# Biochar Combined with Organic and Inorganic Fertilizers Promoted the Rapeseed Growth and Improved the Soil Quality in Purple Soil

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# Abstract

**Background** Biochar is one kind of organic matter that can be added into soil as a soil amendment to improve its quality. To study the effect of biochar addition combined with organic and inorganic fertilizers on growth and fertility and microbial community in purple soil, a completely randomized block design was designed with three levels of biochar [B0: no biochar, B1: low-rate biochar (35 t/ha) , B2: high-rate biochar (50 t/ha)]; two levels of inorganic fertilizers [F1: low-rate inorganic fertilizer (30 kg/ha N, 87.5 kg/ha $P_2O_5$ and 60 kg/ha $K_2O$); F2: high-rate inorganic fertilizer (60 kg/ha N, 175 kg/ha $P_2O_5$ and 120 kg/ha $K_2O$)]; and two levels of organic fertilizers [M1: no organic fertilizer; M2: with organic fertilizer (4.5 t/ha)].

**Results** With the application of biochar, the plant height and stem diameter of rapeseed were increased by 6.15%-9.80% and 16.37%-20.11%, respectively. The photosynthetic capacity increased by 20.25% to 35.83%, and the yield increased by 16.40% to 19.11%, respectively. It also promoted the absorption and utilization of nitrogen, phosphorus and potassium by rapeseed to varying degrees. At the same time, the combined application of biochar and organic and inorganic fertilizers could improve soil pH, nitrogen, phosphorus and potassium content and soil microbial community richness: The pH of B1F2M1 increased 0.41 compared with the control, the nitrogen, phosphorus and potassium content in the soil increased by 103.95%, 117.88% and 99.05%, respectively. Meanwhile, soil microbial community richness was also improved.

**Conclusions** Our research showed that biochar could promote the growth and development of rapeseed, and the combined application of biochar with organic and inorganic fertilizers could improve soil fertility and increase microbial diversity. Low-rate biochar combined with organic fertilizer and low-rate inorganic fertilizer was the most suitable application mode in rapeseed production in purple soil area of Southwest China.

# 1. Background

Agriculture production faces the challenge to feed 9.8 billion humans in 2050 as well as climate change (Bruun et al., 2014). The healthy soil is attentively correlated with agricultural production. Management of soil quality is critical to maintaining agro-ecosystem services (Lal, 2015).

The challenges of the agriculture sector to escalating production are natural resources degradation, small landholder, and the effect of climate change (Lal and Steward, 2015). Cui et al. (2014) reported that from 1961 to 2011, China's agricultural production had been strengthened by the Green Revolution with cereal production escalating by 3.9 times. Increased yield in the Green Revolution was associated with the high application of chemical fertilizers, pesticides, and irrigations as well as with the critical problems of soil degradation, water contamination and air pollution (Lal, 2018; Ren et al, 2021). Overuses of inorganic fertilizers, particularly P and N that mobilized and flowed into groundwater through runoff and leaching, have determined water eutrophication and soil acidification (Wu et al., 2018). In a short time, the Green Revolution boosted food production worldwide, but it stimulated harmful ecological effects due to over-fertilization and nutrient losses (Tilman et al., 2011). In the face of increasing food demand, there is a need to change the concept of maintaining high productivity while reawakening the protection of natural resources such as soil, water and air (Lal, 2018). Sustainable soil management is a must for control and development of modern agriculture (Lavelle et al., 2014).

Soil quality is the competence of soil to provide ecosystem services (Weil and Magdoff, 2004). Soil organic carbon (SOC) is crucial to soil quality and plant production (McBratney et al., 2014). Agriculture needs to maintain the stability of organic carbon content (Lefèvre et al., 2017), as organic carbon is depleted in most croplands (Lal, 2005). Furthermore, Wilson (2014) stated that SOC had two types, namely recalcitrant carbon and labile carbon. Labile carbon, such as compost or manure, is significantly depleted through decomposition in soil over months to at most decade, depending mainly on climate (Magdoff and Van Es, 2009, Wilson, 2014). To maintain organic carbon in the soil, activated carbon must be routinely added. Meanwhile recalcitrant carbon in soil is appraised to be ten times more than labile carbon (Wilson, 2014). Biochar is one of recalcitrant carbon that can be added to the soil (Lehmann et al., 2009). Biochar, a high-carbon material, is charred by biomass such as wood, grass, manure, and agricultural wastes through pyrolysis process (Lehmann et al., 2009, Tammeorg et al., 2014). Due to the recalcitrance of its chemical structure, biochar provides more stable soil C and stays in the soil for a longer time (Lehmann, 2006; Wilson, 2014; Paustian, 2016). Moreover, biochar not only improved soil properties but also mitigated climate change with soil carbon sequestration (Bolan, N et al., 2016; Wu et al., 2020). Lehmann et al. (2009) described that the stability of biochar could escalate nutrient availability beyond a fertilization effect. Biochar has been widely proposed as a strategy to improve soil quality, to increase crop productivity and to address climate change and soil degradation (Van Zwieten et al., 2010; Downie et al. 2011; Ok et al., 2016; Brandy et al., 2008; Hagemann et al., 2017).

At present, many studies of biochar have already been done by researchers. Study by Van Zwieten et al. (2010) revealed that biochar has improved soil quality and crop growth. Meanwhile, Shamim et al. (2015) showed that application of biochar together with full rate of NPK fertilizer in alkaline soil increased biomass and seed yield of rapeseed by 391% and 377%, respectively compared with no biochar or inorganic fertilizer. Bruun et al. (2014) has proved that biochar significantly increased the density of roots in the 40–80 cm depth interval in barley. Biochar amendment also increased the root biomass and developed root system extensively in sandy and sandy loam soil (Abvien et al., 2015; Feng et al., 2021). Recently, Qian et al. (2016) demonstrated that elongation of the wheat roots was promoted by adding 5% biochar processed from rice straw by pyrolysis at 400$^{o}$C. Especially in recent years, the researches of biochar in soil improvement, especially in the soil environment of farmland and fruit trees, had become more and more extensive (Srivastava, A.K. et al., 2022). Mousavi, S.M et al., (2022) showed that biochar was the most promising option for addressing environmental problems such as soil degradation and food production, and highlighted the response of biochar in the soil-plant-environment continuum.

Rapeseed is an important edible oil crop in China. The rapeseed planting area is 1.35 billion hectares and the output is about 4.5-5 million tons, but the supply of rapeseed oil is insufficient (Fu et al., 2016). Inorganic fertilizers, especially nitrogen fertilizers, had controlled rapeseed production (Hu et al., 2017). Excessive use of chemical fertilizers had affected the environment, human health and increased production costs (Zheng et al., 2011). Improved management practices were needed to reduce the application of inorganic fertilizers. Biochar acts as a sorbent for organic and inorganic fertilizers, which could increase crop yields and reduce fertilizer use (Widowati and Asnah, 2014). Although many field experiments and greenhouse experiments had confirmed that biochar improves crop yield, there was a lack of research on the effects of biochar combined with inorganic and organic fertilizers on the growth and development of rapeseed in purple soil. Purple soil is a special soil type in China. It is the primary soil on the purple-red sandstone and shale rich in calcium carbonate in the subtropical region. The purpose of this study was: (1) to evaluate the effects of biochar application on the growth, development and yield of rapeseed in purple soil areas, and (2) to investigate the effects of combined application of biochar and inorganic and organic fertilizers on soil fertility and microbial communities in purple soil.

# 2. Results

## 2.1 The effects of biochar on rapeseed growth, photosynthesis, yield, and yield components

During the budding and flowering stages, the application of biochar had a significant effect on the plant height and stem diameter of rapeseed. At the bud stage, the plant heights of B1 and B2 were increased by 50.40% and 50.51%, respectively, compared to B0; at the flowering stage, the plant heights of B1 and B2 were increased by 27.04% and 17.33%, respectively, compared to B0 (Table 1). The variation trend of stem diameter was consistent with the plant height. The stem diameter of B1 and B2 increased by 57.52% and 43.95%, respectively, compared to B0 at the bud stage. At the flowering stage, the stem diameter of B1 and B2 increased by 29.97% and 25.11%, respectively, compared to B0. At maturity, the increase in stem diameter was smaller than that at flowering. The stem diameter of B1 and B2 increased by 20.10% and 16.39%, respectively, compared to B0.

Table 1
Effects of biochar on plant height and stem diameter of rapeseed

| Treatment | Plant height (cm) | | | | Stem Diameter (mm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Seedling | Bud | Flowering | Maturity | Seedling | Bud | Flowering | Maturity |
| B0 | 9.27 a | 32.63 b | 84.78 b | 98.72 a | 7.64 a | 7.44 b | 10.51 b | 12.83 b |
| B1 | 8.83 b | 49.41 a | 107.71 a | 104.79 a | 7.69 a | 11.72 a | 13.66 a | 15.41 a |
| B2 | 9.08 a | 49.12 a | 99.48 a | 108.39 a | 7.89 a | 10.71 a | 13.15 a | 14.93 a |
| Significance | ns | ** | ** | ns | ns | ** | ** | * |
| CV% | 22.91 | 29.21 | 14. 134 | 27.8 | 14.37 | 15.49 | 14.65 | 14.43 |

B0, B1, and B2 refers to the biochar application rates at 0, 35, 50 t $ha^{-1}$, respectively; Means value followed by the same letter within columns are not significantly different according to LSD test (*P = 0.05, **P = 0.001), CV is coefficient of variation (%).

Similar to plant height, application of biochar increased leaf and stem dry weight (Table 2). At the bud stage, the application of biochar significantly increased the leaf dry weight, and the leaf dry weight of B1 and B2 was 116% and 75% higher than that of B0, respectively. The increase in stem dry weight occurred mainly at the bud and flowering stages. At the bud stage, the stem dry weight of B1 and B2 increased by 126.06% and 90.70%, respectively, compared to that of B0; at the flowering stage, the stem dry weight of B1 and B2 increased by

43.14% and 21.32%, respectively, compared to that of B0. Meanwhile, at the mature stage, the stem dry weight of B1 and B2 decreased by 17.51% and 10.4%, respectively, compared to that of B0 (Table 2).

Table 2
Effects of biochar on dry weight of rapeseed leaves and stems

| Treatment | Leaf weight (g) | | | Stem weight (g) | | | |
|---|---|---|---|---|---|---|---|
| | Seedling | Bud | Flowering | Seedling | Bud | Flowering | Maturity |
| B0 | 2.88 b | 3.90 c | 6.79 c | 0.73 a | 2.23 b | 6.34 b | 14.27 a |
| B1 | 2.81 b | 8.46 a | 8.83 a | 0.73 a | 5.16 a | 9.41 a | 13.69 a |
| B2 | 3.38 a | 6.95 b | 7.68 b | 0.76 a | 4.16 a | 7.85 b | 12.81 b |
| Significance | ns | ** | ns | ns | ** | ** | ns |
| CV% | 33.97 | 32.11 | 29.56 | 39.41 | 39.34 | 22.77 | 23.92 |

B0, B1, and B2 refers to the biochar application rates at 0, 35, 50 t $ha^{-1}$, respectively; Means value followed by the same letter within columns are not significantly different according to LSD test (*P = 0.05, **P = 0.001), CV is coefficient of variation (%).

The yield components of rapeseed were significantly improved by the application of biochar. The number of pods in B1 and B2 increased by 31.70% and 28.90%, respectively, compared to B0. In addition, the number of effective pods in B1 and B2 increased by 33.73% and 18.16%, respectively, compared to B0. While the dry weight of pods in B1 and B2 increased by 16.38% and 18.90%, respectively (Table 3).

Table 3
Effects of biochar on the components of rapeseed yield

| Treatment | Total pod number (unit) | The effective Pod number (unit) | Thousand Seeds Weight (g) | Pod weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|
| B0 | 271.58 b | 118.83 b | 5.08 a | 9.63 b | 2304.6 b |
| B1 | 357.67 a | 158.92 a | 6.08 a | 11.21 a | 2687.5 a |
| B2 | 350.08 a | 140.42 a | 5.27 a | 11.47 a | 2763.4 a |
| Significance | ** | * | ns | * | * |
| CV% | 20.29 | 25.78 | 26.02 | 27.04 | 23.79 |

B0, B1, and B2 refers to the biochar application rates at 0, 35, 50 t $ha^{-1}$, respectively; Means value followed by the same letter within columns are not significantly different according to LSD test (*P = 0.05, **P = 0.001), CV is coefficient of variation (%).

