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N-enriched biochar increases carbon, nitrogen, and phosphorus accumulation associated with changes in plant ecological stoichiometry in subtropical rice paddy fields

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Summary

N-enriched biochar can increase the accumulation of carbon (C), nitrogen (N), phosphorus (P) and biomass in rice plants. On the other hand, the biomass and C, N and P contents of plant organs are important indicators to reflect plant C, N and P storages. We established control, 4 t ha⁻¹, and 8 t ha⁻¹ N-enriched biochar treatment plots in a subtropical paddy field in China to investigate the effect of these treatments on C, N, and P storages, ecological stoichiometry in various rice-plant organs, and their relationships with edaphic factors. The application of N-enriched biochar increased the biomass and storages of C, N and P in rice roots, stems, leaves and grains, mainly at 4 t ha⁻¹. The application of N-enriched biochar decreased the C/N and C/P ratios of rice organs, but increased their N/P ratio. Changes in C/N were mainly due to the changes in storage, while N/P was positively correlated with N storage of stems, leaves and grains, and negatively correlated with P storage in roots. Pearson correlation analysis revealed that pH was negatively correlated and soil N content was positively correlated with P storage in various organs of rice. In addition, soil P content and chlorophyll were positively correlated with N storage. In conclusion, we found that the application of N-enriched biochar improved plant N- and P-storage and stoichiometrical relations among rice organs.

Keywords: N-enriched biochar; Rice organs; Soil environmental factors; Carbon, nitrogen and phosphorus; Ecological stoichiometry

Introduction

The balance and cycles operating among structural elements (such as C), limiting elements (as N and P), and their ecological stoichiometric ratio in plant organs have always been a focus of interest (Jia *et al.*, 2018). C/N and C/P ratios determine the plant ability to assimilate C and P, to a certain extent, reflecting N and P use efficiency, respectively (Sun *et al.*, 2016). N/P ratio can serve as an index to establish N and P saturations and nutrient supply from soils to plants (von Oheimb *et al.*, 2010), as N and P are essential and critical for improving biomass and productivity, but limiting elements in agricultural ecosystems, forests, and wetlands (Elser *et al.*, 2007). Fertilization and agricultural management are traditional ways of boosting crop productivity and promoting ecosystem health (Sanaullah *et al.*, 2020). Awareness and use of biochar and fertilizer mixtures are fast gaining interest as alternatives amongst modern-day sustainable agricultural practices (Wang *et al.*, 2020). Biochar has a special pore structure and singular physicochemical properties, which can improve physical and chemical properties of soils, and consequently increase nutrient use whilst reducing greenhouse gas emissions (Yin *et al.*, 2021a). Intrinsically, biochar is of immense value to both agriculture and the environment (Xu *et al.*, 2016). However, further research is required in defining the nutrient contents, storage and ecological stoichiometry of plant

organs under a combination of nitrogen fertilizers and biochar. Studies of the stoichiometric ratios among C, N, and P contents and storages in plant organs following the application of N-enriched biochar can help develop a robust theoretical basis for understanding the drivers of biogeochemical cycles.

The root system is an important and environmentally sensitive organ involved in nutrient absorption and storage (Cao *et al.*, 2020). Stems are vital for nutrient transport and coordination among plant organs (Fortunel *et al.*, 2012), while leaves are primary sites of photosynthesis (He *et al.*, 2020). Then, the contents and distribution of N and P among plant organs affect the photosynthesis of plants (Dordas, 2009). Plant organs have different nutrient use strategies and ecological functions, and cooperate with each other in terms of nutrition and function (Kleyer and Minden, 2015; Zhao *et al.*, 2019). The contents of N and P in plant organs will be different due to organ function, as organs with intense metabolism tend to enrich more in N and P and form higher biomass (Zhang *et al.*, 2018). In addition, agricultural management patterns and global changes will affect the accumulation of C, N and P by plants and change the ecological stoichiometric ratio (Elser *et al.*, 2010; Sardans *et al.*, 2021). For example, C/N changes can reflect changes in N use efficiency by plants (Zhang *et al.*, 2020). In fact, the lack of studies on disentangling nutrient allocation strategies from ecological stoichiometry of plant organs have hindered our understanding.

Organ function, climate, latitude, and soil nutrients all affect the allocation of C, N, and P and the stoichiometric ratios in plant organs (He *et al.*, 2015). Generally, climate and latitude determine nutrient allocation patterns in plants. For example, plants in temperate regions at high latitudes are most limited by N, while in tropical regions at low latitudes are limited by P (Reich and Oleksy, 2004). Soil nutrient concentrations also affect root absorption and use efficiencies (Zeng *et al.*, 2016). From a macroscopic perspective, climate and soil environment will jointly affect plant growth (Lin *et al.*, 2022). Soil nutrient content and plant nutrient use strategies can determine plant growth more than climate factors in desert areas (Luo *et al.*, 2021). Regardless of the environment, chlorophyll is of great significance for biomass production and crop yield in the ecosystem (Cai *et al.*, 2012; Li *et al.*, 2018), being its content affected by N and P in leaves (Allen and Williams, 1998).

China's subtropical region is an important rice production area with a large demand for N and P fertilizers (Li *et al.*, 2010; Wang *et al.*, 2016). As a new soil conditioner, biochar has a positive effect on plant growth in subtropical acidic soil (Li *et al.*, 2012; Hossain *et al.*, 2020). In our previous studies, the application of N-enriched biochar in subtropical rice fields increased the soil concentrations of C, N, P and available P, changing the composition of microbial communities and reducing greenhouse gas emissions (Yin *et al.*, 2021a; Yin *et al.*, 2021b). The C, N, P contents of plant organs constitute good proxies of overall plant nutrient status and can provide information of how crop amendments impact on plant nutritional status (Ogle *et al.*, 2012; Yu *et al.*, 2018). However, little is known about how C, N and P storages in rice organs are changed when soil C, N and P contents are increased as well as about the relationships

among ecological stoichiometric ratios of C, N and P, soil and plant factors. We collected samples of rice roots, stems, leaves and grains at different growth stages along a year to investigate whether N-enriched biochar would change the concentrations and storage of C, N and P, and C/N/P ratios in different rice organs; and also how soil environmental factors can influence C, N, P storage of rice organs and the regulation of stoichiometric ratio.

