another for carbon and nitrogen determination were collected and stored at −80 °C and 4 °C, respectively. Water pH (wpH) and water temperature (wT) were measured with a portable pH meter. Water dissolved oxygen (wDO) was determined with a portable dissolved oxygen meter. The depth of the water (wH) was also determined with a tape measure.

2.2. Determination of Physicochemical Properties of Sediment and Plant

A portion of the fresh sediment samples stored at 4 °C was passed through a 2 mm sieve and used for the detection of ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_x^-–N) and sediment dissolved organic carbon (DOC). Another portion of the fresh sediment samples was air-dried and then passed through 2 mm and 0.25 mm sieves respectively for testing the carbon and nitrogen components of the sediment. Plants were washed with deionized water, dried, ground and passed through a 0.25 mm sieve for subsequent analysis. The sediment NH_4^+–N, NO_x^-–N, and DOC were separated with a 2 M potassium chloride solution and determined by a total organic carbon analyzer (TOC-5000, Shanghai METASH, China) and a continuous flow analyzer (Scalars San++, Skylar Analytical B.V., Breda, The Netherlands), respectively [39]. The specific gravity method and elemental analyzer (Unicube, Elementar, Germany) were used to determine the sediment heavy fraction organic carbon (HFOC) and light fraction organic carbon (LFOC) [39,41]. The total carbon and the nitrogen content of the plants and sediments were also measured by the elemental analyzer. The calculation of the sediment carbon to nitrogen (C/N) ratio was based on the ratio of sediment organic carbon (SOC) to sediment total nitrogen (TN) [42]. The plant carbon to nitrogen (C/N) ratio was calculated by the ratio of plant carbon content (pC) to plant nitrogen content (pN). The sediment moisture content (mc) was determined with drying method. Sediment pH (spH) was measured using a PHS-3E pH meter, with a water to sediment ratio of 2.5:1 to test the sediment leachate.

2.3. Prediction of Microbial Function

The microbial genome sequencing of the sediment samples was done by Novogene (Beijing, China) and the Illumina Nova sequencing platform was used to perform the ITS1 and 16S rDNA high-throughput sequencing analysis [39]. The barcode and prime removal data of the two constructed wetlands sediments obtained from Novogene (Beijing, China) were spliced, quality controlled, dechimerized, clustered, and annotated to generate operational taxonomic units (OTUs) table and species annotation table by the SSUrRNA and the database Mothur method [43] of SILVA138 (http://www.arb-silva.de/) [44]. Using the FULL NAME (FAPROTAX) database, the function potentials of the bacterial community were annotated [45]. The functions related to carbon, nitrogen, and sulfur cycling in the top 20 most abundant functions were selected to uncover elemental metabolism potentials of bacterial communities in different habitats.

2.4. Data Analysis

R (v.4.2.2), OriginPro (v. 2021), and SPSS (v.25.0) were used to process and analyze the data and draw figures. One-way analysis of variance (ANOVA) was utilized for exploring the variability of sediment carbon and nitrogen components, environmental factors and plant carbon and nitrogen fractions in different plant habitats and different constructed wetlands. The effect of environmental factors and plant species on plant carbon–nitrogen coupling was explored using two-way ANOVA. The correlations between the studied factors were detected using Spearman correlation analysis. Wilcoxon tests were employed to determine the difference in the microbial diversity and taxonomic abundance among different habitats. Microbial beta-diversity among different habitats was analyzed with a non-metric multi-dimensional scaling (NMDS). A structural equation model (SEM) was built to analyze the effects of plants, water environment, sediment microorganisms, and sediment physicochemical properties on sediment carbon–nitrogen coupling. A multiple linear regression model was used to identify the environmental drivers of sediment microbial functions related to carbon and nitrogen metabolisms.

3. Results

3.1. Differences of Carbon and Nitrogen Components in Sediments and Plants in the Two Constructed Wetlands

The study found that in Luohe Constructed Wetland, there were significant differences in carbon and nitrogen components in different habitats, except DOC. Among them, organic carbon components (SOC, LFOC, and HFOC) were generally higher in the sediments of plant habitats than in bare land plots (Figure 1). In addition, the NH_4^+–N contents in the sediments of bare land plots were significantly higher than in plant-covered plots, but the NO_x^-–N contents in the sediment of plant habitats were higher than in bare land plots (Figure 1). And the sediment C/N ratios were significantly different between bare land plots and plant-covered plots (Figure 1). In Xinxue River Constructed Wetland, except for the sediment total carbon (TC), contents in the bare land plots were lower than that in the *T. angustifolia*-covered plots, there were no significant differences in other carbon and nitrogen fractions among the three habitats (Figure 1). Comparing the two constructed wetlands, there were differences in their carbon and nitrogen fractions in the sediment of plant habitats (Figure 1), while in the sediment of bare land, except for DOC, TC, and NH_4^+–N, there were no significant differences in other components (Figure 1). Furthermore, the sediment C/N ratios in the plant habitats in Luohe Constructed Wetland were significantly higher than those in Xinxue River Constructed Wetland, but sediment C/N ratios in the bare land were not significantly different between the two constructed wetlands (Figure 1), which shows that plants are one of the factors influencing sediment carbon–nitrogen coupling in the constructed wetlands, but their effects varied in different wetlands.