Application of biochar significantly affected the root growth of rapeseed (Fig. 1). The application of biochar significantly increased the root total length of rapeseed throughout the growth period (Fig. 1a). Especially at the bud stage, the root total length of B1 and B2 increased by 143.09% and 190.44%, respectively, compared to B0. At the flowering stage, the root total length of B1 and B2 increased by 67.57% and 59.20%, respectively, compared to B0. Similar to root total length, biochar also affected the root surface area of rapeseed (Fig. 1b). During the vegetative stage, the application of biochar had little effect on the root surface area of rapeseed, but at other stages, biochar increased it significantly. At the bud stage, the root surface area of B1 and B2 increased by 125.07% and 161.43%, respectively, compared to B0. At the flowering stage, the root total length of B1 and B2 increased by 62.83% and 49.95%, respectively, compared to B0. Application of biochar slightly reduced the average root diameter of rapeseed (Fig. 1c). During the vegetative growth period, the average root diameter of B1 and B2 increased by 17.28% and 11.11%, respectively, compared to B0. At the flowering stage, the average root diameter of B1 and B2 increased by 62.83% and 49.95%, respectively, compared to B0. At the seedling stage, the average root diameter of B1 and B2 was 17.28% and 11.11% lower than B0, respectively. However, the largest decrease of the average root diameter was observed under B0, being 42.30% from flowering stage to mature stage, while B1 and B2 decreased it by 26.86% and 23.61%, respectively. The root volume of rapeseed showed the same trend as the root surface area (Fig. 1d). At the seedling stage, biochar had no significant effect on the root volume of rapeseed, but at other stages, the addition of biochar increased it significantly.

The PCoA results showed that both agronomic traits and yield components accounted for more than 50% of the first two axes, suggesting that the results were reasonably usable (Fig. 2). Moreover, there was a significant aggregation among the samples of different treatments, indicating that the agronomic traits and yield components within the treatments with different biochar application rates did not differ clearly, but the differences between the treatments with different biochar application rates were significant. Anosim Analysis also proved this. Meanwhile, the similarity between B1 and B2 was high, and the similarity of B1, B2 with B0 was low.

At the seedling and bud stages, biochar had a significant effect on the chlorophyll content of rapeseed leaves (Table 4). At the seedling stage, Chl a, Chl b and Chl a + b of B1 and B2 were decreased by 16.82% and 14.49%, 22.85% and 20.00%, and 18.66% and 15.33%, respectively, compared to B0. At the bud stage, the Chl a, Chl b and Chl a + b of B1 and B2 were 24.15% and 13.52%, 25.71% and 12.85%, and 25.51% and 13.79% higher than B0, respectively. The chlorophyll content of B1 was significantly higher than that of B2 and B0.

Table 4
Effect of biochar on chlorophyll content of rapeseed

| Treatment | Seedling stage (mg/g) | | | Bud stage (mg/g) | | |
|---|---|---|---|---|---|---|
| | Chlorophyll a | Chlorophyll b | Chlorophyll a + b | Chlorophyll a | Chlorophyll b | Chlorophyll a + b |
| B0 | 2.14 a | 0.70 a | 3.00 a | 2.07 c | 0.70 c | 2.90 c |
| B1 | 1.78 a | 0.54 b | 2.44 b | 2.57 a | 0.88 a | 3.64 a |
| B2 | 1.83 b | 0.56 b | 2.54 b | 2.35 b | 0.79 b | 3.30 b |
| Significance | ** | ** | ** | ** | ** | ** |
| CV% | 9.95 | 11.05 | 10.58 | 9.95 | 11.05 | 10.58 |

B0, B1, and B2 refers to the biochar application rates at 0, 35, 50 t $ha^{-1}$, respectively; Means value followed by the same letter within columns are not significantly different according to LSD test (*P = 0.05, **P = 0.001), CV is coefficient of variation (%).

Application of biochar affected the photosynthetic capacity of rapeseed at seedling, bud and flowering stages (Table 5). At the seedling stage, the photosynthetic rates of B1 and B2 were 20.24% and 35.82% higher than B0, respectively. At the bud stage, the photosynthetic rates of B1 and B2 were 65.73% and 50.14% higher than B0, respectively. Similar to the photosynthetic rate, the transpiration rate under biochar application was increased slightly during the vegetative period and then increased sharply during the budding period. At the seedling stage, the transpiration rates of B1 and B2 were 25.26% and 17.89% higher than B0, respectively. At the bud stage, the transpiration rates of B1 and B2 were 59.34% and 46.15% higher than B0, respectively. Compared to photosynthetic rate and transpiration rate, biochar had different effects on water use efficiency. There was no significant difference in water use efficiency among B1, B2 and B0 at the seedling and bud stages. At the flowering stage, the water use efficiency of B1 and B2 was 50.73% and 46.22% higher than that of B0, respectively. Meanwhile, the results of PCoA and Anosim were similar to those of agronomic and yield components (Fig. 3), with high similarity between B1 and B2, and low similarity between B1, B2 and B0.

Table 5
Effects of biochar on photosynthetic capacity of rapeseed

| Stages | Treatment | Photosynthetic rate (μmol/m2· s) | Stomatal conductance (mol/m2· s) | Intercellular $CO_2$ concentration (μmol/mol) | Transpiration rate (mmol/m2·s) | Water use efficiency |
|---|---|---|---|---|---|---|
| Seedling stage | B0 | 8.15b | 0.15b | 325.66b | 0.95b | 8.96ab |
| | B1 | 9.80ab | 0.19a | 337.59a | 1.19a | 8.38b |
| | B2 | 11.07a | 0.18a | 324.01b | 1.12a | 10.04a |
| | Significance | ** | ns | ns | ** | ** |
| | CV% | 21.37 | 24.60 | 8.76 | 13.60 | 15.78 |
| Bud stage | B0 | 6.04b | 0.14a | 297.48 c | 0.73b | 8.56b |
| | B1 | 10.01a | 0.13a | 324.15a | 1.16a | 9.36a |
| | B2 | 9.07a | 0.09b | 303.02b | 1.06a | 8.79b |
| | Significance | ** | ns | ns | ** | ** |
| | CV% | 33.55 | 39.77 | 10.86 | 24.32 | 22.67 |
| Flowering stage | B0 | 7.11b | 0.23a | 333.13a | 0.69a | 13.76b |
| | B1 | 9.69a | 0.23a | 318.66b | 0.63b | 20.74a |
| | B2 | 9.36a | 0.20b | 312.16b | 0.62b | 20.12a |
| | Significance | ** | ns | ns | ns | ** |
| | CV% | 19.23 | 25.79 | 9.73 | 15.2 | 14.75 |

B0, B1, and B2 refers to the biochar application rates at 0, 35, 50 t $ha^{-1}$, respectively; Means value followed by the same letter within columns are not significantly different according to LSD test (*P = 0.05, **P = 0.001), CV is coefficient of variation (%).

## 2.2 The effects of biochar on rapeseed nutrients and oleic acid content

The application of biochar, inorganic and organic fertilizers had different effects on various nutrients and oleic acid in pods (Fig. 4). At the flowering stage, the contents of nitrogen, phosphorus and potassium in the pods of B2F2M1 were the highest, being 38.22%, 20.18% and 35.14% higher than those of B0F1M0, respectively. Under the same amount of biochar application, the nitrogen, phosphorus and potassium contents in the pods under different fertilizer treatments were: organic and inorganic fertilizers > inorganic fertilizer > no fertilizer. The nitrogen content of the pods of B0F2M1 increased by 16.41% compared to B0F1M0, the nitrogen content of pods of B1F2M1 increased by 16.70% compared to B1F1M0, and the nitrogen content of pods of B2F2M1 increased by 20.59% compared to B2F1M0 (Fig. 4a). The phosphorus content of pods of B0F2M1 increased by 14.39% compared to B0F1M0, the phosphorus content of pods of B1F2M1 increased by 6.66% compared to B1F1M0, and the phosphorus content of pods of B2F2M1 increased by 18.73% compared to B2F1M0 (Fig. 4b).

The potassium content of pods of B0F2M1 increased by 18.54% compared to B0F1M0, the potassium content of pods of B1F2M1 increased by 12.39% compared to B2F1M0, and the potassium content of pods of B2F2M1 increased by 16.72% compared to B2F1M0 (Fig. 4c).

This study showed that the application of biochar combined with organic and inorganic fertilizers increased the oleic acid content of rapeseed at maturity (Fig. 4d), and the oleic acid content of B2F2M1 was significantly higher than B2F1M0, being increased by 13.22%.

## 2.3 The effects of combined application of biochar and organic and inorganic fertilizers on soil fertility

The application of biochar, inorganic and organic fertilizers had an effect on soil pH during flowering stage (Fig. 5a). The application of biochar significantly increased the soil pH. The soil pH of the high-rate application of biochar was significantly higher than that of the other treatments, and the soil pH of B2F2M1 was 7.78% higher than that of B0F1M0. This study showed that the application of organic fertilizer could raise the pH of soil. The pH of B0F2M1 was 0.16 higher than that of B0F2M0, and the pH of B1F2M1 was also 0.16 higher than that of B1F2M0. Meanwhile, the application of biochar significantly increased the CEC of the soil (Fig. 5b). The soil CEC of B1 and B2 was 2.27% and 6.53% higher than B0, respectively.

The application of biochar with inorganic and organic fertilizers significantly affected soil nitrogen content at flowering stage (Fig. 6a; Fig. 6b). The application of biochar significantly increased the total nitrogen content in the soil. The total nitrogen in the soil treated with high rate of biochar and low rate of biochar were 101.96% and 62.71% higher than that without biochar treatment, respectively. Meanwhile, the total nitrogen content of B2F2M1 was the highest, being 160.97% higher than B0F1M0. The application of biochar combined with organic and inorganic fertilizers had a great effect on soil available nitrogen. Compared with B0F1M0, the soil available nitrogen of B0F1M1 and B0F2M0 increased by 37.93% and 81.09% respectively, and the soil available nitrogen of B1F1M1 increased by 75.86% compared with B0F1M0.

The application of organic fertilizer had a significant effect on the content of soil total phosphorus and soil available phosphorus (Fig. 6c; Fig. 6d). The total phosphorus content of the soil treated with organic fertilizer was significantly higher than that of other treatments. The soil total phosphorus content of B0F1M1 and B0F2M1 were 21.51% and 36.04% higher than B0F1M0, respectively. The soil total phosphorus content of B1F1M1 and B1F2M1 were 29.03% and 45.16% higher than B1F1M0, respectively; and the soil total phosphorus content of B2F1M1 and B2F2M1 were 43.39% and 28.09% higher than B2F1M0, respectively. Similar to soil total phosphorus, the soil available phosphorus content in the treatment with organic fertilizer was significantly higher than that in the other treatments. The soil available phosphorus content of B0F1M1 and B0F2M1 were 103.03% and 147.45% higher than B0F1M0, respectively. The soil available phosphorus content of B1F1M1 and B1F2M1 were 115.75% and 147.24% higher than B1F1M0, respectively; and the soil available phosphorus content of B2F1M1 and B2F2M1 were 55.56% and 87.30% higher than B2F1M0, respectively.

The application of biochar and inorganic and organic fertilizers significantly affected soil potassium content at flowering stage (Fig. 6e; Fig. 6f). The total soil potassium content in B0F2M1, B1F2M1 and B2F2M1 were 30.59%, 48.64% and 63.24% higher than B0F1M0, respectively. Meanwhile, different biochar application rates also had effect on soil total potassium content, and the soil total potassium content of B1 and B2 was 7.57% and 19.33% higher than B0, respectively. The effect of biochar on soil available potassium content was different from that of biochar on soil total potassium content: soil available potassium exhibited significant differences under different biochar application. The soil available potassium content of B1 and B2 were 71.81% and 179.28% higher than B0, respectively. The application of biochar greatly increased the available potassium content in the soil.