Materials and Methods

Experimental area

The study was performed in the Wufeng Experimental Base of the Rice Research Institute of the Fujian Academy of Agricultural Sciences, China (26.1°N, 119.3°E, 3 to 5 m altitude) in the subtropical marine monsoon climate region. The early and late rice (*Oryza sativa* L.) cultivars were conventional indica rice Hesheng 10 (Jiangxi Academy of Agricultural Sciences, China) and Qinxiangyou 212 (Fujian Academy of Agricultural Sciences, China). Within a year, the farming practice was two successive rice crops (early and late rice), and then vegetables (*Ipomoea aquatica* Forsk) (Yin *et al.*, 2021a). The growth period of early rice was from April to July, and that of late rice was from July to November. The average air temperature and humidity during the early rice and late rice cycles were 27.1° C and 84.9%, and 26.5° C and 78.9%, respectively (Yang *et al.*, 2022). The rice paddy cultivation soil layer consisted of 28% sand, 60% silt, and 12% clay silty loam. The soil pH was 6.5, and soil contents of C, N, and P were 18.2, 1.9, and 1.8 g kg⁻¹, respectively. The available P, NH₄⁺-N and NO₃⁻+NO₂⁻ in soil were 0.3, 4.6 and 29.6 mg kg⁻¹ (Yin *et al.*, 2021b).

Rice seedlings were transplanted and sown by a transplanter, with plant spacing and line spacing of 14 and 28 cm, respectively. Compound fertilizers (N:P₂O₅:K₂O, 16:16:16) and urea (46% N) were the main fertilizers used to provide 42 kg N ha⁻¹, 40 kg P₂O₅ ha⁻¹, and 40 kg K₂O ha⁻¹ before rice planting, and then 35 kg N ha⁻¹, 20 kg P₂O₅ ha⁻¹, and 20 kg K₂O ha⁻¹ during tillering. After tillering, 18 kg N ha⁻¹, 10 kg P₂O₅ ha⁻¹, and 10 kg K₂O ha⁻¹ were supplied again to plants. Throughout the tillering stage, water levels were maintained at 5 to 7 cm above the soil surface using an automatic water level controller. After the tillering stage, the dry wet alternate mode of intermittent irrigation was implemented. Water was drained from the paddies two weeks prior to harvest.

Field experiments

The experiment was designed based on the planting habits of Chinese subtropical farmers and previous research (Yin *et al.*, 2021a; Yin *et al.*, 2021b). Spring and summer were the main periods for planting early rice, while summer and autumn were for late rice. In April 2017, plants of the same size and height in the early rice experimental field were selected, and control, 4 t ha⁻¹, and 8 t ha⁻¹ N-enriched biochar treatment groups were established. Three plots per treatment were established in a total of nine plots over an area of 10 m², 0.5-cm thick and 30-cm high PVC plates were placed

between each plot to isolate replicate areas.

The experimental N-enriched biochar had total carbon concentration (TC) 6%, total nitrogen concentration (TN) 8%, and total phosphorus concentration (TP) 0.4%, with other components as detailed in Suppl. Material Tab. S1, and the electron microscopy image of N-enriched biochar in Suppl. Material Fig. S1. The process of preparation of biochar includes slowly pyrolysis of biomass at 600 °C for 90 min, and thereafter mixing with nitrogen fertilizer at a ratio of about 1:3 (Yin *et al.*, 2021b). The main reason for considering application of N-enriched biochar is based on the characteristics of subtropical rice fields, where soil N is an important limiting factor (Inamura *et al.*, 2009; Yin *et al.*, 2021a). In particular, the role of eutrophic biochar (N, P, etc.) is increasingly valued because it is designed for specific areas and may have great potential for sustainable agricultural development (Karim *et al.*, 2022).

Plant analyses

Three samples of plant roots, stems, leaves, and grains from the control, 4 t ha⁻¹, and 8 t ha⁻¹ treatment groups were randomly collected during the tillering and maturity stages from both early and late rice crops. Fresh leaves were also collected and used to measure chlorophyll concentration, while the remainder of the plant was oven-dried to constant weight for biomass measurement. The leaf surface area was calculated through the equation 1 by measuring the leaf length and maximum leaf width (Umashankar *et al.*, 2005).

$$\text{Leaf surface area} = \text{maximum leaf width} * \text{leaf length} * 0.75 \quad (1)$$

Biomass of rice roots, stems, and leaves was determined by drying at 60 °C. The dried roots, stems, leaves and grains were ground with a ball mill (KZ-II, China). The plant C and N contents were measured by dry combustion using CN element analyzer (Vario EL, Germany) following Leuschner *et al.* (2006), while plant P content was determined after sulfuric acid and perchloric acid digestion, using a continuous flow analyzer (Skalar analytical SAN++, Netherlands), as reported by Van Staaldin *et al.* (2010).

For chlorophyll determination, leaf samples were ground to homogenate, and chlorophyll was extracted with anhydrous ethanol to a fixed volume of 25 mL. The chlorophyll concentrations in rice leaves were measured using an ultraviolet visible spectrophotometer (Shimadzu UV-2450, Kyoto, Japan) and estimated as follows (Zou, 2000):

$$Chla (mg L^{-1}) = 13.95 * D_{665} - 6.88 * D_{649} \quad (2)$$

$$Chlb (mg L^{-1}) = 24.96 * D_{665} - 7.32 * D_{649} \quad (3)$$

$$Chl (mg g^{-1}) = (Chla + Chlb) * \frac{V}{M} \quad (4)$$

where *Chla* and *Chlb* are the concentrations of chlorophyll *a* and *b* in the extract, *Chl*

is the concentration on mass basis, D_{649} and D_{665} represent absorbance at a given wavelength (665 nm and 649 nm), M is the sample weight, and V is the volume (mL).

Soil analyses

Soil C and N contents were determined by dry combustion using CN element analyzer (Vario MAX CN, Elementar, Germany), following Amishev and Fox (2006). Soil P content was digested by sulfuric acid and perchloric acid, and determined by continuous flow analyzer (Skalar analytical SAN++, Netherlands) (Yu *et al.*, 2010). Soil salinity, pH, and temperature were measured at a depth of 15 cm with a conductivity meter (2265FS EC Meter, Spectrum Technologies Inc., USA) and pH/temperature meter (Starter 300, USA), respectively.

Allometric growth

The proportional allometric model $y = ax^k$ was used to explore the proportional relationship among C, N, and P in rice plant organs. Firstly, the logarithm of both sides was transformed as follows:

$$k = \frac{\log(y) - \log(a)}{\log(x)} \quad (5)$$

where k is the slope (allometric growth index), and x and y the C, N, and P contents and storage. During the analysis, the original data was converted into a base-10 logarithmic form. When $k = 1$, the ratio of any two elements in the roots, stems, and leaves of the rice fields was isokinetic, whereas when $k > 1$ or $k < 1$, the ratio of the two elements was allokinetic. Regression analysis was used to analyze the model parameters (Niklas and Cobb, 2005).

Calculation of storage of C, N, and P in rice organs

The C, N, and P storages per unit area ($Y_{storage}$) in both early and late rice were estimated according to Ogle *et al.* (2012):

$$Y_{storage} = X_{concentration} * B_{biomass} \quad (6)$$

where $X_{concentration}$ is the content of C, N, or P in a given organ and $B_{biomass}$ is the biomass of a given organ.