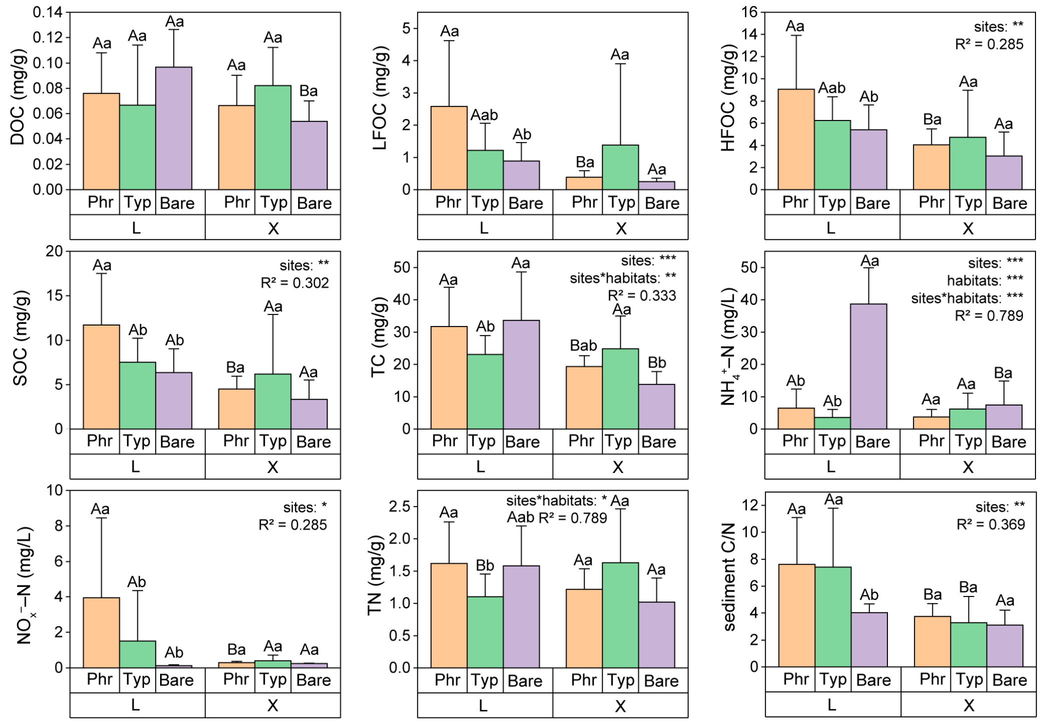

Figure 1. Comparison of sediment carbon and nitrogen fractions in the two constructed wetlands. The impacts of sites and habitats on sediment carbon and nitrogen components were tested with two-way ANOVA. The significant effects were labeled in the corner. (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Phr = *P. australis*, Typ = *T. angustifolia*, Bare = bare land, X = Xinxue River Constructed Wetland, L = Luohe Constructed Wetland, DOC = dissolved organic carbon, LFOC = light fraction organic carbon, HFOC = heavy fraction organic carbon, SOC = sediment organic carbon, TC = sediment total carbon, NH_4^+–N = ammonium nitrogen, NO_x^-–N = nitrate and nitrite nitrogen, TN = sediment total nitrogen, sediment C/N = ratio of sediment organic carbon to sediment total nitrogen. Different superscript capital letters mean the significant differences between the two constructed wetlands; Different superscript lowercase letters mean the significant differences between different habitats ($p < 0.05$).

In Luohe Constructed Wetland, contrasting with *T. angustifolia*, the plant carbon and plant nitrogen contents of *P. australis* were significantly higher, but the plant C/N ratios of *P. australis* were significantly lower (Figure 2). In Xinxue River Constructed Wetland, the plant carbon content of the two species did not significantly differ, but compared with *T. angustifolia*, the nitrogen content of *P. australis* was significantly higher, and the plant C/N ratios of *P. australis* were significantly lower (Figure 2). Comparing the two wetlands, the carbon content of *P. australis* and the *T. angustifolia* did not significantly differ, but in Luohe Constructed Wetland, the content of nitrogen in *P. australis* was significantly higher than that in Xinxue River Constructed Wetland (Figure 2). But the differences in plant C/N ratios of *P. australis* and *T. angustifolia* were consistent across the two constructed wetlands. Two-way ANOVA indicated that plant species had a substantial effect on plant carbon and nitrogen fractions compared to the variability of environmental factors (Figure 2).

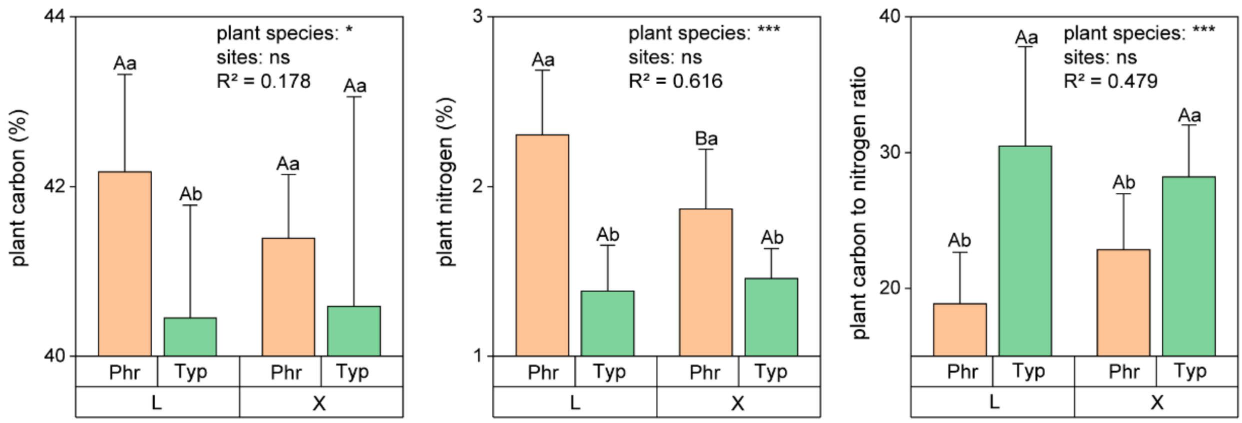

Figure 2. Comparison of plant carbon and nitrogen fractions in two constructed wetlands. The impacts of sites and plant species on plant carbon, plant nitrogen and plant C/N ratios were tested with two-way ANOVA. (*** $p < 0.001$, * $p < 0.05$). Phr = *P. australis*, Typ = *T. angustifolia*, X = Xinxue River Constructed Wetland, L = Luohe Constructed Wetland. Different superscript capital letters mean the significant differences between the two constructed wetlands; Different superscript lowercase letters mean the significant differences between different habitats ($p < 0.05$).

3.2. Differences in Environmental Factors and Their Correlation with Carbon and Nitrogen Fractions of Sediments and Plants

The environmental factors of the two constructed wetlands were tested. It was found that in Luohe Constructed Wetland, there were no significant differences in water environmental factors among the three habitats, but sediment pH and sediment moisture content in the bare land plots were significantly higher than in the plant-covered plots (Figure 3). The reason for this difference may be the influence of plant root activities on the sediment in the plant habitats. In Xinxue River Constructed Wetland, except for depth of water, other environmental factors had no significant differences among the three habitats (Figure 3). However, compared with the two constructed wetlands, there were significant differences among the water environmental factors such as water pH, water dissolved oxygen, and depth of water, and the sediment pH significantly differed between the two constructed wetlands (Figure 3).

Spearman correlation analysis showed that in Luohe Constructed Wetland, sediment moisture content and plant nitrogen were correlated with sediment carbon and nitrogen components, indicating that sediment physicochemical properties and plant nitrogen have an influence on sediment carbon and nitrogen components (Figure 4a). In Xinxue River Constructed Wetland, sediment moisture content, sediment pH, and dissolved oxygen of water environment were significantly correlated with sediment carbon and nitrogen fractions, indicating that sediment physicochemical properties and water environment conditions have an influence on sediment carbon and nitrogen fractions (Figure 4b). In the two constructed wetlands, significant correlations were observed between sediment carbon and sediment nitrogen fractions (Figure 4a,b).