The application of biochar with inorganic and organic fertilizers significantly affected the content of SOM (Fig. 7). The application of biochar significantly affected SOM: the SOM of B1 and B2 were 80.17% and 124.55% higher than B0, respectively. At the same time, the application of organic fertilizer also significantly increased the SOM: the SOM of B0F1M1 and B0F2M1 were 25.86% and 37.00% higher than B0F1M0; the SOM of B1F1M1 and B1F2M1 were 16.21% and 17.41% higher than B1F1M0; and the SOM of B2F1M1 and B2F2M1 were 9.72% and 13.98% higher than B2F1M0.

### 2.4 The effects of combined application of biochar and organic and inorganic fertilizers on soil microbial community

The application of biochar with inorganic and organic fertilizers significantly affected the soil PLFA (Fig. 8). First, the application of biochar significantly increased the soil PLFA: the soil PLFA of B1 and B2 was 84.99% and 112.16% higher than that of B0, respectively. At the same time, the application of organic fertilizers also significantly increased the total soil PLFA: the soil PLFA of B0F1M1 and B0F2M1 were 34.22% and 48.68% higher than B0F1M0; the soil PLFA of B1F1M1 and B1F2M1 were 48.15% and 51.11% higher than B1F1M0; and the soil PLFA of B2F1M1 and B2F2M1 were 23.64% and 17.74% higher than B2F1M0.

The application of biochar with inorganic and organic fertilizers significantly affected soil microbial community composition (Fig. 9). The application of biochar significantly increased the number of eukaryotes (Fig. 9a): the eukaryotes of B1 and B2 were 90.51% and 120.30% higher than B0, respectively. At the same time, the application of organic fertilizer also increased the number of eukaryotes: the eukaryotes of B0F1M1 and B0F2M1 were 14.72% and 15.06% higher than B0F1M0; the eukaryotes of B1F1M1 and B1F2M1 were 36.74% and 57.37% higher than B1F1M0; and the eukaryotes of B2F1M1 and B2F2M1 were 23.08% and 26.48% higher than B2F1M0. The application of biochar significantly increased the number of fungi (Fig. 9b): the fungi of B1 and B2 were 80.38% and 159.85% higher than those of B0, respectively. The application of biochar and inorganic and organic fertilizers had similar effects on actinomycetes as eukaryotes. Both biochar and organic fertilizer could significantly increase the number of actinomycetes (Fig. 9c). The actinomycetes of B1 and B2 were 56.45% and 111.59% higher than B0, respectively. The actinomycetes of B0F1M1 and B0F2M1 were 18.12% and 17.90% higher than B0F1M0, respectively; the actinomycetes of B1F1M1 and B1F2M1 were 22.34% and 39.05% higher than B1F1M0, respectively; and the actinomycetes of B2F1M1 and B2F2M1 were 11.34% and 25.62% higher than B2F1M0, respectively.The application of biochar with inorganic and organic fertilizers significantly affected the gram-negative bacteria and the gram-positive bacteria (Fig. 9d; Fig. 9e). The low-rate application of biochar significantly increased the number of the gram-negative bacteria and the gram-positive bacteria: the gram-negative bacteria and the gram-positive bacteria of B1 increased by 114.42% and 50.91%, respectively, compared with B0. The low-rate application of biochar also significantly increased the number of protozoa (Fig. 9f): the protozoa of B1 increased by 70.08% compared to B0.

### 2.5 Linking PLFA and soil fertility properties to the combined application of biochar and organic and inorganic fertilizers

Relationships of different treatments and microbial community compositions and soil fertility properties were illustrated by RDA analysis (Fig. 10). Soil microbial community compositions accounted for 96.2% of the soil fertility. Specifically, the fungi, actinobc, eukaryot and PLFA were significant positively correlated with soil fertility. The protozoa, gram-negative and gram-positive bacteria were not strongly correlated with soil fertility. Moreover, the contributions of different application rates of biochar to soil fertility were also different, which was ordered as: B2 > B1 > B0.

## 3. Discussions

# 3.1 Effect of biochar application on growth, yield and yield components, photosynthesis, and nutrient uptake.

This study showed that the application of biochar especially the low rate of biochar could significantly increase the plant height, stem diameter and dry matter weight of rapeseed. This result was consistent with the conclusions obtained by previous studies on rice and oats, which showed that biochar had a positive impact on plant growth (Bair et al. 2020; Wang et al. 2012; Schulz and Glaser 2012; Wang et al. 2014). One of the reasons might be that biochar could improve the physicochemical properties of soil, increase the soil permeability and soil fertility (Fageria, 2013; Lehmann, 2015; Yan et al., 2021), which was suitable for the growth and development of rapeseed roots, thereby promoting the growth and development of rapeseed shoot.

In the research on roots, Amonette and Joseph (2009) pointed out that the application of biochar could improve soil permeability in upland rice plantations, thereby promoting root growth. Fageria, (2013) showed that, during the vegetative growth stage, the growth and development of the crop root system with biochar was better than that without biochar. The addition of biochar to the soil resulted in faster root growth than the treatment of no biochar. We also found that the application of biochar could significantly promote root growth in various stages of rapeseed, and this study pointed out that low-rate biochar could better promote root growth. The reason might be that too much biochar application leaded to the inability of rapeseed root to contact enough soil. At the same time, due to the characteristics of biochar, such as containing a large amount of aromatic hydrocarbons (Godlewska et al., 2021), directly exposure to too much biochar might hinder the growth of the root system.

The application of biochar to promote dry matter weight and root growth of rapeseed resulted in an increase in yield. Compared with no application of biochar, the low-rate biochar increased the total number of pods, the number of effective pods, the thousand-seeds weight and yield. Agegnehu et al. (2016) reported a similar trend in barley, which showed that the application of biochar with compost promoted straw growth and increased the yield. Furthermore, Qian et al. (2014) explained that the application of biochar with organic matter was a new method for improving rice yield and developing carbon fertilizer carriers in soil.

Chlorophyll a fluorescence represents the photosynthesis process (Stirbet et al. 2014), and Chlorophyll b is essential for the assembly, stability and function of light-harvesting proteins (Apostolova and Misra, 2014), both of which are required for photosynthesis. This study showed that the application of biochar could significantly promote the synthesis of chlorophyll. The application of biochar could significantly increase the chlorophyll content of rapeseed at the bud stage, and the chlorophyll content was higher under the low-rate of biochar treatment. However, excessive application of biochar would affect crop growth, thereby reducing chlorophyll content and reducing photosynthetic capacity. Previous studies had shown that leaf chlorophyll content might be affected by the plant environment (Meskini-Vishkaee et al., 2015). Changes in chlorophyll content might be the result of changes in soil nutrients (Dudeja and Chaudhary, 2005).

The impact of biochar on the soil environment extended throughout all stages of plant growth, and the presence of biochar increased soil moisture and nutrients, making the soil more suitable for plant growth (Suppadit et al., 2012; Amoah et al., 2020). This condition promoted the increase of stomatal conductance and intercellular $CO_2$ concentration, thereby enhancing photosynthetic rate and water use efficiency (Xu et al., 2015). This study pointed out that the application of biochar increased the photosynthetic capacity of rapeseed, especially the low-rate biochar significantly increased the photosynthetic rate and intercellular $CO_2$ concentration. The higher photosynthetic capacity of rapeseed under low-rate biochar application also explained the increase in yield from another perspective.

This study showed that the combined application of biochar, inorganic fertilizer and organic fertilizer could improve the absorption and utilization of nutrients in rapeseed. Widowati and Asnah (2014) found that biochar application increased crop uptake of potassium by 128%. Xiang et al. (2017) explained that biochar modified the soil environment thus promoting crop root growth and soil nutrient absorption. Nigussie (2012) et al. found that biochar significantly increased nutrient absorption by crops. This study showed that the application of biochar could improve the absorption and utilization of nitrogen and potassium in rapeseed, but there was no significant improvement effect on the absorption and utilization of phosphorus. At the same time, the application of organic fertilizers and inorganic fertilizers could improve the absorption and utilization of nitrogen and phosphorus in rapeseed, and the application of organic fertilizers could significantly improve the absorption and utilization of potassium.

Oleic acid is a fatty acid that is a healthier source of fat in the diet (Fu et al., 2016). This study found that the application of biochar had little effect on the oleic acid in rapeseed, but the application of organic fertilizer could significantly increase the oleic acid content in rapeseed, indicating that the application of biochar did not reduce the quality of rapeseed while promoting crop growth and development, and the combination of organic fertilizer could significantly improve the quality of rapeseed.

### 3.2 Effect of combined application of biochar and organic and inorganic fertilizers on soil fertility and soil microbial communities.

This study showed that the application of biochar in combination with inorganic and organic fertilizers could improve soil fertility. Application of biochar was able to significantly increase soil pH and CEC. This finding was consistent with Martinsen et al. (2015) who explained that biochar application in the soil could react a liming effect. It could be understood because biochar was alkaline in nature and dissociation of phenolic –OH groups in biochar increased its net negative surface charge (Nanda et al., 2016). The presence of organic fertilizer increased soil pH, because plant absorbed more cations (positive charges) than anions (negative charges), and the combined application of inorganic and organic fertilizers were generally useful to sustain pH and soil fertility (Osman, 2013).

This study showed that the application of biochar could increase the nitrogen and potassium content in the soil and reduce the loss of nitrogen and potassium, but had little effect on phosphorus. The application of organic fertilizer could increase the content of nitrogen, phosphorus and potassium, especially the content of soil available phosphorus. These findings were consistent with Widowati and Asnah (2014), who found that biochar could act as a sorbent for both organic and inorganic fertilizers, thereby increasing crop yields and reducing fertilizer requirement. This was because biochar could attract and retain soil nutrients (Lehmann et al., 2009). However, for phosphorus, exchangeable aluminum in acidic soils would combine with phosphorus to form insoluble aluminum phosphorus, thereby reducing the availability of phosphorus. The purple soil was alkaline soil, so this might be the reason why the use of biochar had little effect on the phosphorus content (Jin et al., 2019). Van Zwieten et al. (2010) showed that the positive effects of biochar treatment on plant growth were due to its consequences on plant nutrition. This study showed that the application of biochar in combination with inorganic and organic fertilizers could significantly increase SOM. This was because biochar itself was an organic material with a high carbon content, and its application to the soil increased the organic matter in the soil (Liu et al., 2016). At the same time, the application of organic fertilizer also significantly increased the soil organic matter content. As soil conditioners with high organic matter content, biochar and organic fertilizer have a very significant impact on soil organic matter, and the impact of their own organic matter on soil even exceeds the synergistic effect of biochar, organic fertilizer and inorganic fertilizer.

Biome diversity was critical to soil function and had important implications for controlling nutrient cycling, increasing water use efficiency, enhancing soil aeration, and maintaining soil structure and stability (Taketani et al., 2013). This study showed that biochar application could significantly increase the PLFA of the soil. With the application of biochar and organic fertilizer, the PLFA of the soil increased significantly. Li et al. (2020) found that although the effects of biochar on soil microbial diversity were different, it was certain that biochar application had increased microbial biomass. This study found that eukaryotes, fungi and actinomycetes under high-rate biochar application were higher than those under low-rate biochar application; but for gram-negative bacteria, gram-positive bacteria and protozoa, they were higher under low-rate biochar than that under high-rate biochar. This was inconsistent with the study of Yuan et al. (2022), possibly due to differences in soil type and the type of biochar applied. The study of Jin (2018) showed that different types of biochar, when applied in different types of soil, had different effects on soil microbes. However, it was certain that the combination of biochar and organic and inorganic fertilizers could improve the richness of soil microbial communities. Lehmann et al. (2011) elucidated that the efficacy of biochar on soil microorganism might be determined by both physical and chemical properties of biochar. We demonstrated that biochar could significantly increase soil pH, CEC, SOM and TOC, and could significantly increase the nitrogen, phosphorus and potassium content in soil and plants. This result implied that soil microbial community was encouraged by availability of nutrients in the soil. Gul et al. (2015) explained that the presence of inorganic and organic fertilizer as co-amendment prevented nutrient deficiency for microbial growth. Moreover, DeLuca et al. (2009) proved that in farming practice, biochar was frequently applied together with fertilizer, as biochar was not fertilizer and it did not carry a lot of readily available nutrients. In brief, biochar application in soil could change soil physiochemical properties and mediate electron transfer processes, thereby improving metabolism of microbial community (Wilson, 2014; Keppler et al., 2014; Yang et al., 2019)

This study showed that soil fertility was related to soil microbial communities, being consistent with previous studies (L R Bulluck et al., 2002; Wang et al., 2021; Niu et al., 2021), in which soil fertility could affect soil microbial growth, and soil microbial richness could also affect soil fertility. Luo (2016) found that microbial communities were more active in high-yielding soils in a study on two types of dryland paddy soils. This study showed that there was a strong relation between the amount of biochar application and soil fertility. This study showed that soil fertility, especially SOM, had the highest contribution to microbial community diversity. Li et al. (2021) showed that SOM could be used to predict soil microbial activity. Stefanowicz et al (2020) believed that SOM had the strongest positive effect on most microbial parameters. This study supported the conclusion of previous studies, and this study also pointed out that K was a large contributor to the effects of fungi and actinobc, while gram-negative bacteria and gram-positive bacteria were weakly correlated with soil fertility.