Statistical analysis

All the experimental data were tested for normality and homogeneity of variance before analysis and inconsistent data were standardized. Data were plotted using Excel 2019 (Microsoft, USA), Origin 2020 (OriginLab, USA) and SPSS 20.0 (SPSS Inc., USA) statistical analysis software. A one-way analysis of variance (ANOVA) and repeated-measures ANOVA in SPSS 22.0 were used to analyze differences in C, N, and P contents, storages and ecological stoichiometric ratio of rice roots, stems, leaves, and grains after application of the N-enriched biochar. While one-way ANOVA was used to analyze the treatment effects in each sampling time, repeated-measures ANOVA was used to analyze the overall treatment effect along time and the interactions of time with treatment. The minimum significant difference (LSD, at $p < 0.05$) was used for comparisons. The storages, ecological stoichiometric ratios, contents of C, N, and P in

roots, stems, leaves, and grains were mapped using a linear fitting of the allometric growth in Origin 2020. The redundancy analysis (RDA), the Pearson correlation analysis, and the mapping of the environmental factors, plant C, N, and P contents, storages and their stoichiometric ratios were carried out with *Canoco 5* and *R* language *corrplot* package (McKenna *et al.*, 2016).

Results

N-enriched biochar, phenotypic parameters and biomass of rice organs

During the tillering and maturity stages, 4 t ha⁻¹ N-enriched biochar increased leaf surface area in both early and late rice (Fig. 1a), and the chlorophyll concentrations in early rice (Fig. 1b). Applying 4 t ha⁻¹ N-enriched biochar during the tillering stage increased root biomass in early and late rice, and at maturity stage, 4 t ha⁻¹ N-enriched biochar increased root biomass of only early rice (Fig. 1c). The 4 t ha⁻¹ N-enriched biochar increased stem biomass at maturity for both early and late rice (Fig. 1d). The 4 t ha⁻¹ N-enriched biochar during the tillering and maturity stages in both early and late rice increased leaf biomass (Fig. 1e) and increased grain biomass in both early rice and late rice (Fig. 1f). In comparison, application of 8 t ha⁻¹ N-enriched biochar (cf. control) generally had no effect on the measured phenotypic parameters, except chlorophyll concentrations of early rice at tillering.

N-enriched biochar and C, N, and P storages in rice organs

In early rice, 4 t ha⁻¹ N-enriched biochar dose increased C storage in roots and leaves at the tillering stage (Fig. 2a,c), and in stems, leaves, and grains at the maturity stage (Fig. 2b-d). In late rice, 4 t ha⁻¹ biochar increased C storage in stems and leaves at the tillering and maturity stages (Fig. 2b,c). Yet, 8 t ha⁻¹ biochar dose generally had no effect on C storage in roots and stems, but it did increase C storage in leaves both in early and late rice.

In early rice, 4 and 8 t ha⁻¹ doses of N-enriched biochar increased N storage in roots, stems, and leaves at the tillering stage (Fig. 2e-g), and in stems and leaves, at the maturity stage (Fig. 2f,g). In late rice, 8 t ha⁻¹ of N-enriched biochar increased N storage in roots and stems at the tillering stage (Fig. 2e,f), and 4 t ha⁻¹ and 8 t ha⁻¹ doses increased N storage in leaves at the tillering and maturity stage (Fig. 2g). In both early and late rice, 4 t ha⁻¹ N-enriched biochar dose increased N storage in grains (Fig. 2h).

In both early and late rice, 4 and 8 t ha⁻¹ doses of N-enriched biochar increased P storage in leaves during the tillering and maturity stages (Fig. 2k). In early rice, 4 and 8 t ha⁻¹ doses increased P storage in stems at the maturity stage (Fig. 2g). In both early and late rice, 4 t ha⁻¹ biochar dose increased P storage in grains (Fig. 2l). In addition, repeated-measures ANOVA showed N-enriched biochar treatment had a great impact on C, N and P storages in rice organs as a whole, while time and biochar × time interaction had a great impact on N storage of rice roots, stems and leaves (Suppl. Material Tab. S2).

N-enriched biochar and the ecological stoichiometric ratios

In early rice, 4 and 8 t ha⁻¹ doses of N-enriched biochar reduced the C/N in roots at tillering and in stems and leaves at both tillering and maturity stages (Fig. 3a-c). At the maturity stage, 4 and 8 t ha⁻¹ doses of N-enriched biochar decreased the C/N in grains of early rice (Fig. 3d). Four and 8 t ha⁻¹ doses of N-enriched biochar increased root C/P in early rice at tillering (Fig. 3e), but decreased stem and leaf C/P at maturity (Fig. 3f,g). The 4 and 8 t ha⁻¹ doses of N-enriched biochar reduced leaf C/P of late rice at the maturity stage (Fig. 3g). At the tillering stage, 4 and 8 t ha⁻¹ doses of N-enriched biochar significantly increased N/P in roots, stems, and leaves in early rice (Fig. 3i-k). In early rice, 8 t ha⁻¹ N-enriched biochar dose increased N/P in all rice organs at the maturity stage (Fig. 3i-l). On the other hand, 8 t ha⁻¹ N-enriched biochar dose increased N/P only in stems and leaves of late rice at the tillering stage (Fig. 3j,k). Overall, N-enriched biochar treatment had a great impact on the C/N and C/P of rice roots and stems, while time and biochar × time interaction impacted N/P of rice roots and stems (Suppl. Material Tab. S2).

Based on the principal component analysis, we found significant differences between C, N and P storages of roots, stems and leaves and the ecological stoichiometric ratio at the tillering stage. Among them, the difference between stems and roots was the most obvious (Fig. 4a,b). C, N and P storages of stems and grains were higher than those of roots and leaves in both early and late rice (Suppl. Material Fig. S2).

Allometric growth, C, N and P storages and ecological stoichiometric ratio

The allometric growth model of ecological stoichiometric ratio and C, N and P storages revealed that root N storage was negatively correlated with C/N (Fig. 5a) and root P storage was negatively correlated with C/N and positively correlated with N/P (Fig. 5a,c). Root P storage was negatively correlated with C/N and positively correlated with N/P (Fig. 5a,c). Root C storage was negatively correlated with C/P and N/P (Fig. 5b,c). The N storage of stems, leaves and grains were negatively correlated with C/N and positively correlated with N/P (Fig. 5d,f,g,i,j,l), while grain P storage was negatively correlated with C/P (Fig. 5k).