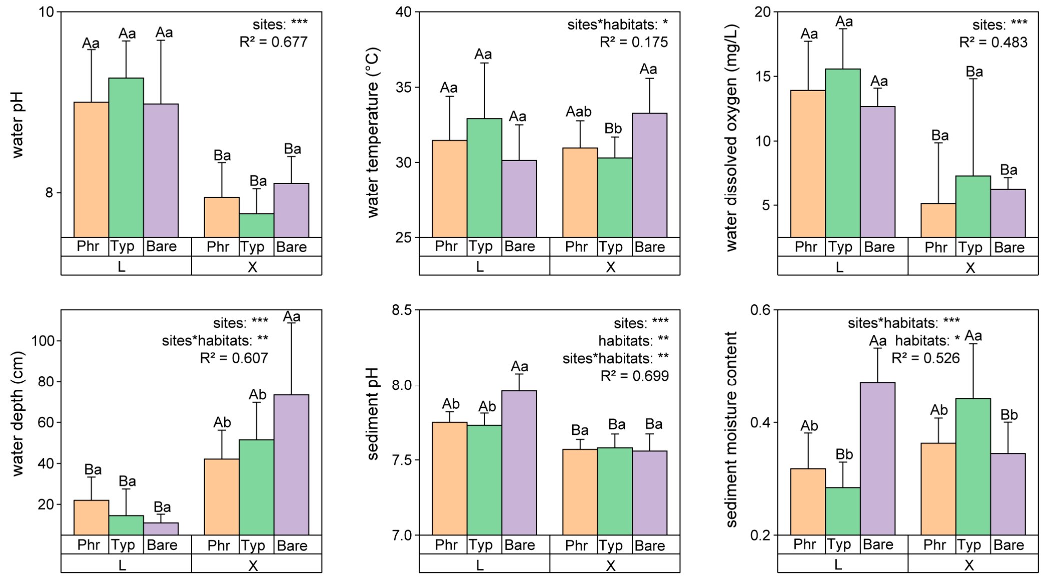

Figure 3. Comparison of environmental physicochemical properties in two constructed wetlands. The impacts of sites and habitats on environmental factors were tested with two-way ANOVA. The significant effects were labeled in the corner. (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Phr = *P. australis*, Typ = *T. angustifolia*, Bare = bare land, X = Xinxue River Constructed Wetland, L = Luohe Constructed Wetland. Different superscript capital letters mean the significant differences between the two constructed wetlands; Different superscript lowercase letters mean the significant differences between different habitats ($p < 0.05$).

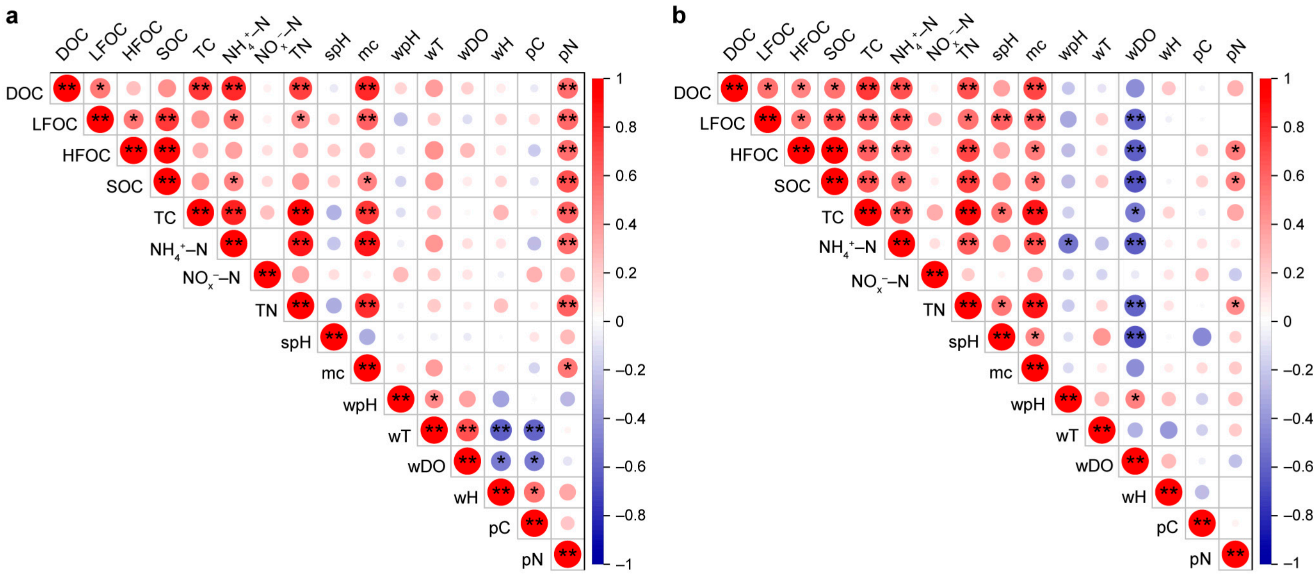

Figure 4. Spearman correlations between sediment and plant carbon and nitrogen fractions and environmental factors in Luohe Constructed Wetland (**a**) and Xinxue River Constructed Wetland (**b**). Red and blue indicate positive and negative correlations, respectively, with the gradient of color reflecting the degree of correlation. (** $p < 0.01$, * $p < 0.05$).

3.3. Microbial Richness and Community Composition in Different Habitats and Factors Affecting Sediment Carbon to Nitrogen Ratio

Significant differences existed in the richness of microbes in the different habitats (Figure 5a,d): the abundance of bacteria and fungi differed significantly between the plant habitats and the bare land in Luohe Constructed Wetland but not in Xinxue River Constructed Wetland. The abundance of bacteria in Xinxue River Constructed Wetland was significantly higher than that of the Luohe Constructed Wetland, while the abundance of fungi showed little difference between the two constructed wetlands. As shown in Figure 5b,e, the composition of the bacterial community differed significantly between the plant habitats and bare land in Luohe Constructed Wetland but not in Xinxue River Constructed Wetland; the composition of the fungal community did not differ significantly between the plant habitats and bare land in the two constructed wetlands. NMDS also revealed that the composition of the microbial community was significantly different between the two constructed wetlands (Figure 5b,e). The main bacterial phyla detected included *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, and *Chloroflexi* (Figure 5c). Significant differences existed in *Chloroflexi* between the two constructed wetlands (Figure S1). The main fungal phyla detected included *Ascomycota*, *Basidiomycota*, *Rozellomtcota*, and *Chytridiomycota* (Figure 5f). Significant differences existed in *Ascomycota* and *Chytridiomycota* between the two constructed wetlands (Figure S2).