# 4. Conclusion

The application of biochar in combination with organic and inorganic fertilizers could promote the growth of rapeseed and improve the soil fertility and microbial environment. Low-rate of biochar could promote rapeseed growth, improve soil fertility, and increase soil PLFA. However,excessive biochar application was not conducive to sustainable farming. Therefore, we recommend the application of low-rate biochar (35 t/ha) in combination with organic fertilizer (4.5 t/ha) and low-rate inorganic fertilizers (30 kg/ha N, 87.5 kg/ha $P_2O_5$ and 60 kg/ha $K_2O$) in rapeseed production in purple soil area of Southwest China.

# 5. Materials And Methods

## 5.1 Experimental area

The experiment was conducted using pot experiment in the green house of College of Agronomy and Biotechnology (CAB), Southwest University (SWU), China, during September 2016 to May 2017. There was no additional supplementary light in the greenhouse, and the light intensity and photo-period in the greenhouse were consistent with the external environment. The research area was located at 220m in altitude, 29°49 32 N in latitude, and 106°26 02 E in longitude. Measurement of soil, biochar and plant parameters was carried out in Key Laboratory of Crop Quality Improvement of Chongqing Municipality, CAB, SWU, China. The main physical and chemical properties of the soil are: soil bulk density 1.21 $g{\cdot}cm^{-3}$, pH value 6.47, organic matter content 28.00 $g{\cdot}kg^{-1}$, total nitrogen content 1.68 $g{\cdot}kg^{-1}$, total phosphorus content 1.46 $g{\cdot}kg^{-1}$, the total potassium content is 34.54 $g{\cdot}kg^{-1}$, the available phosphorus is 18.13 $mg{\cdot}kg^{-1}$, the available potassium is 270.23 $mg{\cdot}kg^{-1}$, and the alkaline hydrolyzable nitrogen is 35.23 $mg{\cdot}kg^{-1}$.

## 5.2 Experimental design

A pot experiment was conducted in Randomized Complete Block Design (RCBD)with 3×2×2 treatments, with three replications. The first treatment was rate of biochar(B): B0, B1, and B2. The second treatment was fertilizer (F): F1, and F2. The third treatment was organic fertilizer (M): M0 and M1. Table 6 showed the twelve treatment combinations.

Table 6. Treatment combinations of biochar, inorganic fertilizer, and organic fertilizer

| Treatment | Inorganic fertilizer (30 N, 87.5 $P_2O_5$,60 $K_2O$) kg/ha) F1 | | Inorganic fertilizer (60 N, 175 $P_2O_5$,120 $K_2O$) kg/ha (F2 | |
|---|---|---|---|---|
| | Organic fertilizer 0 t/ha (M0) | Organic fertilizer 4.5 t/ha (M1) | Organic fertilizer 0 t/ha (M0) | Organic fertilizer 4.5 t/ha (M1) |
| Biochar 0 t/ha(B0) | B0F1M0 | B0F1M1 | B0F2M0 | B0F2M1 |
| Biochar 35 t/ha(B1) | B1F1M0 | B1F1M1 | B1F2M0 | B1F2M1 |
| Biochar 50 t/ha(B2) | B2F1M0 | B2F1M1 | B2F2M0 | B2F2M1 |

Total weight of soil, biochar, and organic fertilizer in each pot was 5000 g. They were mixed with inorganic fertilizer as treatment before transplanting. The seeds of rapeseed were provided by Rapeseed Research Institute, CAB, SWU, China. According to local planting traditions, 'two-cropping' system was uesd in this study: rapeseed was sown in September 2016, and the rapeseed variety was 'Zhongshuang No. 11', and soybean was sown in April 2017 after rapeseed harvest, and the soybean variety was 'Zhechun No.3'. The rapeseed and soybean varieties were artificially cultivated varieties and supplied by the Engineering Research Center of South Upland Agriculture, Ministry of Education, Chongqing, China. Each pot was transplanted with 1 plant. The plants were watered once every 5 days according to the daily temperature. The amount of water added in each pot was the same, and the amount of water added in each pot was 200 – 600 ml, to made sure the relative soil moisture content was controlled at about 60% of the saturated moisture. The temperature of glasshouse ranged from 20 to 25$^{o}$C and relative humidity was 50 to 90% during the entire growth period.

Biochar (carbonized corncob) was obtained commercially from Nanjing Qinfeng Straw Technology Co., LTD., China. Based on the information from the manufacturer, corncob was prepared at a pyrolysis temperature of 400℃. Inorganic fertilizers used were urea, $P_2O_5$, and $K_2O$ as sources of mineral nitrogen, phosphate, and potassium, respectively. The organic fertilizer used in the experiment was the ZhenGeng biological organic fertilizer provided by Beijing Xingpeng Agricultural Development Co., Ltd. The main component of organic fertilizer was pig manure, and the main technical parameters were: organic matter content was greater than 50%, Amino acid + nucleotide was greater than 3%, moisture was lower than 18.0%, effective viable count was greater than 50 million/g, Zn + B + Fe + Mn + Mo + Si was greater than 0.1%. The tested purple soil was collected from the Experiment Farm of SWU, China. The soil was air-dried and sieved to pass through 2mm. The plastic pots used in the experiment were 23 cm in diameter and 22 cm tall with a total soil volume of 5000 gram. The basic physical and chemical properties of biochar were: pH was 9.90 ± 0.04, EC was 0.94 ± 0.01 $ms{\cdot}cm^{-1}$, total nitrogen was 5.76 ± 0.23 $g{\cdot}kg^{-1}$, total phosphorus was 3.75 $g{\cdot}kg^{-1}$, total potassium was 21.15 ± 0.17 $g{\cdot}kg^{-1}$, CEC was 34.49 ± 5.16 $cmol{\cdot}kg^{-1}$, and organic carbon was 439.01 ± 40.06 $g{\cdot}kg^{-1}$.

## 5.3 Growth, yield, and yield components and photosynthesis

The data of rapeseed plants were collected at four stages of growth: vegetative stage, bud stage, flowering stage, and maturity stage. Plant height was measured with a ruler. Stem diameter was measured with a calliper. Plant dry weight was measured gravimetrically with an electronic scale.

Rapeseed plants were harvested at the end of May, 2017. The plants were removed from the pot and divided into stems and pods while roots were washed thoroughly. Dry matter of plant samples was obtained after being dried in an oven at 75 °C for 48 h.

The plant root was collected at flowering stage, and was slowly washed to remove soil. Total of root length, root surface, volume of root and mean diameter of root was determined by root scanner (Epson Perfection V700, 6400 dpi optical resolution film scanning) in transparent plastic tray filled with a few water using WinRhizo Pro 3.10, 2007 (Regent Instrument Inc., Quebec, Canada).

Chlorophyll contents, including chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophyll a+b (Chl a+b), were assessed at seedling and bud stages by using the method of Weng (2020). A leaf sample (0.10 g) was taken from fresh leaves of rapeseed, made into small size and transferred into a 15 mL centrifuge tube, which was filled with 10 mL of solution by 95.5 % acetone and absolute ethyl alcohol in ratio of 1:1. Then the tube was stored at dark place to avoid from light for 24 hour or until the color of leaf sample was changed into white. The absorbance concentrations of Chl a, Chl b and Chl a+b were measured at 645 nm, 652 nm and 663 nm, respectively using a UV-visible spectrophotometer.

Photosynthesis gas exchange was determined with a portable infrared gas analysers (IRGAs) based photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) during 8:30–10:30 am at seedling and bud stages (Yao et al., 2017). Nine leaves were needed for each sample with the following arrangement: molar flow of air per unit leaf area was 400.00 mmol/$m^2$·s, water vapor pressure into leaf chamber was 3.7 mbar, the photosynthetically active radiation (PAR) at leaf surface was up to 978 mmol/$m^2$·s, leaf temperature was ranged from 29.9°C to 37.5°C, ambient temperature was ranged from 30.1 to 37.8°C, ambient $CO_2$ concentration was 399.9 mol/mol and relative humidity (RH) was 59.78%. The parameters of photosynthesis gas exchange consisted of net photosynthesis (Pn), transpiration (Tr), stomata conductance (Gs), and intercellular $CO_2$ concentration (Ci). The ratio between Pn and Tr was known as water use efficiency (WUE).

## 5.4 Plant and soil analysis

Kjeldahl method was used for determination of total nitrogen content in plant tissues. Phosphorus content was determined by vanadium molybdenum yellow absorbance method. Potassium content was determined by flame photometer method (Zhou et al., 2019).

Soil samples were collected at the same stages as plant samples. They were air-dried and then sieved to pass through 2mm. Soil pH ($H_2O$) was measured in 1:5 soil to water ratio with pH digital apparatus. Kjeldahl method was used for determination of soil total nitrogen content. Total phosphorus was determined using molybdenum antimony anticolorism method. Soil total potassium was determined using flame photometer method. (Athalye-Jape et al., 2020; Tian et al., 2020) The ready kits provided by Nanjing Jiancheng Bioengineering Institute, China was used for determining soil organic matter (SOM). The test of SOM was carried out following the procedure mentioned with the detection kits. The 1 M $NH_4OAc$ saturation method at pH7 was used to measure cation exchange capacity (CEC)(Cui et al., 2021).

The soil samples for soil microbial community were restored in -80°C fridge to keep fresh before test. Phospholipid fatty acids (PLFA) method was used for measurement of soil microbial diversity. Phospholipid fatty acids were extracted from fresh sieved soil at the maturity stage. Procedure standard method of PLFA followed the literature described by Zalles and Bai (1993) , Zelles (1999) , Ringelberg (1997) and Buyer and Sasser (2012) . The recognized PLFAs were appointed into a microbial group, such as eukaryotes, Poly-unsaturated fatty acids, gram-positive bacteria (branched saturated fatty acids), gram-negative bacteria (monounsaturated fatty acids) and protozoa labeled as 20:3 and 20:4 .

## 5.5 Statistical analysis

The factorial ANOVA technique was used to analyze the data. Only the significant ANOVA results were fixed with Least Significant Different (LSD) test with probability of 0.05, which was performed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Graphics of the data was made using Microsoft Excel. In order to evaluate the compositional differences of various indicators in rapeseed under different biochar application rates, principal coordinate analysis (PCoA) and Anosim analysis were performed for each indicator in this study. The PCoA was conducted using the *factoextra* package in the R and the Anosim analysis was conducted using the *vegan* package in the R. PCoA was based on weighted Unifrac distances. Redundancy analysis (RDA) was conducted using CANOCO 5.0.

# Declarations

***Ethics approval and consent to participate:***

The plants (rapeseed and soybean) involved in this study wrer artificially cultivated varieties and supplied by the Engineering Research Center of South Upland Agriculture, Ministry of Education, Chongqing, China. The study complies with relevant institutional, national, and international guidelines and legislation.

***Consent for publication:***

Not applicable.