Contents, storages, and ecological stoichiometric ratio as affected by environmental factors

The cumulative interpretation degrees of soil environmental factors to the contents, storages and ecological stoichiometric ratio of C, N and P in rice roots, stems, leaves and grains were 27.9%, 59.9%, 59.9% and 91.5%, respectively (Suppl. Material Fig. S3a-d). Among them, soil pH was the main factor affecting roots, and soil pH, bulk density and water content were the main factors affecting stems. The soil P content, bulk density and leaf surface area were the main factors affecting leaves, and soil C content and leaf surface area were the main factors affecting grains (Suppl. Material Fig. S3a-d). From the relationship between soil environmental factors and C, N, P

330 storages and ecological stoichiometric ratio, soil pH was negatively correlated with P
331 storage in various rice organs (Fig. 6a-d), negatively correlated with C/P in roots, stems
332 and leaves (Fig. 6a-c), and negatively correlated with grain N/P in various organs (Fig.
333 6d). Soil salinity was positively correlated with N storage in roots, stems and leaves,
334 and negatively correlated with C/N (Fig. 6a-c). Soil N content was positively correlated
335 with P storage and C/P in roots, stems and leaves of rice (Fig. 6a-c). Soil P content was
336 positively correlated with N storage and negatively correlated with C/N in roots and
337 stems (Fig. 6a,b). Leaf surface area was positively correlated with C storage and
338 chlorophyll was positively correlated with N storage and negatively correlated with
339 C/N in various organs (Fig. 6a-d).

341 Discussion

342 *C, N, and P storages and ecological stoichiometry in rice organs as affected by N-* 343 *enriched biochar*

345 Biochar can affect plant growth by changing the composition and then physical
346 and chemical properties of soil microbial community (Liao *et al.*, 2016), which is an
347 important additive affecting the cycling of nutrients such as N and P (Hossain *et al.*,
348 2020). During both early and late rice cultivation, 4 t ha⁻¹ N-enriched biochar increased
349 biomass, and storage of C, N, and P in grains, and 4 and 8 t ha⁻¹ N-enriched biochar
350 increased storages of C, N and P in leaves (Figs. 1c-f and 2c,d,g,h,k,l). During early
351 rice cultivation, 4 and 8 t ha⁻¹ N-enriched biochar increased the content of N in roots,
352 stems and grains, increased the contents of C, N and P in leaves, and increased C, N
353 storages of roots and N storage of stems (Fig. 2a,e,f and Suppl. Material Fig.
354 S4c,e,f,g,h,k). These changes supported the hypothesis that N-enriched biochar would
355 increase the contents and storages of C and N in some rice tissues. Our results are also
356 in agreement with previous reports on the application of P-enriched biochar and other
357 types of biochar (Biederman and Harpole, 2013; Wang *et al.*, 2014). The most direct
358 influencing factor is that N-enriched biochar improves the plant accumulation of N, P
359 and biomass (Yuan *et al.*, 2018). Because the application of N-enriched biochar
360 increased the contents of soil C, N and P through the increase of fungal and bacterial
361 abundance and the improvement of soil physics and chemistry, P sequestration in
362 subtropical paddy soil was increased and N limitation alleviated (Yin *et al.*, 2021b). In
363 addition, the N-enriched biochar applied in this experiment contained elements such as
364 iron, aluminium, manganese, and silicon (Suppl. Material Tab. S1), which can meet
365 other nutrient needs of plants, combine with cations in soil minerals and reduce nutrient
366 loss (Yin *et al.*, 2021a).

367 N-enriched biochar has unique pore structure and high specific surface area, which
368 improve soil physical and chemical conditions to increase the nutrient retention
369 capacity of soils, and then improve the nutrient use efficiency of plants (Jin *et al.*, 2019;
370 Hossain *et al.*, 2020). For example, N-enriched biochar can improve soil cation and
371 anion exchange capacity to reduce nutrient gaseous volatilization and leaching loss

(Tomczyk *et al.*, 2020), and reduce soil bulk density to improve soil water holding capacity and aggregate stability (Shaheen and Turaib Ali Bukhari, 2018). Therefore, N-enriched biochar can play a role in nutrient fixation and provide potential nutrient sources for plants. However, due to the influence of environmental factors such as soil physics and chemistry and temperature, the relationship between the application amount of biochar and the absorption of nutrients by plants is not linear (Yuan *et al.*, 2018). Our study revealed that the application of 4 t ha⁻¹ N-enriched biochar had better effects on C, N and P storage and phenotypic parameters of rice than 8 t ha⁻¹ (Figs. 1 and 2). In fact, excessive fertilization can cause nutrient overload or even disrupt the ecological stoichiometric balance between C, N, and P in plant organs, negatively affecting plant growth (Hoegberg *et al.*, 2006; Penuelas *et al.*, 2013).

The ecological stoichiometric ratio of C, N, and P elements in plants can reveal nutritional limitations, as well as changes in use of nutrients and growth rate (Luo *et al.*, 2020). Herein, we found that after the application of N-enriched biochar, the C/N of rice organs decreased in early rice (Fig. 3a-d), which also supported the hypothesis that N-enriched biochar would reduce the C/N in some rice tissues. This finding indicates that the limitation of N in rice organs decreased, and the N use-efficiency increased, which was conducive to the provision of biomass and yield (Hurtado *et al.*, 2020; He *et al.*, 2015). In addition, N and C/N, P and C/P in rice organs were negatively correlated, suggesting that the nutritional limitation imposed by N and P decreased after the application of N-enriched biochar (Fig. 6). Plant N/P can indicate the degree of limitation of N and P. When N/P<14, plant growth tends to be affected by N restrictions, whereas N/P>16 suggests plant growth tends to be restricted by P. When N/P is between 14 and 16, plant growth may be affected by N and P, or not limited by them (Koerselman and Meuleman, 1996). Our study showed that N/P of rice-plant organs was less than 14 (Fig. 3). During early rice cultivation at tillering stage, 4 and 8 t ha⁻¹ N-enriched biochar increased N/P of rice roots (1.7 and 2.8 times, respectively), stems (97% and 1.4 times, respectively) and leaves (32% and 33%, respectively), as shown in Fig. 3i-k. During early rice cultivation at maturity stage, 8 t ha⁻¹ N-enriched biochar increased the N/P of grains in 62% (Fig. 3l). Taken together, our data suggest that rice was mainly affected by N, but the application of N-enriched biochar increased the N/P ratio in rice and so compensated for the N limitation.