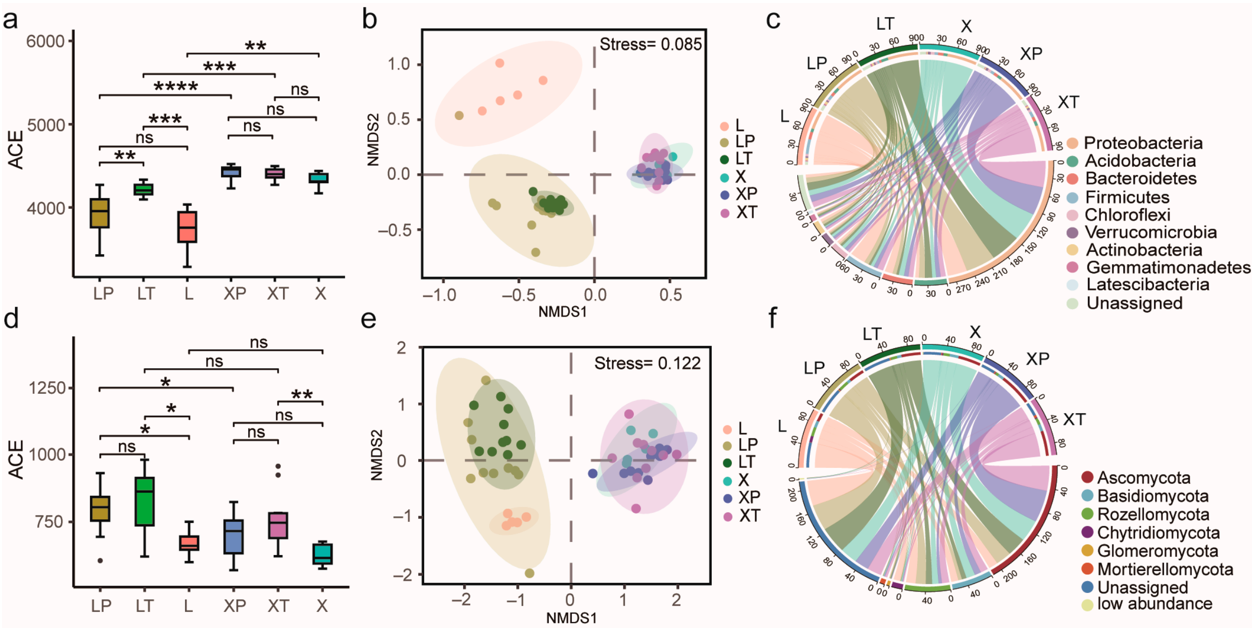

Figure 5. Differences of sediment bacterial (**a**) and fungal (**d**) richness among different habitats. NMDS analysis shows the structural differences of sediment bacterial (**b**) and the fungal (**e**) communities in the different habitats. Circos plots show the composition of bacterial (**c**) and fungal (**f**) communities at the phylum level in the different habitats. LP = *P. australis*-covered sites in Luohe Constructed Wetland, LT = *T. angustifolia*-covered sites in Luohe Constructed Wetland, L = bare land in Luohe Constructed Wetland, XP = *P. australis*-covered sites in Xinxue River Constructed Wetland, XT = *T. angustifolia*-covered sites in Xinxue River Constructed Wetland, X = bare land in Xinxue River Constructed Wetland. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

The differences in bacterial richness and community composition were the same as the differences in sediment C/N ratios, therefore, this study selected bacteria for functional prediction and used them as microbial functions and other factors for structural equation modeling analysis. The results showed that the sediment C/N ratios were dominantly and directly affected by the microbial function in the sediment, regardless of the plant habitat (Figure 6a). The sediment C/N ratios were indirectly influenced by water environmental conditions and sediment physicochemical properties with standardized effects of 0.466 and 0.272, respectively (Figure 6a,b). In addition, the water conditions also affect microbial function indirectly by influencing soil physicochemical properties (Figure 6a). A total of 53.4% of the top 20 predicted bacterial functions were related to carbon and nitrogen metabolism (Figure S3). Effects of water environmental factors and soil physicochemical properties on the sediment microbial carbon and nitrogen metabolism potentials were investigated by multiple linear regression models. The results show that the explanatory values of water environmental factors and soil physicochemical properties on carbon and nitrogen metabolism functions were 42.35% and 23.67%, respectively (Figure 6c,d). In addition, water pH, sediment moisture content, water DO, and water depth explained more variations in carbon metabolism potential, while sediment moisture content had the highest explanatory value for nitrogen metabolism function (Figure 6c,d).