***Availability of data and materials:***

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

***Competing interests:***

The authors declare that they have no competing interests.

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***Author Contributions***

LCW, CL, SMM and ML contributed to conception and design of the study. CL and SMM organized the database and performed the statistical analysis, ML wrote the manuscript. QM, WFS, LXS, KDC, MZS and YLZ participated in the experiment and collected data. All authors read and approved the final manuscript.

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***Footnotes:***

Not applicable.

# References

1. Abiven, S., Hund, A., Martinsen, V., & Cornelissen, G. (2015). Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. Plant and soil, *395*(1), 45–55.
2. Agegnehu, G., Nelson, P. N., & Bird, M. I. (2016). The effects of biochar, compost and their mixture and nitrogen fertilizer on yield and nitrogen use efficiency of barley grown on a Nitisol in the highlands of Ethiopia. Science of the Total Environment, *569*, 869–879.
3. Amoah-Antwi, C., Kwiatkowska-Malina, J., Thornton, S. F., Fenton, O., Malina, G., & Szara, E. (2020). Restoration of soil quality using biochar and brown coal waste: A review. Science of the Total Environment, *722*, 137852.
4. Amonette, J. E., & Joseph, S. (2009). Characteristics of biochar: microchemical properties. Biochar for Environmental Management: Science and Technology, *33*.
5. Apostolova, E. L., & Misra, A. N. (2014). Alterations in structural organization affect the functional ability of photosynthetic apparatus. Handbook of Plant and Crop Physiology, 103–118.
6. Athalye-Jape, G., Nettleton, M., Lai, C.T., Nathan, E., Geddes, D., Simmer, K., Patole, S., 2020. Composition of coloured gastric residuals in extremely preterm infants-a nested prospective observational study. *Nutrients 12 (9)*, 2585.
7. Bair, D. A., Anderson, C. G., Chung, Y., Scow, K. M., Franco, R. B., & Parikh, S. J. (2020). Impact of biochar on plant growth and uptake of ciprofloxacin, triclocarban and triclosan from biosolids. Journal of Environmental Science and Health, Part B, *55*(11), 990–1001.
8. Brady, N. C., & Weil, R. R. (2008). *The Nature and Properties of Soils* (Vol. 13, pp. 662–710). Upper Saddle River, NJ: Prentice Hall.
9. Bruun, E. W., Petersen, C. T., Hansen, E., Holm, J. K., & Hauggaard-Nielsen, H. (2014). Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. Soil Use and Management, *30*(1), 109–118.
10. Bulluck Iii, L. R., & Ristaino, J. B. (2002). Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology, *92*(2), 181–189.
11. Buyer, J. S., & Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. Applied Soil Ecology, *61*, 127–130.

12. Cornelissen, G., Nurida, N. L., Hale, S. E., Martinsen, V., Silvani, L., & Mulder, J. (2018). Fading positive effect of biochar on crop yield and soil acidity during five growth seasons in an Indonesian Ultisol. Science of the Total Environment, *634*, 561–568.
13. Cui, Z., Dou, Z., Chen, X., Ju, X., & Zhang, F. (2014). Managing agricultural nutrients for food security in China: past, present, and future. Agronomy Journal, *106*(1), 191–198.
14. Cui, X., Mao, P., Sun, S., Huang, R., Fan, Y., Li, Y., … Li, Z. (2021). Phytoremediation of cadmium contaminated soils by Amaranthus Hypochondriacus L.: The effects of soil properties highlighting cation exchange capacity. Chemosphere, *283*, 131067.
15. DeLuca, T. H., Gundale, M. J., MacKenzie, M. D., & Jones, D. L. (2015). Biochar effects on soil nutrient transformations. Biochar for Environmental Management: Science, Technology and Implementation, *2*, 421–454.
16. Downie, A., Crosky, A., & Munroe, P. (2009). Physical properties of biochar. Biochar for Environmental Management: Science and Technology, 1.
17. Dudeja, S. S., & Chaudhary, P. (2005). Fast chlorophyll fluorescence transient and nitrogen fixing ability of chickpea nodulation variants. Photosynthetica, *43*(2), 253–259.
18. Fageria, N. K.. (2012). *The role of plant roots in crop production*. CRC Press.
19. Feng, L., Xu, W., Tang, G., Gu, M., & Geng, Z. (2021). Biochar induced improvement in root system architecture enhances nutrient assimilation by cotton plant seedlings. BMC Plant Biology, *21*(1), 1–14.
20. Fu, D. H., JIANG, L. Y., Mason, A. S., Xiao, M. L., Zhu, L. R., LI, L. Z., & Huang, C. H. (2016). Research progress and strategies for multifunctional rapeseed: A case study of China. Journal of Integrative Agriculture, *15*(8), 1673–1684.
21. Godlewska, P., Ok, Y. S., & Oleszczuk, P. (2021). The dark side of black gold: Ecotoxicological aspects of biochar and biochar-amended soils. Journal of hazardous materials, *403*, 123833.
22. Hagemann, N., Joseph, S., Schmidt, H. P., Kammann, C. I., Harter, J., Borch, T., & Kappler, A. (2017). Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. Nature Communications, *8*(1), 1–11.
23. Hu, Q., Hua, W., Yin, Y., Zhang, X., Liu, L., Shi, J., & Wang, H. (2017). Rapeseed research and production in China. The Crop Journal, *5*(2), 127–135.
24. Jin, Z., Chen, C., Chen, X., Hopkins, I., Zhang, X., Han, Z., & Billy, G. (2019). The crucial factors of soil fertility and rapeseed yield-A five year field trial with biochar addition in upland red soil, China. Science of the Total Environment, *649*, 1467–1480.
25. Jin, Z., Chen, C., Chen, X., Jiang, F., Hopkins, I., Zhang, X., & Benavides, J. (2019). Soil acidity, available phosphorus content and optimum fertilizer N and biochar application rate-a six-year field trial with biochar and fertilizer N addition in upland red soil, China. Field Crop Res, *232*, 77–87.
26. Kappler, A., Wuestner, M. L., Ruecker, A., Harter, J., Halama, M., & Behrens, S. (2014). Biochar as an electron shuttle between bacteria and Fe (III) minerals. Environmental Science & Technology Letters, *1*(8), 339–344.
27. Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota–a review. Soil Biology and Biochemistry, *43*(9), 1812–1836.

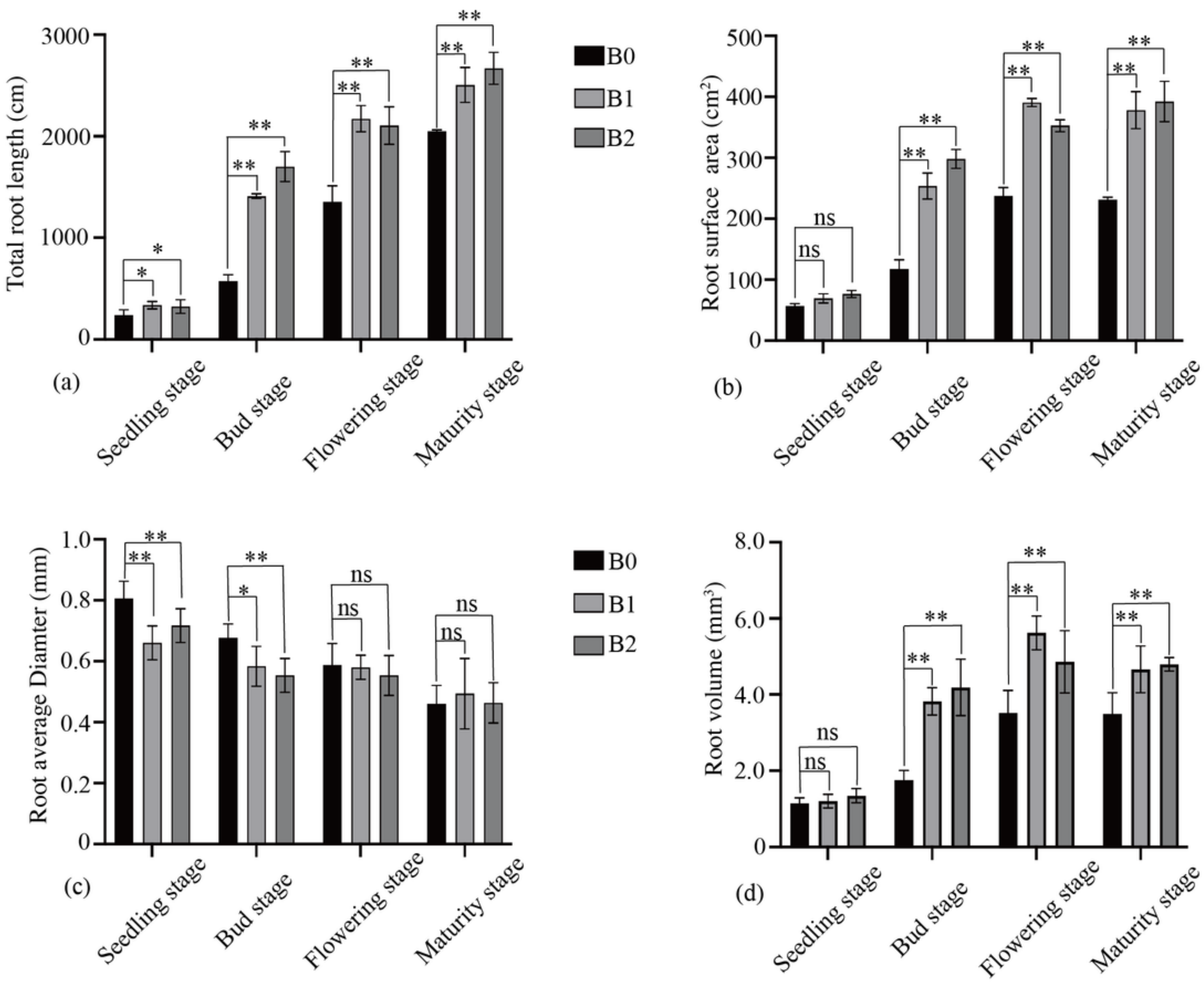
28. Lal, R. (2018). Sustainable intensification of China's agroecosystems by conservation agriculture. International Soil and Water Conservation Research, *6*(1), 1–12.
29. Lal, R., & Stewart, B. A. (Eds.). (2014). *Soil Management of Smallholder Agriculture* (Vol. 21). CRC Press.
30. Lavelle, P., Moreira, F. F., & Spain, A. (2014). Biodiversity: Conserving biodiversity in agroecosystems. Encyclopedia of agriculture and food systems, 41–60.
31. Lefèvre, C., Rekik, F., Alcantara, V., & Wiese, L. (2017). *Soil Organic Carbon: the Hidden Potential*. Food and Agriculture Organization of the United Nations (FAO).
32. Lehmann, J., Czimczik, C., Laird, D., & Sohi, S. (2009). Stability of biochar in soil. Biochar for Environmental Management: Science andTechnology, 183–206.
33. Lehmann, J., Gaunt, J., & Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems–a review. Mitigation and Adaptation Strategies for Global Change, *11*(2), 403–427.
34. Lehmann, J., Kuzyakov, Y., Pan, G., & Ok, Y. S. (2015). Biochars and the plant-soil interface. Plant and Soil, *395*(1), 1–5.
35. Li, H., Yang, S., Semenov, M. V., Yao, F., Ye, J., Bu, R., & Kuzyakov, Y. (2021). Temperature sensitivity of SOM decomposition is linked with a K-selected microbial community. Global Change Biology, *27*(12), 2763–2779.
36. Li, X., Wang, T., Chang, S. X., Jiang, X., & Song, Y. (2020). Biochar increases soil microbial biomass but has variable effects on microbial diversity: A meta-analysis. Science of the Total Environment, *749*, 141593.
37. Liu, M., Linna, C., Ma, S., Ma, Q., Guo, J., Wang, F., & Wang, L. (2022). Effects of Biochar With Inorganic and Organic Fertilizers on Agronomic Traits and Nutrient Absorption of Soybean and Fertility and Microbes in Purple Soil. Frontiers in Plant Science, *13*, 871021–871021.
38. Liu, Y., Lu, H., Yang, S., & Wang, Y. (2016). Impacts of biochar addition on rice yield and soil properties in a cold waterlogged paddy for two crop seasons. Field Crops Research, *19*1, 161–167.
39. Luo, X., Fu, X., Yang, Y., Cai, P., Peng, S., Chen, W., & Huang, Q. (2016). Microbial communities play important roles in modulating paddy soil fertility. Scientific Reports, *6*(1), 1–12.
40. Martinsen, V., Alling, V., Nurida, N. L., Mulder, J., Hale, S. E., Ritz, C., & Cornelissen, G. (2015). pH effects of the addition of three biochars to acidic Indonesian mineral soils. Soil Science and Plant Nutrition, *61*(5), 821–834.
41. McBratney, A. B., Stockmann, U., Angers, D. A., Minasny, B., & Field, D. J. (2014). *Challenges for soil organic carbon research*. Springer, Cham.
42. Meskini-Vishkaee, F., Mohammadi, M. H., Neyshabouri, M. R., & Shekari, F. (2015). Evaluation of canola chlorophyll index and leaf nitrogen under wide range of soil moisture. International Agrophysics, *29*(1).
43. Mousavi, S.M., Srivastava, A.K., & Cheraghi, M. (2022). Soil health and crop response of biochar: an updated analysis. Archives of Agronomy and Soil Science, *68*, (*4*), https://doi.org/10.1080/03650340.2022.2054998.
44. Nanda, S., Dalai, A. K., Berruti, F., & Kozinski, J. A. (2016). Biochar as an exceptional bioresource for energy, agronomy, carbon sequestration, activated carbon and specialty materials. Waste and Biomass Valorization, *7*(2), 201–235.