C, N and P storages and ecological stoichiometric allocation strategies in rice organs as affected by N-enriched biochar

The ecological stoichiometric ratio of C, N and P among plant organs can reflect nutrient acquisition strategies, functional differentiation and nutrient cycle patterns (Luo *et al.*, 2021). The results of PCoA showed that the distance between roots and leaves and grains were greater than that of stems. Differences in C, N and P storages among roots, leaves and grains due to N-enriched biochar application (Fig. 4) are consistent with a general change in functioning of rice organs and with the ecological stoichiometry paradigms (Sardans *et al.*, 2017). The allometric growth theory indicates

that C, N, and P contents in leaves have positive allometric correlation (Suppl. Material Fig. S5). As important metabolic organs, leaves capture CO₂ through photosynthesis and increase the accumulation of plant organic matter (Gao *et al.*, 2020). In structural organs such as roots and stems, P is stored to meet the needs of rice growth (Schreeg *et al.*, 2014). During the maturation, grain growth needs more nutrients to promote rice heading and high yields (Deng *et al.*, 2015). Therefore, physiological functions and nutrient use strategy enable plants to selectively adjust their nutrients to support growth (Yan *et al.*, 2016; Schreeg *et al.*, 2014). Our results also show that plants can coordinate the distribution and accumulation of nutrients in organs through the ecological chemometrics of C, N and P (Fig. 5), thus supporting our hypothesis that N-enriched biochar changes the stoichiometry of C, N and P accumulation among rice organs. There was a negative allometric relationship between N storage and C/N in all organs of rice (Fig. 5a,d,g,j). Herein, low C/N corresponded to higher N storage (Fig. 5d,e,g,j), which was caused by N limitation in subtropical rice fields where rice with fast growth rate but short cycle should absorb more nutrients (Wang *et al.*, 2015). In addition, N/P of rice stems, leaves and grains showed positive allometric correlation with N storage, and N/P of roots showed negative allometric correlation with P storage (Fig. 5c,f,i,l). Enrichment of N in stems, leaves and grains after the application of N-enriched biochar would alleviate N limitation and increase N stock. The enrichment of P in roots would increase P stock as well. This may be related to the differential distribution of N and P among rice organs (Suppl. Material Fig. S2b,c). The aboveground parts (stems, leaves and grains) usually have richer chlorophyll concentration, and then N should be concentrated to meet the photosynthetic requirement in a shorter life cycle (Ullah *et al.*, 2019). Because of P limitation, roots should absorb more P in subtropical soil to promote rice growth (Elser *et al.*, 2007; Hurtado *et al.*, 2020). Therefore, the accumulation of C, N and P is affected by the nutrient allocation strategy and the C, N and P ecological stoichiometry of rice organs.

C, N, and P storages in rice organs as affected by environmental factors

Our results showed that soil environmental factors such as soil pH, salinity, soil N and P contents affected the C, N and P storages and ecological stoichiometric ratio of rice organs (Fig. 6). In order to adapt to a changing environment, the nutrient dynamics and nutrient absorption strategy of plants should change and improve allocation to match growth (Leishman *et al.*, 2007). Correlation analysis revealed that low pH was associated to reduced P storage in roots, stems and leaves of rice (Fig. 6a-c). Soil P is easily fixed by iron and aluminium in acid soil, making it difficult for plants to use this nutrient (Elser *et al.*, 2007). However, the N-enriched biochar contains alkaline metal ions, which improve soil pH, improve the acidic soil environment, and help rice to obtain more available P from the soil (Hossain *et al.*, 2020). For example, P content in rice roots, stems and leaves was positively correlated with soil pH (Fig. 6a-c). In addition, soil salinity had a positive relationship with N storage of rice roots, stems and leaves (Fig. 6a-c). Higher investment in P mineralization and uptake is common when

N availability increases in terrestrial plant communities due to higher capacity of phosphatases and root growth (Sardans and Peñuelas 2012). Based on the specific surface area and pore structure, N-enriched biochar increases the content of soluble NO_3^- , K^+ and Ca^{2+} by affecting soil water holding capacity and pH (Igalavithana *et al.*, 2017). However, excessive salinity would reduce the absorption of nutrients such as N and P and this effect should be considered when applying N-enriched biochar. Previously, the application of N-enriched biochar increased the contents of soil C, N and P (Yin *et al.*, 2021b). Our results showed that N-enriched biochar increased and strengthened the relationships between soil N and P contents and the N and P storages in rice organs (Fig. 6). Soil N and P contents were interrelated with plant N and P storages, and leaf surface area was positively correlated with C storage in rice organs while chlorophyll was positively correlated with N storage in rice organs (Fig. 6). In fact, rice with short growth cycle needs more chlorophyll – and then N – to accumulate organic matter (Wang *et al.*, 2015). In conclusion, we found that organs responded differently to environmental factors, most likely due to different nutrient use strategies.

Overall impact of N-enriched biochar on economic and environmental benefits in rice paddy fields

The application of N-enriched biochar can change the physical and chemical properties of soil, leaf surface area and chlorophyll and increase the storages of C, N and P, which are regulated by the ecological stoichiometric ratio of C, N and P in rice organs (Fig. 7). A negative linear relationship between rice N storage and C/N was found, while N/P has a negative linear relationship with P storage of rice roots, and a positive linear relationship with N storage of rice stems, leaves and grains (Fig. 7). These findings can be explained by nutrient utilization strategy and the optimal ratio among plant organs (Zhang *et al.*, 2020). Our study showed that the application of N-enriched biochar will be conducive to the accumulation of rice biomass and N and P storage and stimulate plants to use and remobilize nutrients from rice organs, especially under 4 t ha^{-1} of N-enriched biochar. At the same time, N-enriched biochar help to reduce costs and accumulation of pollutants in farmland, thereby providing both economic and environmental benefits (Wang *et al.*, 2020). For example, Yin *et al.* (2021a) found that the application of N-enriched biochar in subtropical rice fields reduced greenhouse gas emissions while increasing rice yield. Previous studies have also shown that the application of N-enriched biochar in subtropical rice fields alleviate the limitation of N in soil and increase total and available soil P by improving soil physicochemical properties and microbial community richness, thus promoting rice growth (Yin *et al.*, 2021b).

The preparation of N-enriched biochar in this study used waste straw and wood, with a cost about US\$ 222.40 per ton, while the price of nitrogen fertilizer was about US\$ 355.85 per ton. By using agricultural residues such as waste straw and wood, we can reduce transportation costs and environmental pollution caused by incineration (Galinato *et al.*, 2011). It is worth noting that the carbon trading system may also bring

some economic income to farmers in the future (Pandit *et al.*, 2018). From the perspective of soil environment improvement, plant nutrient absorption and reduction of greenhouse gas emission, the application of 4 t ha⁻¹ N-enriched biochar would promote the sustainable development of subtropical rice production and offer farmers a new way of increasing rice yields. However, we must recognize that the rice response to 8 t ha⁻¹ N-enriched biochar in terms of biomass and yield was low, which indicates that excessive dosage of biochar may produce low economic and ecological benefits. This may be due to the negative effect of high salinity, as commented previously (Biederman *et al.*, 2013; Hossain *et al.*, 2020).

Conclusion

In general, this study comprehensively analyzed the changes of C, N and P storages in rice organs and their responses to soil environmental factors and ecological stoichiometry. The application of 4 t ha⁻¹ N-enriched biochar played a positive role in the nutrient accumulation in rice organs. Our study also revealed that in the subtropical paddy field dominated by N limitation, the storage of C, N and P among organs of rice varied, and the accumulation of C, N and P and their stoichiometric relations were affected mainly by soil pH, salinity, and N and P concentrations. Finally, the application of N-enriched biochar in subtropical areas can promote the absorption and accumulation of plant nutrients such as C, N and P, and provide good economic and ecological benefits.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1 Effects of N-enriched biochar (4 and 8 t ha⁻¹) on leaf surface area (a), chlorophyll concentration (b), and root (c), stem (d), leaf (e) and grain (f) biomass of early and late rice crops. Mean values and standard errors. Uppercase letters indicate differences among treatments in a given growth stage ($p<0.05$). In legend, T stands for tillering stage and M stands for maturity stage.