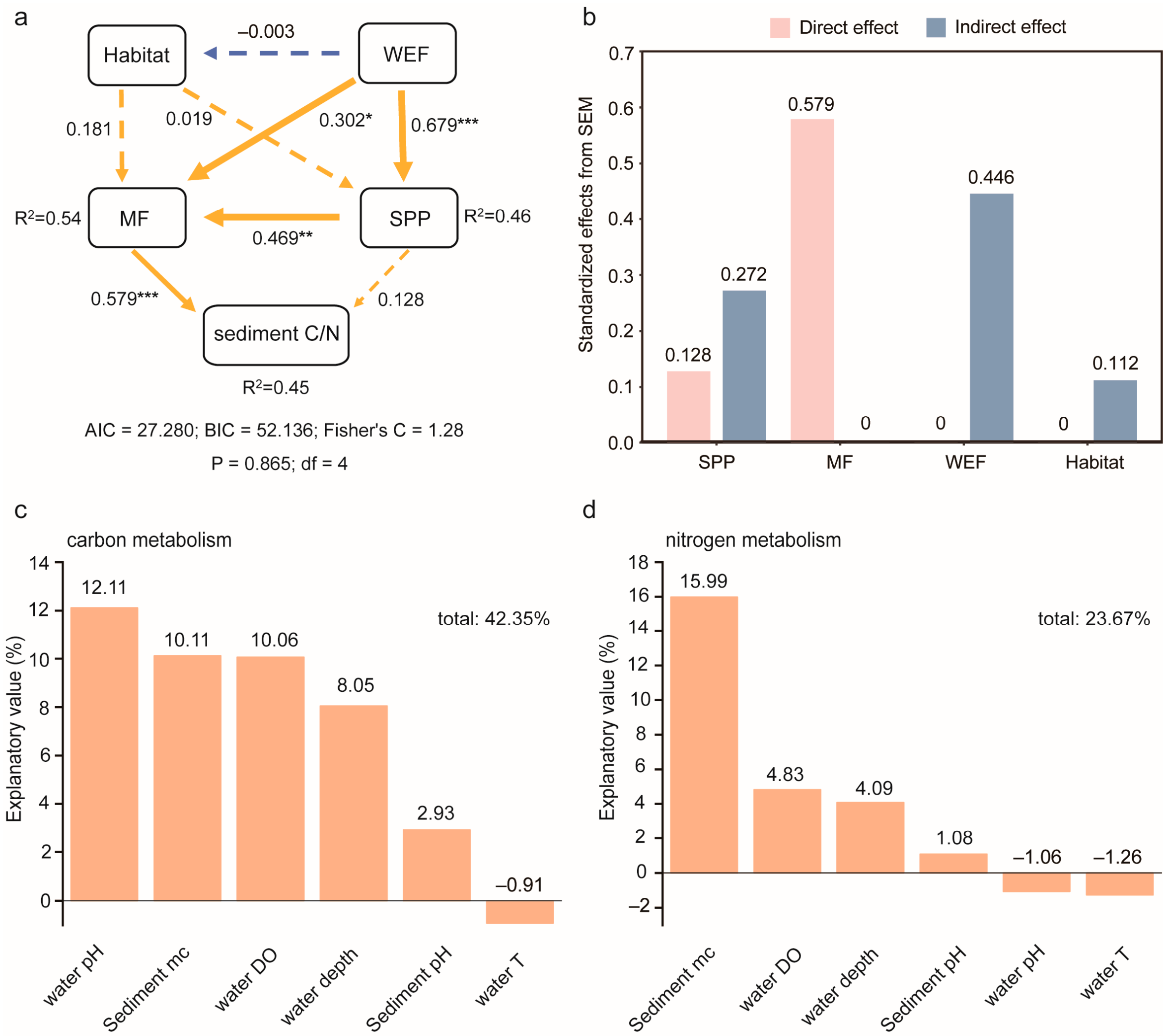

Figure 6. (**a**) Structural equation modeling showing the relative influence of habitat, water environmental factors, microorganisms and soil physicochemical properties on sediment C/N. (**b**) The histogram of their effects on sediment C/N. Habitat = plant species including *P. Australis*, *T. angustifolia* and bare land, WEF = water environmental factors including water pH, water temperature (T), water dissolved oxygen (DO) and water depth, MF = microbial carbon and nitrogen cycle function, SPP = sediment physicochemical properties including sediment pH and moisture content (mc). The yellow and blue arrows represent a positive and negative relationship, respectively. The numbers adjacent to the arrows are path coefficients. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Multiple linear regression model showing the explanatory values of environmental factors on microbial functions related to carbon (**c**) and nitrogen (**d**) metabolism.

4. Discussion

4.1. Carbon–Nitrogen Coupling in Constructed Wetland Plants

This study did not find a significant difference in total carbon content of *P. Australis* and *T. angustifolia* across two constructed wetlands. But in Luohe Constructed Wetland, the nitrogen content of *P. Australis* was significantly higher than that in Xinxue River Constructed Wetland, which may be because the nitrate nitrogen content in the sediment of the studied habitat in Luohe Constructed Wetland was significantly higher than that in Xinxue River Constructed Wetland. As an active nitrogen nutrient required for plant growth [23,46], nitrate nitrogen is crucial for plant growth and development [22]. Wetland plant nitrogen content is limited by the ammonium and nitrate nitrogen content in sediments [47]. Plant response to reactive nitrogen in sediments maintains plant growth [22]. Nitrogen availability in sediments limits plant growth and thus affects plant carbon input to sediments [24]. The sediment nitrogen availability in constructed wetlands may be affected by the nitrogen content of the inflow water, which mainly comes from the preliminarily treated wastewater from the surrounding area.

In addition, the environmental factors and sediment carbon and nitrogen components were significantly different across two constructed wetlands and were correlated with plant carbon and nitrogen. But the plant C/N ratios of *P. Australis* and *T. angustifolia* did not differ across the two constructed wetlands, indicating that environmental factors and sediment carbon and nitrogen components had no significant effects on plant carbon–nitrogen coupling in constructed wetlands. This may be related to the characteristics of plants, according to the homeostatic mechanism, plants can maintain a relatively constant proportion of their chemical composition in a changing environment [48]. Previous research have also revealed that the C/N ratio of plants is determined by the characteristics of plants [49] and that plants will adjust the nitrogen nutrients in the soil according to the allocation of nutrient requirements [30]. This study further verified the influence of environmental factors and plant species on plant carbon–nitrogen coupling through an analysis of variance. The results demonstrated that environmental factors had no significant impact on plant carbon, plant nitrogen and plant C/N ratios of plant (Figure 2), indicating plant carbon–nitrogen coupling was determined by its own characteristics and was unaffected by the variability of environmental factors.

4.2. Carbon–Nitrogen Coupling in Constructed Wetland Sediments

Our study showed that sediment carbon and nitrogen fractions were significantly correlated in the two constructed wetlands, which is consistent with previous research results [50]. This indicated that carbon and nitrogen fractions in the sediment were coupled. In this study, sediment carbon and nitrogen fractions differed significantly among different habitats in Luohe Constructed Wetland (Figure 1), indicating that wetland plant growth could influence sediment carbon and nitrogen fractions, which is consistent with those previous research results [51]. Wetland sediment carbon and nitrogen are mainly obtained from root exudation of plant and decomposition of plant litter both above- and below-ground [16,17]. Plant carbon inputs [20] and plant nitrogen use efficiency [30,52] contribute to the variability of carbon and nitrogen in wetland surface sediments. Plant species can also control the input and loss of nitrogen in sediments [52], thus limiting the carbon input from plants to sediments [24]. In addition, this study showed that nitrogen content in plants correlated significantly with sediment carbon and nitrogen fractions in the two constructed wetlands, indicating that plant growth and nitrogen acquisition might influence sediment carbon–nitrogen coupling because nitrogen is the primary nutrient limiting plant growth [23], and plants acquire nitrogen by distributing carbon to sediments through their root systems [15,27]. Furthermore, the microbial abundance and community composition were significantly different between plant habitats and bare sites in Luohe Constructed Wetland, suggesting that plants affected the sediment carbon and nitrogen components via microorganisms. Plants are important for shaping microbial community diversity and regulating community stability [36], and plant roots stimulate the formation of sediment pores to provide living space for microorganisms [53], while plant root carbon allocation maintains sediment microbial communities [27]. Plant impacts on sediment microbes are primarily root-driven [54]. Sediment microorganisms mediate the conversion of inert nitrogen in sediments to forms that can be absorbed by plants [23] and microbes regulate sediment carbon and nitrogen cycling primarily by influencing the metabolism of reactive carbon and active nitrogen in constructed wetlands [35,55].