45. Nigussie, A., Kissi, E., Misganaw, M., & Ambaw, G. (2012). Effect of biochar application on soil properties and nutrient uptake of lettuces (Lactuca sativa) grown in chromium polluted soils. American-Eurasian Journal of Agriculture and Environmental Science, *12*(3), 369–376.
46. Niu, H., Pang, Z., Fallah, N., Zhou, Y., Zhang, C., Hu, C., & Yuan, Z. (2021). Diversity of microbial communities and soil nutrients in sugarcane rhizosphere soil under water soluble fertilizer. Plos One, *16*(1), e0245626.
47. Ok, Y. S., Uchimiya, S. M., Chang, S. X., & Bolan, N. (Eds.). (2015). *Biochar: Production, Characterization, and Applications*. CRC press.
48. Osman, K. T. (2012). *Soils: Principles, Properties and Management*. Springer Science & Business Media.
49. Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. Nature, *532*(7597), 49–57.
50. Qian, L., Chen, B., & Chen, M. (2016). Novel alleviation mechanisms of aluminum phytotoxicity via released biosilicon from rice straw-derived biochars. Scientific Reports, *6*(1), 1–11.
51. Qian, L., Chen, L., Joseph, S., Pan, G., Li, L., Zheng, J., & Wang, J. (2014). Biochar compound fertilizer as an option to reach high productivity but low carbon intensity in rice agriculture of China. Carbon Management, *5*(2), 145–154.
52. Ren, C., Jin, S., Wu, Y., Zhang, B., Kanter, D., Wu, B., & Gu, B. (2021). Fertilizer overuse in Chinese smallholders due to lack of fixed inputs. Journal of Environmental Management, *293*, 112913.
53. Ringelberg, D. B., Stair, J. O., Almeida, J., Norby, R. J., O'Neill, E. G., & White, D. C. (1997). Consequences of rising atmospheric carbon dioxide levels for the belowground microbiota associated with white oak. Journal of Environmental Quality, *26*(2), 495–503.
54. Savary, S, Willocquet, L. (2020). Climate change and global food security. Annual Review of Phytopathology, *58*(1).
55. Schulz, H., & Glaser, B. (2012). Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. Journal of Plant Nutrition and Soil Science, *175*(3), 410–422.
56. Shamim, M. I. A., DIJKSTRA, F. A., ABUYUSUF, M., & HOSSAIN, A. I. (2015). Synergistic effects of biochar and NPK fertilizer on soybean yield in an alkaline soil. Pedosphere, *25*(5), 713–719.
57. Srivastava, A.K., Wu, Q.S., Mousavi, S.M. and Hota, D., (2021). Integrated soil fertility management in fruit crops: An overview. International Journal of Fruit Science, *21*(*1)*, pp.413–439.
58. Stefanowicz, A. M., Kapusta, P., Zubek, S., Stanek, M., & Woch, M. W. (2020). Soil organic matter prevails over heavy metal pollution and vegetation as a factor shaping soil microbial communities at historical Zn–Pb mining sites. Chemosphere, *240*, 124922.
59. Stirbet, A., Riznichenko, G. Y., & Rubin, A. B. (2014). Modeling chlorophyll a fluorescence transient: relation to photosynthesis. Biochemistry, *79*(4), 291–323.
60. Taketani, R. G., Lima, A. B., da Conceiçao Jesus, E., Teixeira, W. G., Tiedje, J. M., & Tsai, S. M. (2013). Bacterial community composition of anthropogenic biochar and Amazonian anthrosols assessed by 16S rRNA gene 454 pyrosequencing. Antonie van Leeuwenhoek, *104*(2), 233–242.

61. Tammeorg, P., Simojoki, A., Mäkelä, P., Stoddard, F. L., Alakukku, L., & Helenius, J. (2014). Biochar application to a fertile sandy clay loam in boreal conditions: effects on soil properties and yield formation of wheat, turnip rape and faba bean. Plant and Soil, *374*(1), 89–107.
62. Tian, X., Li, Z., Wang, L., Wang, Y., Li, B., Duan, M., & Liu, B. (2020). Effects of biochar combined with nitrogen fertilizer reduction on rapeseed yield and soil aggregate stability in upland of purple soils. International Journal of Environmental Research and Public Health, *17*(*1*), 279.
63. Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences, 108*(50), 20260–20264.
64. Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant and Soil, *327*(1), 235–246.
65. Wang, J., Pan, X., Liu, Y., Zhang, X., & Xiong, Z. (2012). Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. Plant and Soil, *360*(1), 287–298.
66. Wang, J. L., Liu, K. L., Zhao, X. Q., Zhang, H. Q., Li, D., Li, J. J., & Shen, R. F. (2021). Balanced fertilization over four decades has sustained soil microbial communities and improved soil fertility and rice productivity in red paddy soil. Science of The Total Environment, *793*, 148664.
67. Wang, Y., Pan, F., Wang, G., Zhang, G., Wang, Y., Chen, X., & Mao, Z. (2014). Effects of biochar on photosynthesis and antioxidative system of Malus hupehensis Rehd. seedlings under replant conditions. Scientia Horticulturae, *175*, 9–15.
68. Weil, R. R., & Magdoff, F. (2004). Significance of soil organic matter to soil quality and health. Soil Organic Matter in Sustainable Agriculture, 1–43.
69. Widowati, W., & Asnah, A. (2014). Biochar can enhance potassium fertilization efficiency and economic feasibility of maize cultivation. Journal of Agricultural Science, *6*(2), 24–32.
70. Wu, S., Zhang, Y., Tan, Q., Sun, X., Wei, W., & Hu, C. (2020). Biochar is superior to lime in improving acidic soil properties and fruit quality of Satsuma mandarin. Science of The Total Environment, *714*, 136722.
71. Xu, C. Y., Hosseini-Bai, S., Hao, Y., Rachaputi, R. C., Wang, H., Xu, Z., & Wallace, H. (2015). Effect of biochar amendment on yield and photosynthesis of peanut on two types of soils. Environmental Science and Pollution Research, *22*(8), 6112–6125.
72. Yan, T., Xue, J., Zhou, Z., & Wu, Y. (2021). Biochar-based fertilizer amendments improve the soil microbial community structure in a karst mountainous area. Science of the Total Environment, *794*, 148757.
73. Yao, X., Zhou, H., Zhu, Q., Li, C., Zhang, H., Wu, J. J., & Xie, F. (2017). Photosynthetic response of soybean leaf to wide light-fluctuation in maize-soybean intercropping system. *Frontiers in plant scienc*e, 8, 1695.
74. Yang, Z., Sun, T., Subdiaga, E., Obst, M., Haderlein, S. B., Maisch, M., & Kappler, A. (2020). Aggregation-dependent electron transfer via redox-active biochar particles stimulate microbial ferrihydrite reduction. Science of The Total Environment, *703*, 135515.
75. Yuan, J., Wang, Y., Zhao, X., Chen, H., Chen, G., & Wang, S. (2022). Seven years of biochar amendment has a negligible effect on soil available P and a progressive effect on organic C in paddy soils. Biochar, *4*(1), 1–13.
76. Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils, *29*(2), 111–129.

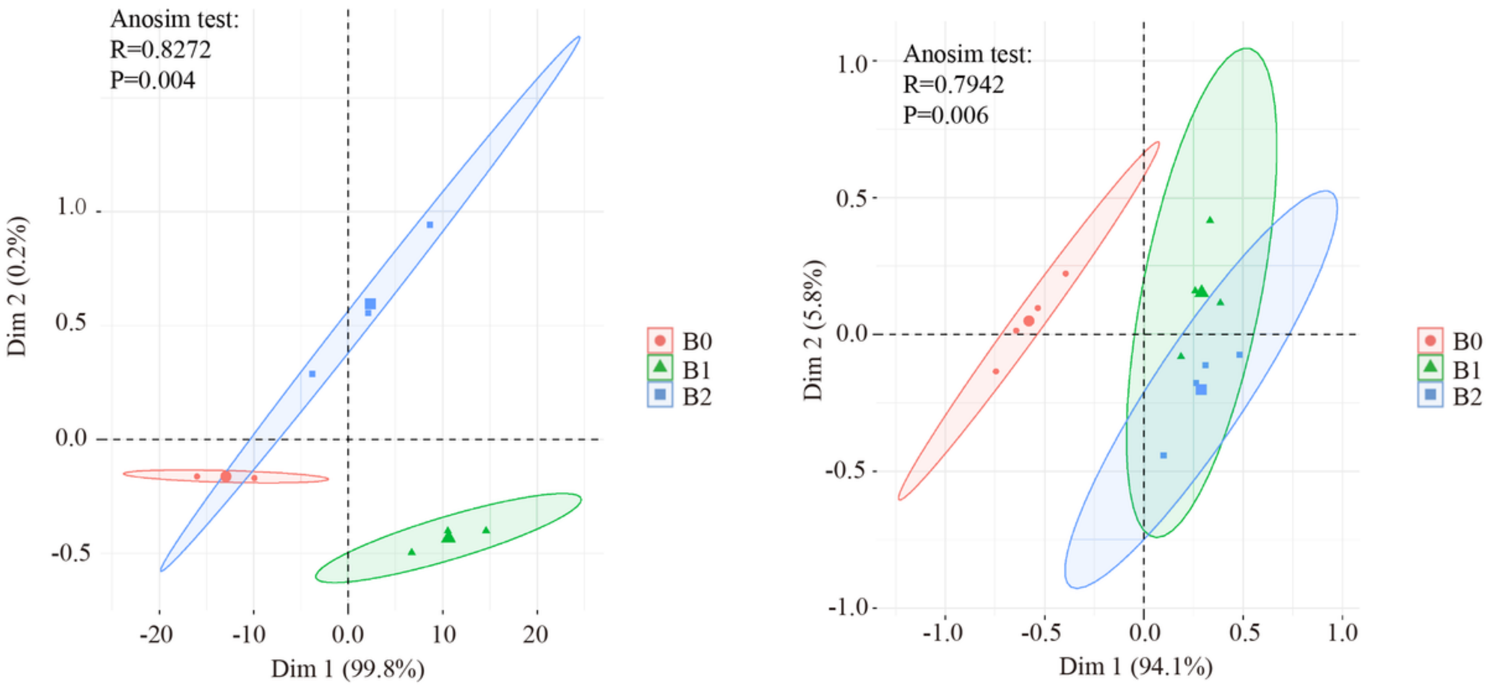
77. Zelles, L., & Bai, Q. Y. (1993). Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biology and Biochemistry, *25*(4), 495–507.
78. Zhengbin, Z., Ziyuan, D., Hongbo, S., Peng, C., & Ping, X. (2011). Establishing a biotech-modern-agriculture for China. African Journal of Biotechnology, 10(72), 16113–16119.
79. Zhou, H., Yao, X., Zhao, Q., Zhang, W., Zhang, B., & Xie, F. (2019). Rapid effect of nitrogen supply for soybean at the beginning flowering stage on biomass and sucrose metabolism. Scientific Reports, 9(1), 1–13.