Fig. 2 Effects of N-enriched biochar (4 and 8 t ha⁻¹) on C (a-d), N (e-h) and P (i-l) storages in root (a,e,i), stem (b,f,j), leaf (c,g,k) and grain (d,h,l) of early and late rice crops. Mean values and standard errors. Uppercase letters indicate differences among treatments in a given growth stage ($p<0.05$). In legend, T stands for tillering stage and M stands for maturity stage.

Fig. 3 Effects of N-enriched biochar (4 and 8 t ha⁻¹) on C/N (a-d), C/P (e-h) and N/P (i-l) ratios in root (a,e,i), stem (b,f,j), leaf (c,g,k) and grain (d,h,l) of early and late rice crops. Mean values and standard errors. Uppercase letters indicate differences among treatments in a given growth stage ($p<0.05$). In legend, T stands for tillering stage and M stands for maturity stage.

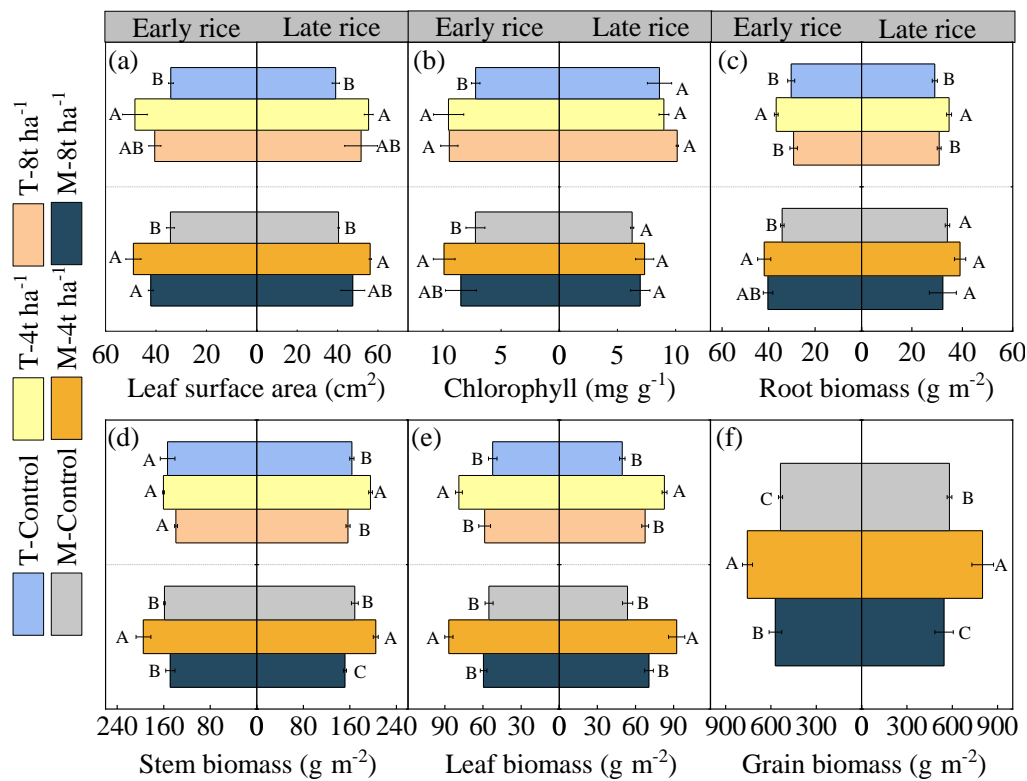
Fig. 4 Principal component analysis of C, N and P storage and ecological stoichiometric ratio in rice organs at the tillering (a) and maturation stages (b). The data includes early and late rice. The horizontal and vertical axes represent the first and second principal coordinates, respectively. The percentage marked on the horizontal and vertical axis is the contribution of the principal coordinate to the difference of the sample matrix data variance.

Fig. 5 Correlations between C, N and P storage (gray square, red circle, and blue triangle, respectively) and ecological stoichiometric ratios C/N (a,d,g,j), C/P (b,e,h,k) and N/P (c,f,i,l) in root (a-c), stem (d-f), leaf (g-i) and grain (j-l) of rice plants. r is the correlation coefficient, * and ** stand for $p<0.05$ and $p<0.01$, respectively.

Fig. 6 Correlation analysis of C, N and P concentrations, storage, ecological stoichiometric ratios and soil environmental factors, considering root (a), stem (b), leaf (c) and grain (d) of rice plants. WC: Soil water content; CL: Chlorophyll; BD: Soil bulk density; T: Soil temperature; SL: Soil salinity; LA: Leaf surface area; SC: Soil C; SN: Soil N; SP: Soil P, CS: C storage, NS: N storage, PS: P storage.

Fig. 7 A conceptual model of the effects of N-enriched biochar on the concentrations, storage and ecological stoichiometric ratios of C, N and P in rice organs.