However, in Xinxue River Constructed Wetland, there were no significant differences in sediment carbon and nitrogen fractions and microbial abundance, as well as microbial community composition among the different habitats (Figure 1). The sediment C/N ratios did not differ significantly between the two constructed wetlands in the bare sites but did in the plant-covered sites, suggesting the distinct role of plants in shifting the carbon–nitrogen coupling in the different constructed wetlands; plants affected the sediment C/N ratios in Luohe Constructed Wetland but did not in Xinxue River Constructed Wetland. Considering that environmental factors, microbial abundance, and microbial community composition were significantly different between the two constructed wetlands, it is reasonable to speculate that the impact of plants on sediment carbon–nitrogen coupling was influenced by environmental factors and microorganisms. Previous studies have proposed that sediment pH is a factor affecting sediment carbon and nitrogen [56], as well as moisture content [54] and temperature [35], both of which affect surface sediment carbon and nitrogen cycling. In addition, environmental factors and sediment properties (moisture content, pH) could be the main factors affecting the sediment nitrogen distribution [32]. Furthermore, the physicochemical properties of sediment (particularly the moisture content) can affect microbial heterotrophic respiration and thus affect the sediment carbon fluxes [34,35].

In this study, the effects of environmental factors on sediment carbon–nitrogen coupling were investigated by SEM analysis (Figure 6a). The results showed that the microbial function directly affected the sediment C/N ratios, and the environmental factors, including the water conditions and sediment physicochemical properties, indirectly affected the sediment C/N ratios by influencing the microbial function. In addition, multiple linear regression analysis showed that environmental factors mainly affected the functions related to carbon and nitrogen metabolism in microbial functions, and the explanatory values were 42.35% and 23.67%, respectively. And water pH, sediment moisture content, water DO, and water depth have a great effect on the function of carbon metabolism, while sediment moisture has the greatest effect on nitrogen metabolism function (Figure 6c,d). Previous studies have mostly shown the influence of sediment physicochemical properties (especially moisture content) on sediment carbon and nitrogen cycles [34,35,54,56], while this study found that water environmental factors (water pH, water DO, and water depth) were equally crucial in explaining the function of carbon and nitrogen metabolism, as well as the physicochemical properties of sediment. The reason for the significant difference in sediment carbon and nitrogen between the two constructed wetlands is mainly due to the significant difference in the water environment of the two constructed wetlands. In Luohe Constructed Wetland, plants were the main influence factor, while in Xinxue River Constructed Wetland, the influence of plants on sediment carbon–nitrogen coupling was not significant because of greater variation in environmental factors. Therefore, in the process of managing and regulating the carbon and nitrogen cycles in constructed wetlands, the plant species and the regulation of environmental factors are both essential to increasing carbon sinks in constructed wetlands.

5. Conclusions

This study found that the carbon–nitrogen coupling of plants was determined by plant species identity and was not affected by the variability of environmental factors in the two studied constructed wetlands. The SEM analysis showed that microbial function significantly affected sediment C/N ratios directly, while water environmental conditions and sediment physicochemical properties affected sediment C/N ratios by influencing the microbial function. A multiple linear regression model showed that environmental factors (water pH, sediment moisture content, water DO, and water depth) drove microbial carbon metabolism potentials, while the sediment moisture content shaped the nitrogen metabolism potentials. These results demonstrate that changes in environmental factors do not affect plant carbon–nitrogen coupling, but they can weaken the impact of plants on sediment carbon–nitrogen coupling and become the main factor affecting sediment carbon–nitrogen coupling by affecting sediment microbial carbon and nitrogen metabolism potentials. This study comprehensively explains the factors influencing the carbon–nitrogen coupling of plants and sediments in constructed wetlands, which provides new ideas for understanding the mechanism of carbon–nitrogen coupling in wetlands and holds great importance for the management of carbon sinks toward mitigating the effects of global warming.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16111550/s1, Figure S1: Differences in relative abundance of main bacterial taxa at the phylum level between different habitats; Figure S2: Differences in relative abundance of main fungal taxa at the phylum level between different habitats; Figure S3: Bacterial functional signature predicted with the FAPROTAX.

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References

1. Merot, P.; Squividant, H.; Aurousseau, P.; Hefting, M.; Burt, T.; Maitre, V.; Kruk, M.; Butturini, A.; Thenail, C.; Viaud, V. Testing a Climato-Topographic Index for Predicting Wetlands Distribution along an European Climate Gradient. *Ecol. Model.* **2003**, *163*, 51–71. [CrossRef]
2. Tiner, R.W. Assessing Cumulative Loss of Wetland Functions in the Nanticoke River Watershed Using Enhanced National Wetlands Inventory Data. *Wetlands* **2005**, *25*, 405–419. [CrossRef]
3. Moomaw, W.R.; Chmura, G.L.; Davies, G.T.; Finlayson, C.M.; Middleton, B.A.; Natali, S.M.; Perry, J.E.; Roulet, N.; Sutton-Grier, A.E. Wetlands in a Changing Climate: Science, Policy and Management. *Wetlands* **2018**, *38*, 183–205. [CrossRef]
4. Nahlik, A.M.; Fennessy, M.S. Carbon Storage in US Wetlands. *Nat. Commun.* **2016**, *7*, 13835. [CrossRef] [PubMed]
5. Roe, S.; Streck, C.; Obersteiner, M.; Frank, S.; Griscom, B.; Drouet, L.; Fricko, O.; Gusti, M.; Harris, N.; Hasegawa, T.; et al. Contribution of the Land Sector to a 1.5 °C World. *Nat. Clim. Chang.* **2019**, *9*, 817–828. [CrossRef]
6. Hu, S.; Niu, Z.; Chen, Y.; Li, L.; Zhang, H. Global Wetlands: Potential Distribution, Wetland Loss, and Status. *Sci. Total Environ.* **2017**, *586*, 319–327. [CrossRef] [PubMed]
7. Davidson, N.C. How Much Wetland Has the World Lost? Long-Term and Recent Trends in Global Wetland Area. *Mar. Freshw. Res.* **2014**, *65*, 934–941. [CrossRef]
8. Vymazal, J. Constructed Wetlands for Treatment of Industrial Wastewaters: A Review. *Ecol. Eng.* **2014**, *73*, 724–751. [CrossRef]
9. Li, Y.; Zhu, G.; Ng, W.J.; Tan, S.K. A Review on Removing Pharmaceutical Contaminants from Wastewater by Constructed Wetlands: Design, Performance and Mechanism. *Sci. Total Environ.* **2014**, *468*, 908–932. [CrossRef]
10. Knicker, H. Soil Organic N—An under-Rated Player for C Sequestration in Soils? *Soil Biol. Biochem.* **2011**, *43*, 1118–1129. [CrossRef]
11. Zhao, X.; Chen, J.; Guo, M.; Li, C.; Hou, N.; Bai, S. Constructed Wetlands Treating Synthetic Wastewater in Response to Day-Night Alterations: Performance and Mechanisms. *Chem. Eng. J.* **2022**, *446*, 137460. [CrossRef]
12. Wu, H.; Wang, R.; Yan, P.; Wu, S.; Chen, Z.; Zhao, Y.; Cheng, C.; Hu, Z.; Zhuang, L.; Guo, Z.; et al. Constructed Wetlands for Pollution Control. *Nat. Rev. Earth Environ.* **2023**, *4*, 218–234. [CrossRef]
13. Zaehle, S.; Dalmonech, D. Carbon-Nitrogen Interactions on Land at Global Scales: Current Understanding in Modelling Climate Biosphere Feedbacks. *Curr. Opin. Environ. Sustain.* **2011**, *3*, 311–320. [CrossRef]
14. Niu, S.; Song, L.; Wang, J.; Luo, Y.; Yu, G. Dynamic Carbon-Nitrogen Coupling under Global Change. *Sci. China Life Sci.* **2023**, *66*, 771–782. [CrossRef] [PubMed]