# Figures




### Figure 1

Effects of biochar on root growth during flowering stage of rapeseed. Bars followed by the same letter are not significantly different according to LSD-test (P≤ 0.05). Bars represent ± standard errors of the means.




## Figure 2

Principal coordinates analysis (PCoA) and Anosin test of agronomic (a) and yield components (b) of rapeseed under different biochar treatments.

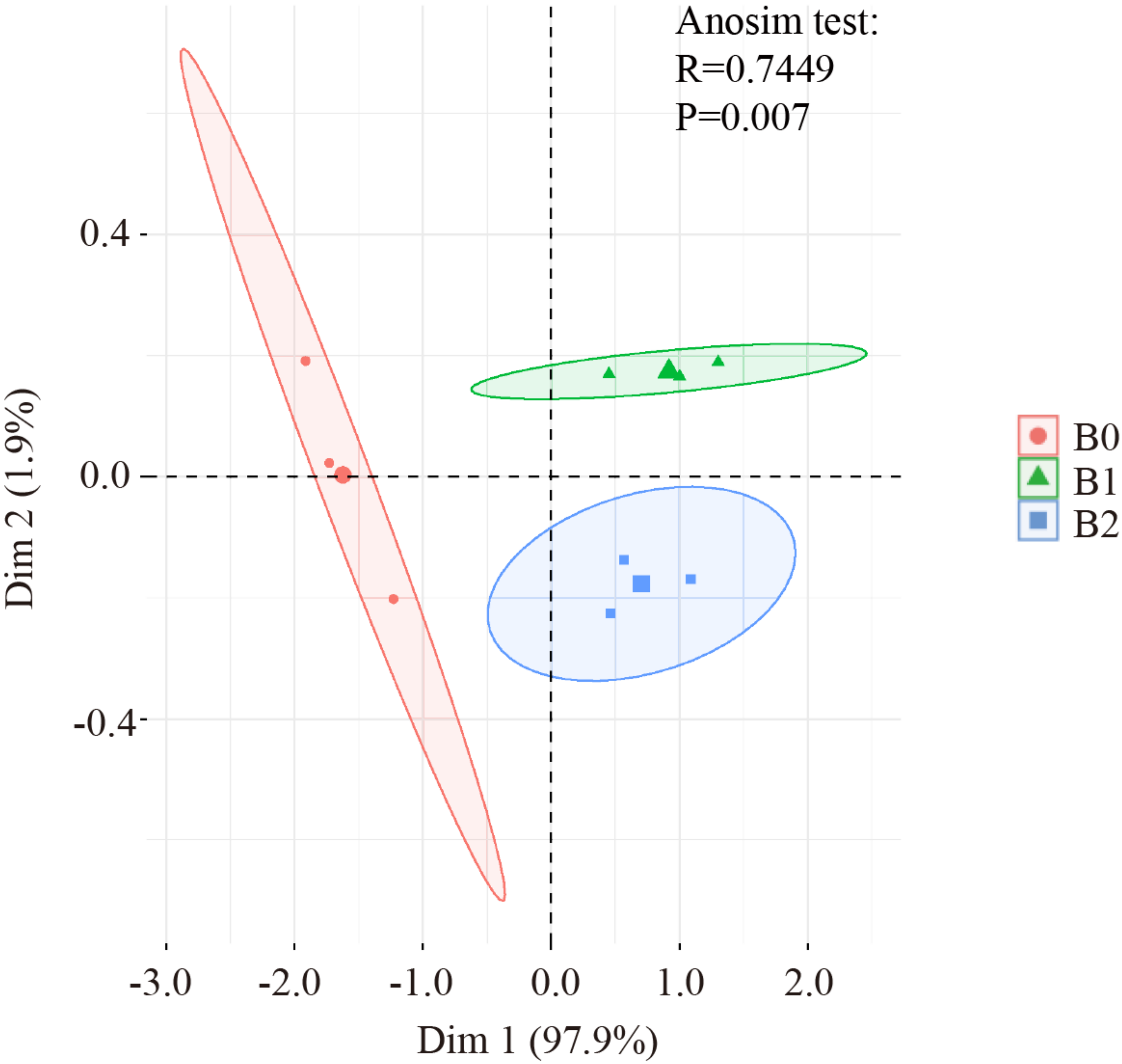
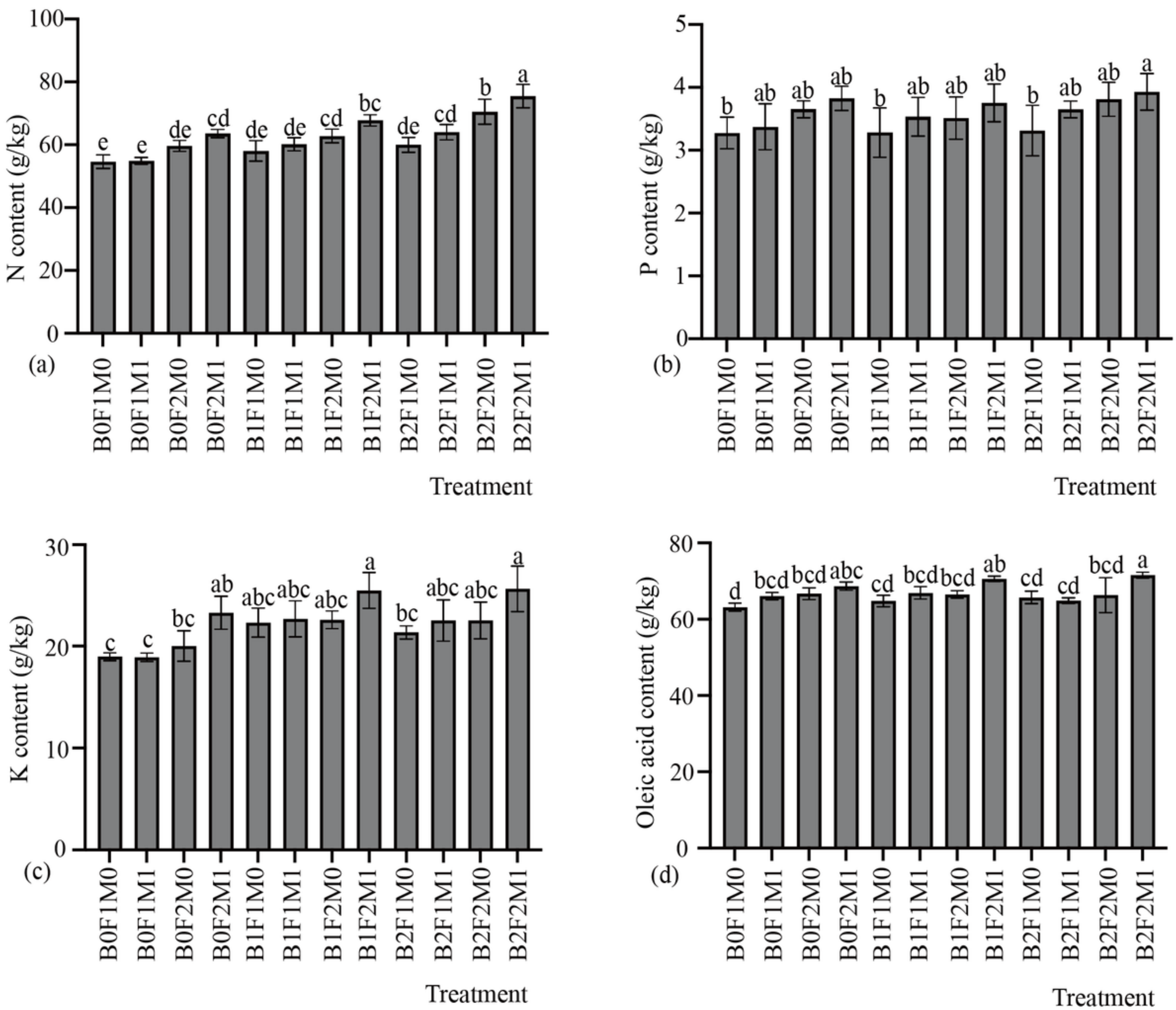


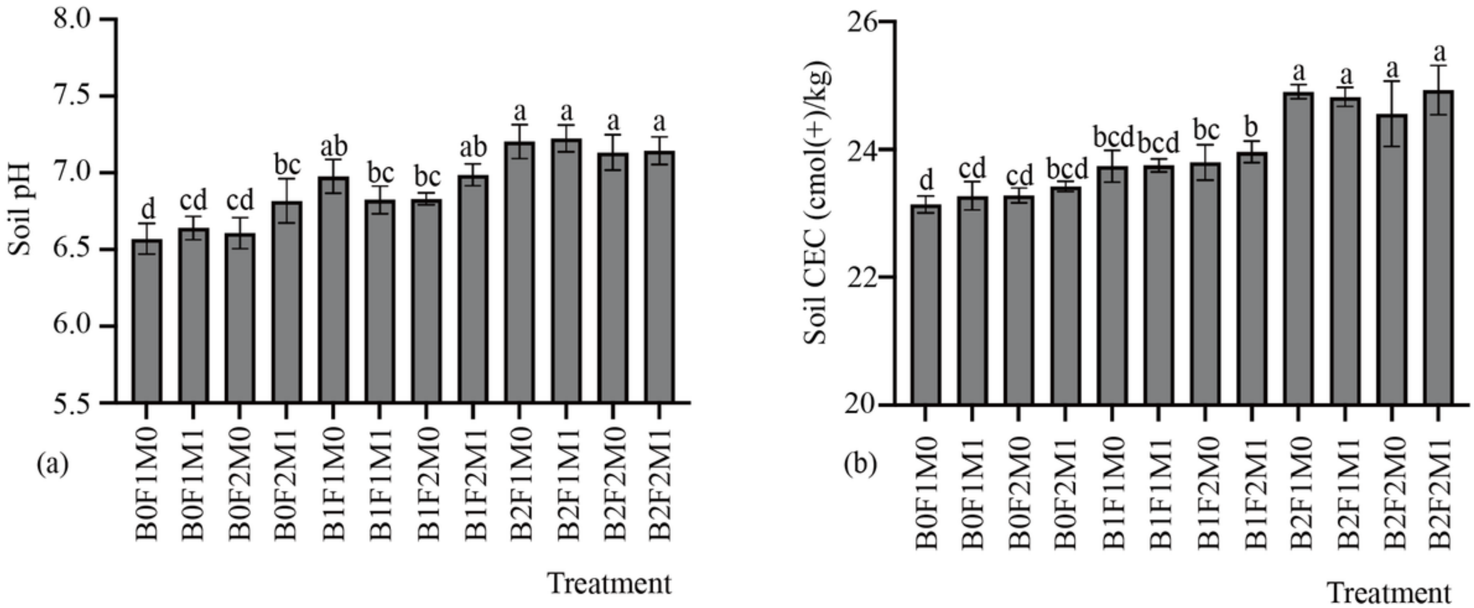

Figure 3

Principal coordinates analysis (PCoA) and Anosin test of photosynthetic traits of rapeseed under different biochar treatments.