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836 **Fig. 1**

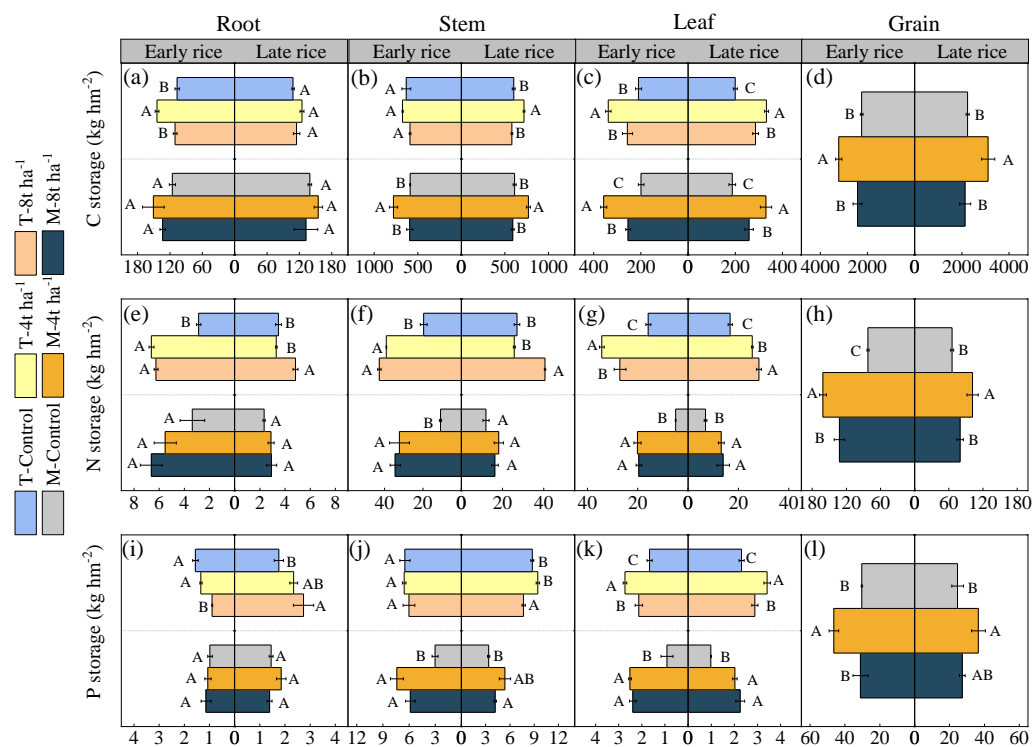


Fig. 2

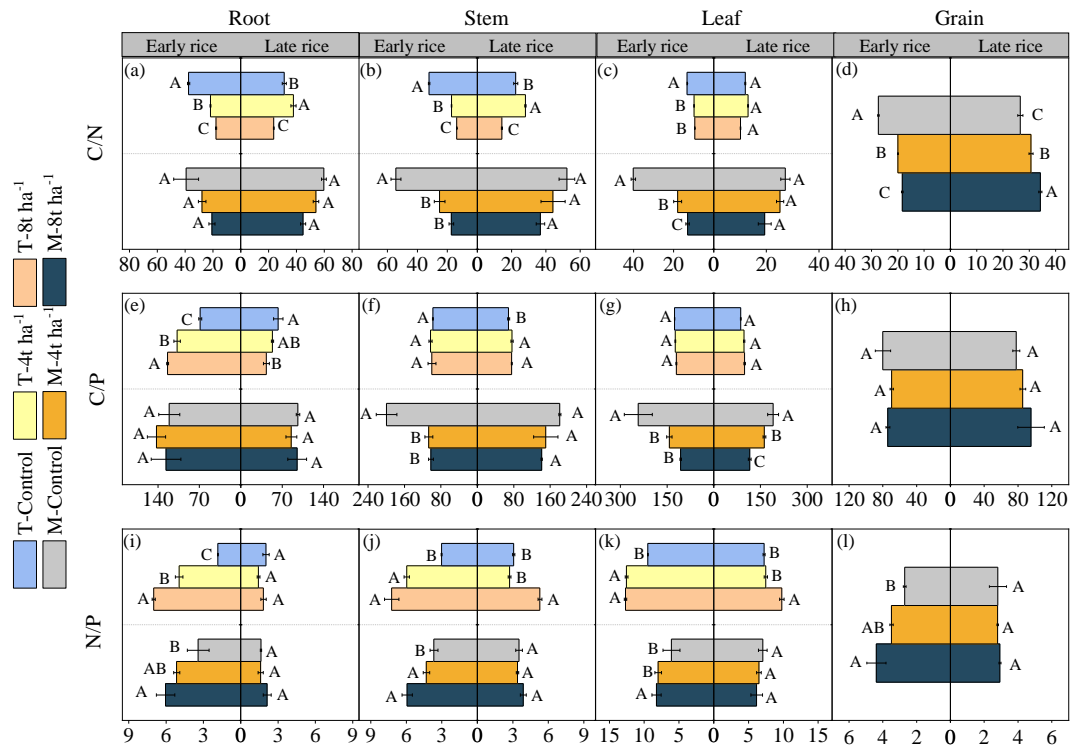
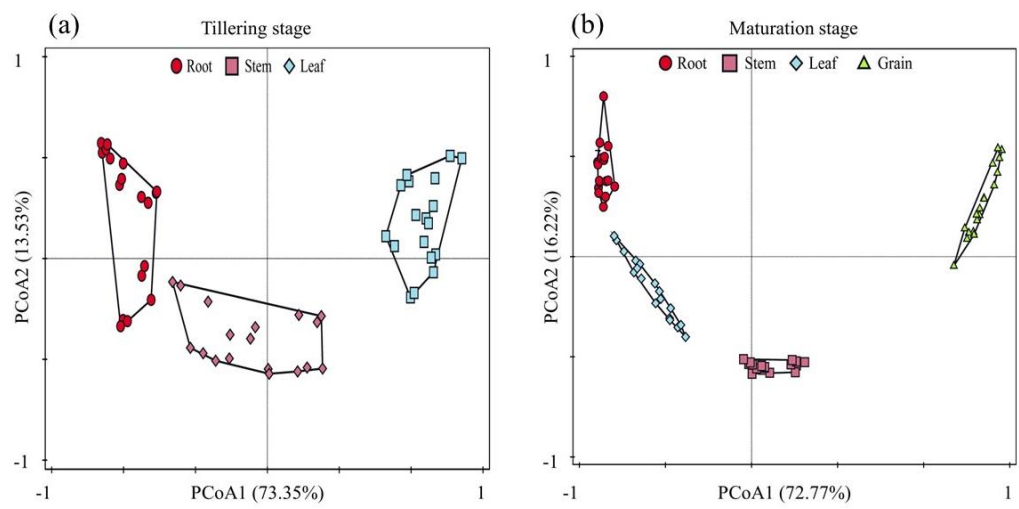