15. Liu, H.Y.; Huang, N.; Zhao, C.M.; Li, J.H. Responses of Carbon Cycling and Soil Organic Carbon Content to Nitrogen Addition in Grasslands Globally. *Soil Biol. Biochem.* **2023**, *186*, 109164. [CrossRef]
16. Cao, Q.; Wang, H.; Zhang, Y.; Lal, R.; Wang, R.; Ge, X.; Liu, J. Factors Affecting Distribution Patterns of Organic Carbon in Sediments at Regional and National Scales in China. *Sci. Rep.* **2017**, *7*, 5497. [CrossRef] [PubMed]
17. Qi, Q.; Zhang, D.; Zhang, M.; Tong, S.; Wang, W.; An, Y. Spatial Distribution of Soil Organic Carbon and Total Nitrogen in Disturbed Carex Tussock Wetland. *Ecol. Indic.* **2021**, *120*, 106930. [CrossRef]
18. Wang, J.; Chen, G.; Fu, Z.; Qiao, H.; Liu, F. Assessing Wetland Nitrogen Removal and Reed (Phragmites Australis) Nutrient Responses for the Selection of Optimal Harvest Time. *J. Environ. Manag.* **2021**, *280*, 111783. [CrossRef] [PubMed]
19. Wu, Y.; Leng, Z.; Li, J.; Yan, C.; Wang, X.; Jia, H.; Chen, L.; Zhang, S.; Zheng, X.; Du, D. Sulfur Mediated Heavy Metal Biogeochemical Cycles in Coastal Wetlands: From Sediments, Rhizosphere to Vegetation. *Front. Environ. Sci. Eng.* **2022**, *16*, 102. [CrossRef]
20. Chen, L.; Fang, K.; Wei, B.; Qin, S.; Feng, X.; Hu, T.; Ji, C.; Yang, Y. Soil Carbon Persistence Governed by Plant Input and Mineral Protection at Regional and Global Scales. *Ecol. Lett.* **2021**, *24*, 1018–1028. [CrossRef]
21. Wang, L.; Mei, W.; Yin, Q.; Guan, Y.; Le, Y.; Fu, X. The Variability in CO_2 Fluxes at Different Time Scales in Natural and Reclaimed Wetlands in the Yangtze River Estuary and Their Key Influencing Factors. *Sci. Total Environ.* **2021**, *799*, 149441. [CrossRef] [PubMed]
22. Wang, Y.; Wang, Y.-M.; Lu, Y.-T.; Qiu, Q.-L.; Fan, D.-M.; Wang, X.-C.; Zheng, X.-Q. Influence of Different Nitrogen Sources on Carbon and Nitrogen Metabolism and Gene Expression in Tea Plants (*Camellia sinensis* L.). *Plant Physiol. Biochem.* **2021**, *167*, 561–566. [CrossRef] [PubMed]
23. Moreau, D.; Bardgett, R.D.; Finlay, R.D.; Jones, D.L.; Philippot, L. A Plant Perspective on Nitrogen Cycling in the Rhizosphere. *Funct. Ecol.* **2019**, *33*, 540–552. [CrossRef]
24. Averill, C.; Turner, B.L.; Finzi, A.C. Mycorrhiza-Mediated Competition between Plants and Decomposers Drives Soil Carbon Storage. *Nature* **2014**, *505*, 543–545. [CrossRef] [PubMed]
25. Zhang, P.; Li, B.; Wu, J.; Hu, S. Invasive Plants Differentially Affect Soil Biota through Litter and Rhizosphere Pathways: A Meta-Analysis. *Ecol. Lett.* **2019**, *22*, 200–210. [CrossRef] [PubMed]
26. Witzgall, K.; Vidal, A.; Schubert, D.I.; Höschen, C.; Schweizer, S.A.; Buegger, F.; Pouteau, V.; Chenu, C.; Mueller, C.W. Particulate Organic Matter as a Functional Soil Component for Persistent Soil Organic Carbon. *Nat. Commun.* **2021**, *12*, 4115. [CrossRef] [PubMed]
27. Perkowski, E.A.; Waring, E.F.; Smith, N.G. Root Mass Carbon Costs to Acquire Nitrogen Are Determined by Nitrogen and Light Availability in Two Species with Different Nitrogen Acquisition Strategies. *J. Exp. Bot.* **2021**, *72*, 5766–5776. [CrossRef] [PubMed]
28. Hoek, T.A.; Axelrod, K.; Biancalani, T.; Yurtsev, E.A.; Liu, J.; Gore, J. Resource Availability Modulates the Cooperative and Competitive Nature of a Microbial Cross-Feeding Mutualism. *PLoS Biol.* **2016**, *14*, e1002606. [CrossRef] [PubMed]
29. Hu, Y.; Chen, M.; Yang, Z.; Cong, M.; Zhu, X.; Jia, H. Soil Microbial Community Response to Nitrogen Application on a Swamp Meadow in the Arid Region of Central Asia. *Front. Microbiol.* **2022**, *12*, 797306. [CrossRef]
30. Montesinos-Navarro, A. Nitrogen Transfer between Plant Species with Different Temporal N-Demand. *Ecol. Lett.* **2023**, *26*, 1676–1686. [CrossRef]
31. Cotrufo, M.F.; Soong, J.L.; Horton, A.J.; Campbell, E.E.; Haddix, M.L.; Wall, D.H.; Parton, W.J. Formation of Soil Organic Matter via Biochemical and Physical Pathways of Litter Mass Loss. *Nat. Geosci.* **2015**, *8*, 776–779. [CrossRef]
32. Xia, S.; Song, Z.; Singh, B.P.; Guo, L.; Bolan, N.; Wang, W.; Lin, G.; Fang, Y.; Wen, X.; Wang, J.; et al. Contrasting Patterns and Controls of Soil Carbon and Nitrogen Isotope Compositions in Coastal Wetlands of China. *Plant Soil* **2023**, *489*, 483–505. [CrossRef]
33. Lin, Y.; Tang, K.W.; Ye, G.; Yang, P.; Hu, H.-W.; Tong, C.; Zheng, Y.; Feng, M.; Deng, M.; He, Z.-Y.; et al. Community Assembly of Comammox Nitrospira in Coastal Wetlands across Southeastern China. *Appl. Environ. Microbiol.* **2023**, *89*, e0080723. [CrossRef] [PubMed]
34. Nissan, A.; Alcolombri, U.; Peleg, N.; Galili, N.; Jimenez-Martinez, J.; Molnar, P.; Holzner, M. Global Warming Accelerates Soil Heterotrophic Respiration. *Nat. Commun.* **2023**, *14*, 3452. [CrossRef]
35. Domeignoz-Horta, L.A.; Pold, G.; Liu, X.J.A.; Frey, S.D.; Melillo, J.M.; DeAngelis, K.M. Microbial Diversity Drives Carbon Use Efficiency in a Model Soil. *Nat. Commun.* **2020**, *11*, 3684. [CrossRef] [PubMed]
36. Chen, K.; Zhang, J.; Muneer, M.A.; Xue, K.; Niu, H.; Ji, B. Plant Community and Soil Available Nutrients Drive Arbuscular Mycorrhizal Fungal Community Shifts during Alpine Meadow Degradation. *Fungal Ecol.* **2023**, *62*, 101211. [CrossRef]
37. Huang, X.; Li, Y.; Lin, H.; Wen, X.; Liu, J.; Yuan, Z.; Fu, C.; Zheng, B.; Gong, L.; Zhan, H.; et al. Flooding Dominates Soil Microbial Carbon and Phosphorus Limitations in Poyang Lake Wetland, China. *Catena* **2023**, *232*, 107468. [CrossRef]
38. Li, J.; Jiang, M.; Pei, J.; Fang, C.; Li, B.; Nie, M.; Scott, T. Convergence of Carbon Sink Magnitude and Water Table Depth in Global Wetlands. *Ecol. Lett.* **2023**, *26*, 797–804. [CrossRef] [PubMed]
39. Wang, Y.; Wu, F.; Li, X.; Li, C.; Zhao, Y.; Gao, Y.; Liu, J. Effects of Plants and Soil Microorganisms on Organic Carbon and the Relationship between Carbon and Nitrogen in Constructed Wetlands. *Environ. Sci. Pollut. Res.* **2023**, *30*, 62249–62261. [CrossRef]
40. Zhang, J.; Zhang, B.; Jing, Y.; Kang, X.; Zhang, C. Design of Constructed Wetland in Xinxue River Estuary into Nansi Lake in Eastern Line of South-to-North Water Transfer Project. *China Water Wastewater* **2008**, *24*, 49–51.

41. Cao, Q.; Wang, R.; Zhang, H.; Ge, X.; Liu, J. Distribution of Organic Carbon in the Sediments of Xinxue River and the Xinxue River Constructed Wetland, China. *PLoS ONE* **2015**, *10*, e134713. [CrossRef] [PubMed]
42. Meng, X.; Zhang, X.; Li, Y.; Jiao, Y.; Fan, L.; Jiang, Y.; Qu, C.; Filimonenko, E.; Jiang, Y.; Tian, X.; et al. Nitrogen Fertilizer Builds Soil Organic Carbon under Straw Return Mainly via Microbial Necromass Formation. *Soil Biol. Biochem.* **2024**, *188*, 109223. [CrossRef]
43. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef] [PubMed]
44. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naïve Bayesian Classifier for Rapid Assignment of RRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [CrossRef] [PubMed]
45. Louca, S.; Parfrey, L.W.; Doebeli, M. Decoupling Function and Taxonomy in the Global Ocean Microbiome. *Science* **2016**, *353*, 1272–1277. [CrossRef] [PubMed]
46. Zhang, Z.; Cao, B.; Chen, Z.; Xu, K. Grafting Enhances the Photosynthesis and Nitrogen Absorption of Tomato Plants Under Low-Nitrogen Stress. *J. Plant Growth Regul.* **2022**, *41*, 1714–1725. [CrossRef]
47. Hu, X.; Wang, X.; Abbas, T.; Fang, T.; Miao, D.; Li, Y.; Chang, S.X.; Li, Y. Higher Ammonium-to-Nitrate Ratio Shapes Distinct Soil Nitrifying Community and Favors the Growth of Moso Bamboo in Contrast to Broadleaf Tree Species. *Biol. Fertil. Soils* **2021**, *57*, 1171–1182. [CrossRef]
48. Pontiggia, D.; Benedetti, M.; Costantini, S.; De Lorenzo, G.; Cervone, F. Dampening the DAMPs: How Plants Maintain the Homeostasis of Cell Wall Molecular Patterns and Avoid Hyper-Immunity. *Front. Plant Sci.* **2020**, *11*, 613259. [CrossRef] [PubMed]
49. Zhou, M.; Jin, X.; Jiang, M.; Lü, X.; Lou, Y. Helophytes Adapt to Water and N-Enrichment Stresses by Adjusting and Coordinating Stoichiometry Characteristics in Main Organs. *Sci. Total Environ.* **2023**, *898*, 165538. [CrossRef]
50. Fang, J.; Zhao, R.; Cao, Q.; Quan, Q.; Sun, R.; Liu, J. Effects of Emergent Aquatic Plants on Nitrogen Transformation Processes and Related Microorganisms in a Constructed Wetland in Northern China. *Plant Soil* **2019**, *443*, 473–492. [CrossRef]
51. Liu, Y.; Shen, J.; Zhang, C.; Chen, Z. Impact of Rubber-Based Land Use Changes on Soil Properties and Carbon Pools: A Meta-Analysis. *Catena* **2023**, *227*, 107121. [CrossRef]
52. Knops, J.M.H.; Bradley, K.L.; Wedin, D.A. Mechanisms of Plant Species Impacts on Ecosystem Nitrogen Cycling. *Ecol. Lett.* **2002**, *5*, 454–466. [CrossRef]
53. Vymazal, J. Plants in Constructed, Restored and Created Wetlands. *Ecol. Eng.* **2013**, *61*, 501–504. [CrossRef]
54. Gutiérrez-Girón, A.; Rubio, A.; Gavilán, R.G. Temporal Variation in Microbial and Plant Biomass during Summer in a Mediterranean High-Mountain Dry Grassland. *Plant Soil* **2014**, *374*, 803–813. [CrossRef]
55. Fierer, N.; Walsh, C.M. Can We Manipulate the Soil Microbiome to Promote Carbon Sequestration in Croplands? *PLoS Biol.* **2023**, *21*, e3002207. [CrossRef]
56. Lehmann, J.; Kleber, M. The Contentious Nature of Soil Organic Matter. *Nature* **2015**, *528*, 60–68. [CrossRef]