Fig. 3

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849 **Fig. 4**

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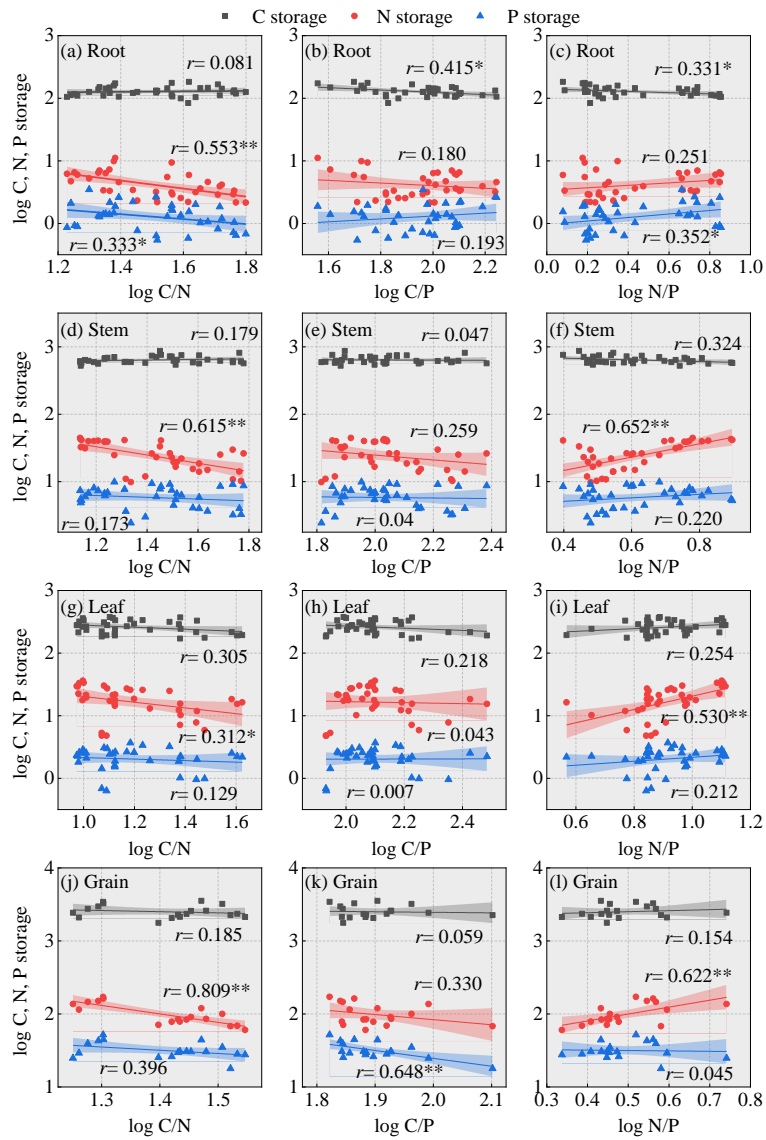


Fig. 5

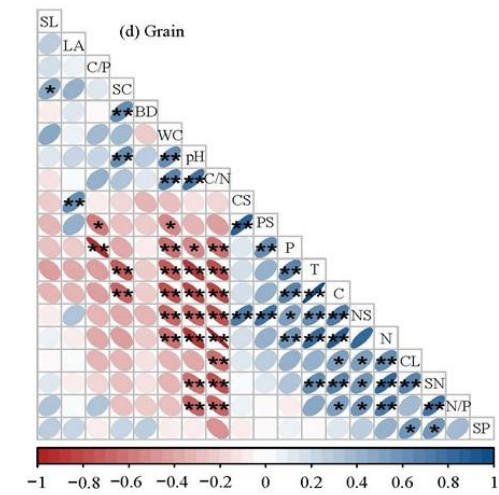
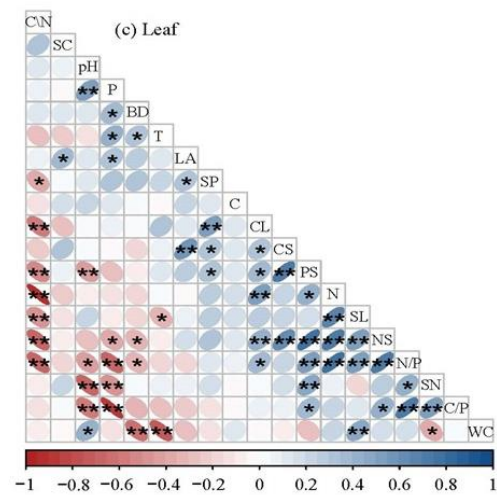
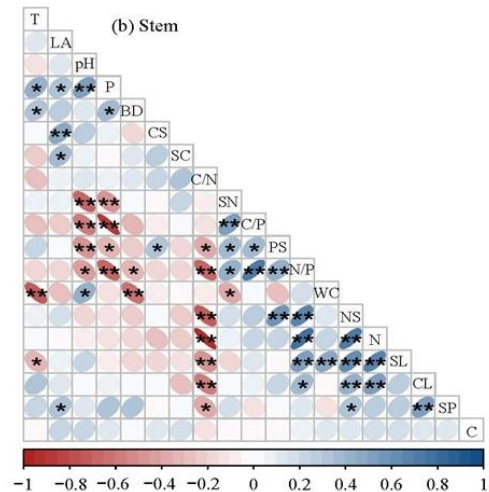
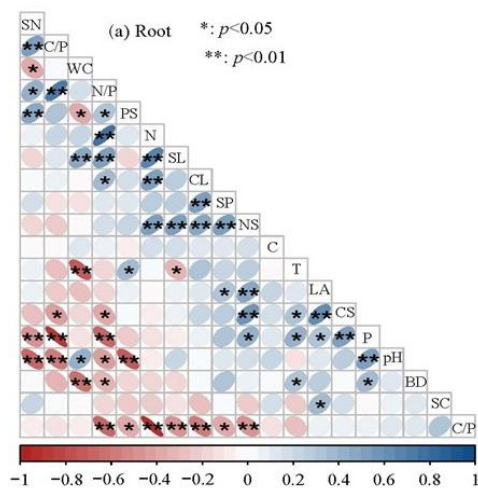
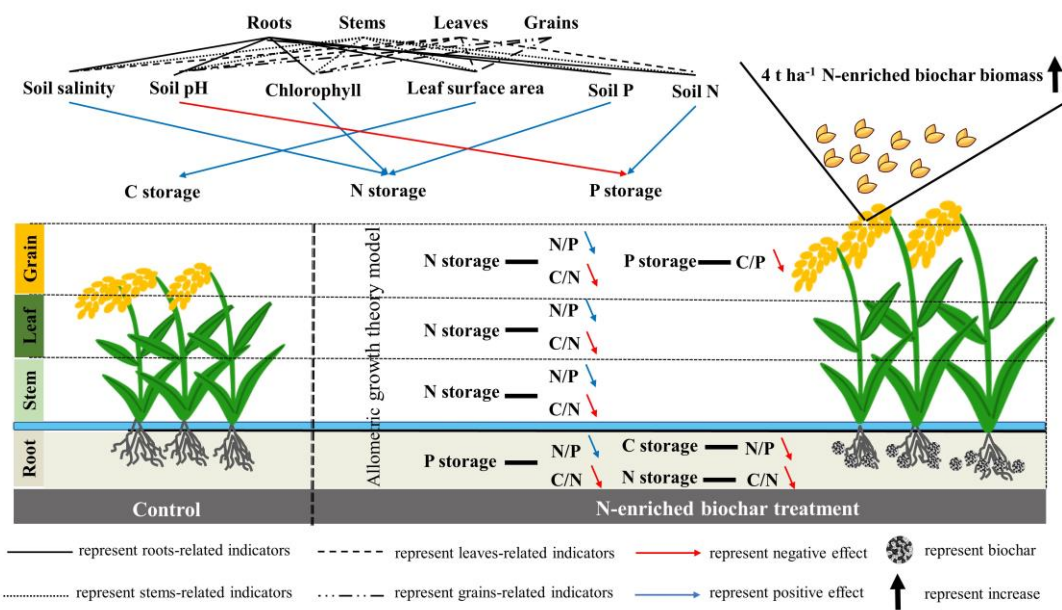


Fig. 6

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866 **Fig. 7**