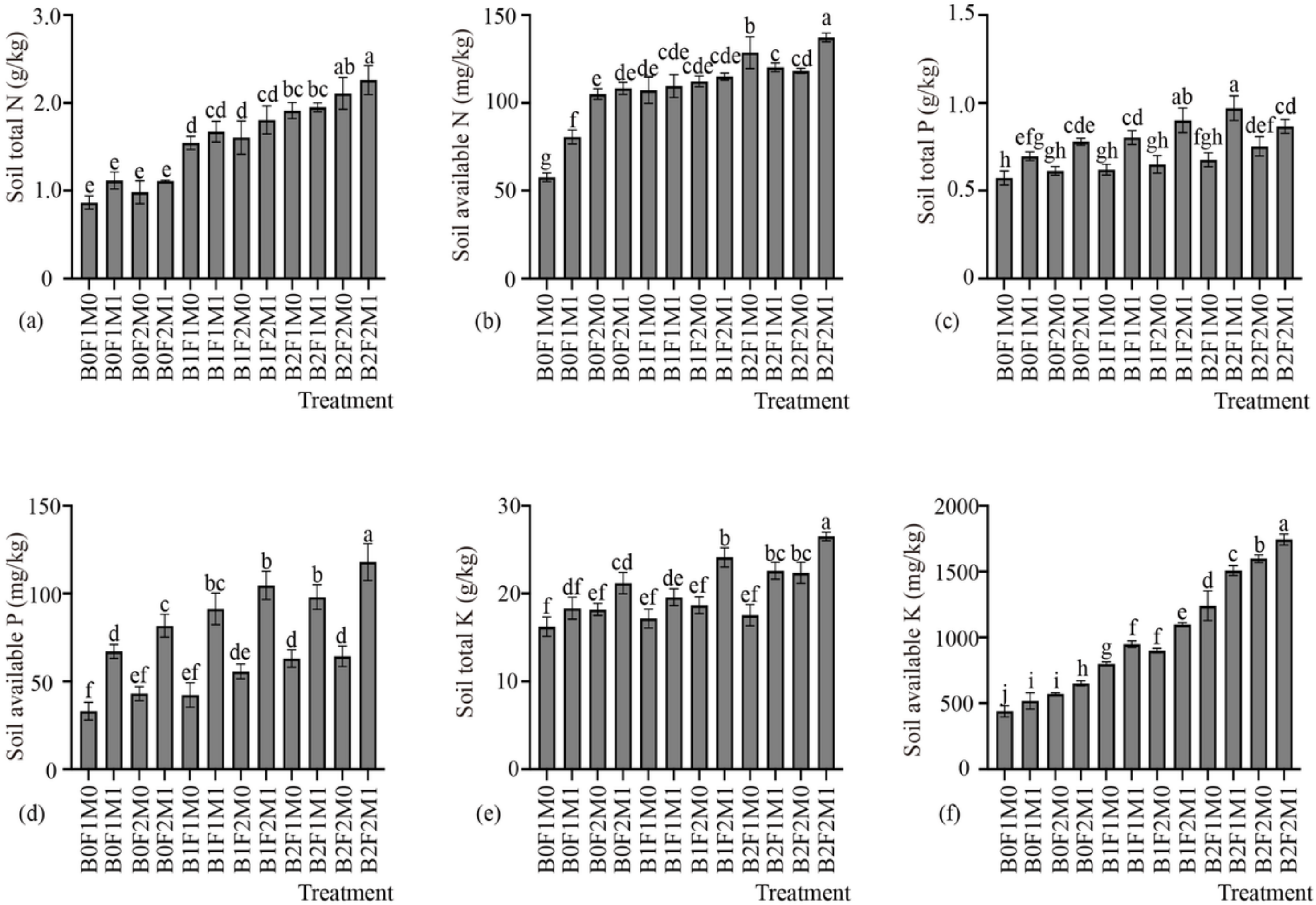

## Figure 4

Effects of biochar, inorganic fertilizer and organic fertilizer on pod nutrients at flowering stage and seed oleic acid at mature stage. Bars followed by the same letter are not significantly different according to LSD-test ($P \leq 0.05$). Bars represent ± standard errors of the means.




### Figure 5

Effects of biochar, inorganic fertilizers and organic fertilizers on soil pH (a) and CEC (b) at flowering stage. Bars followed by the same letter are not significantly different according to LSD-test ($P \leq 0.05$). Bars represent ± standard errors of the means.




## Figure 6

Effects of biochar, inorganic fertilizers and organic fertilizers on soil nutrients at flowering stage. Bars followed by the same letter are not significantly different according to LSD-test ($P \leq 0.05$). Bars represent ± standard errors of the means.

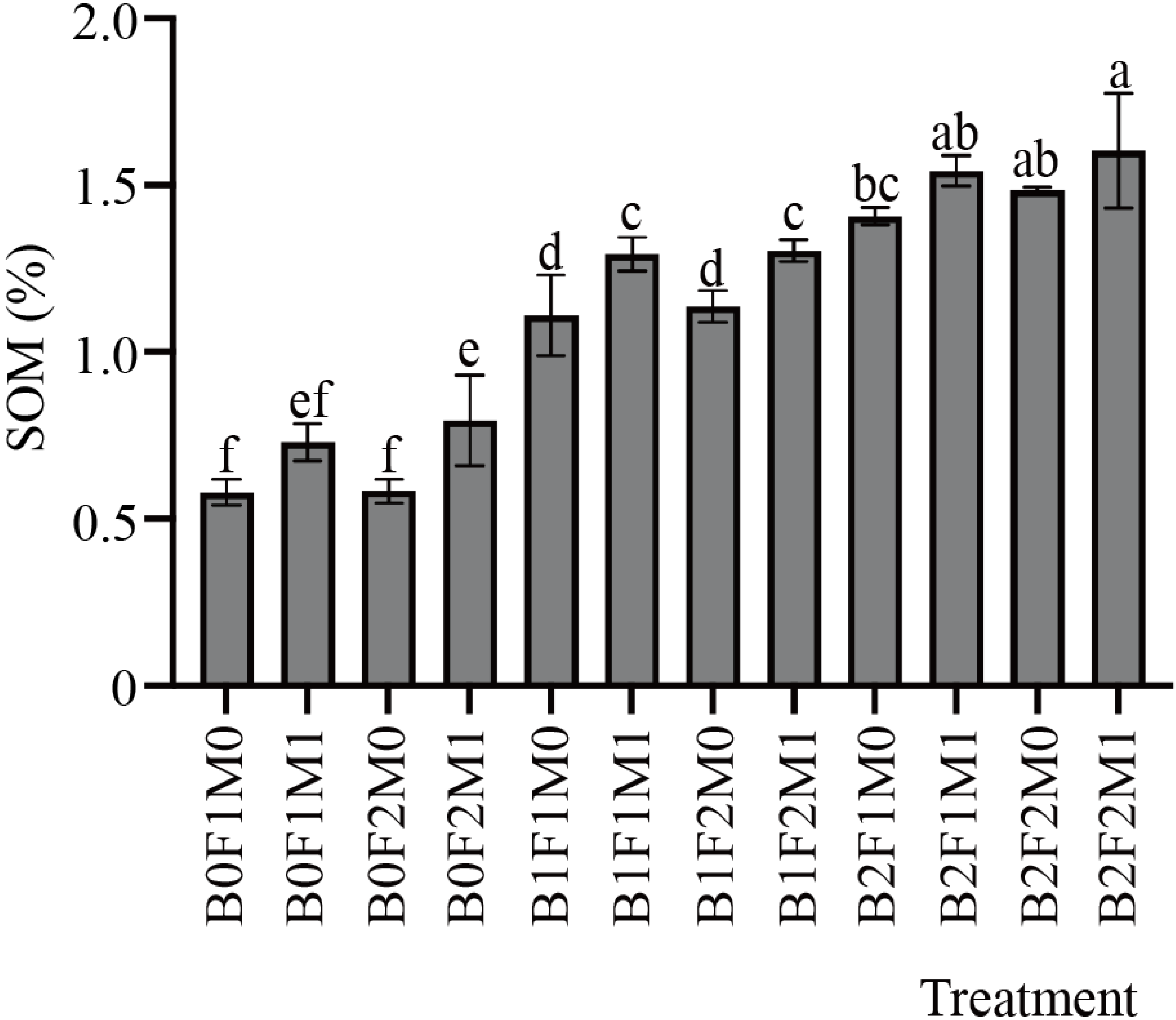



**Figure 7**

Effects of biochar, inorganic fertilizers and organic fertilizers on SOM at flowering stage. Bars followed by the same letter are not significantly different according to LSD-test (P≤ 0.05). Bars represent ± standard errors of the means.

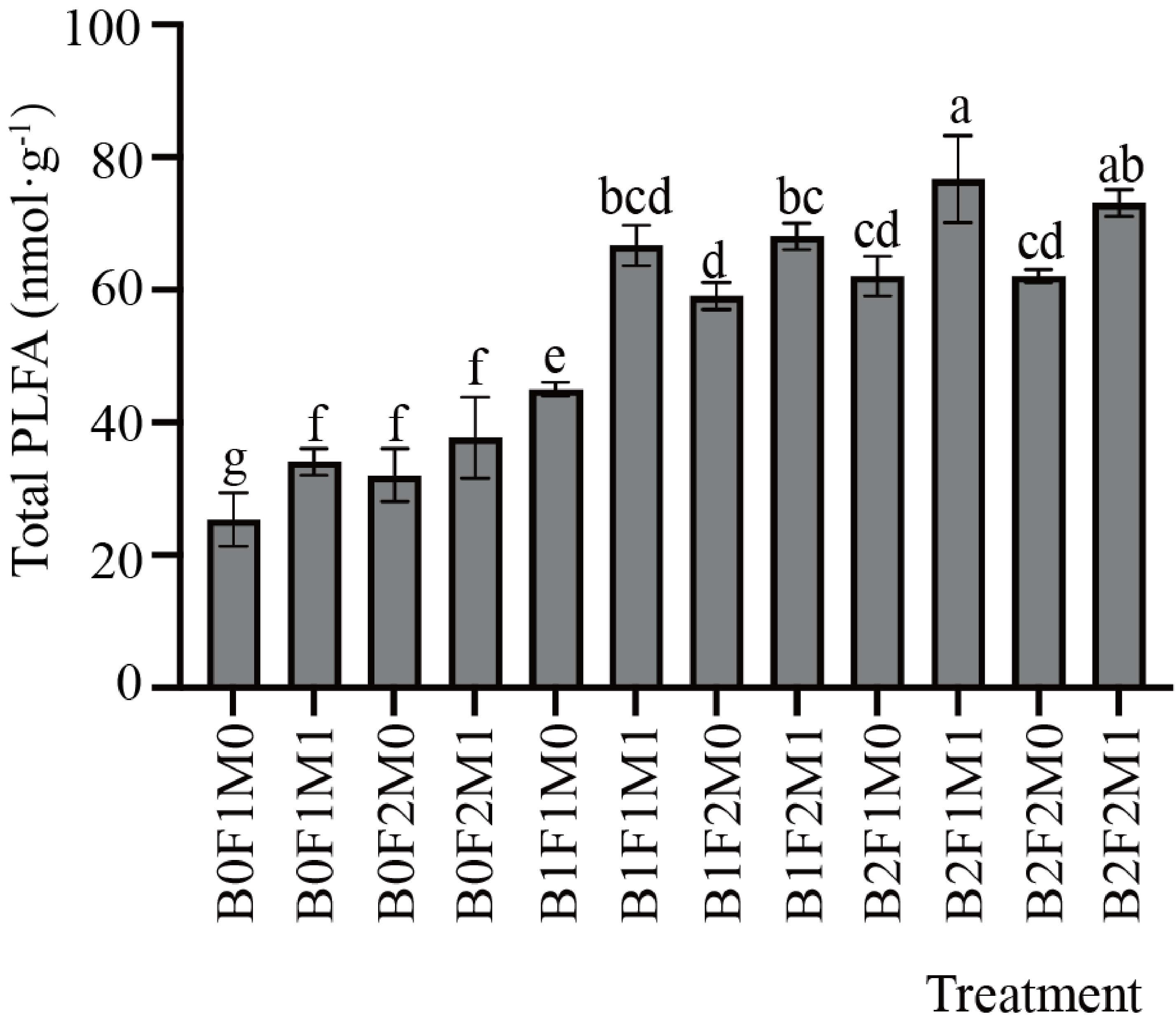



**Figure 8**

Effects of biochar, inorganic fertilizers and organic fertilizers on soil PLFA at flowering stage. Bars followed by the same letter are not significantly different according to LSD-test ($P \leq 0.05$). Bars represent ± standard errors of the means.

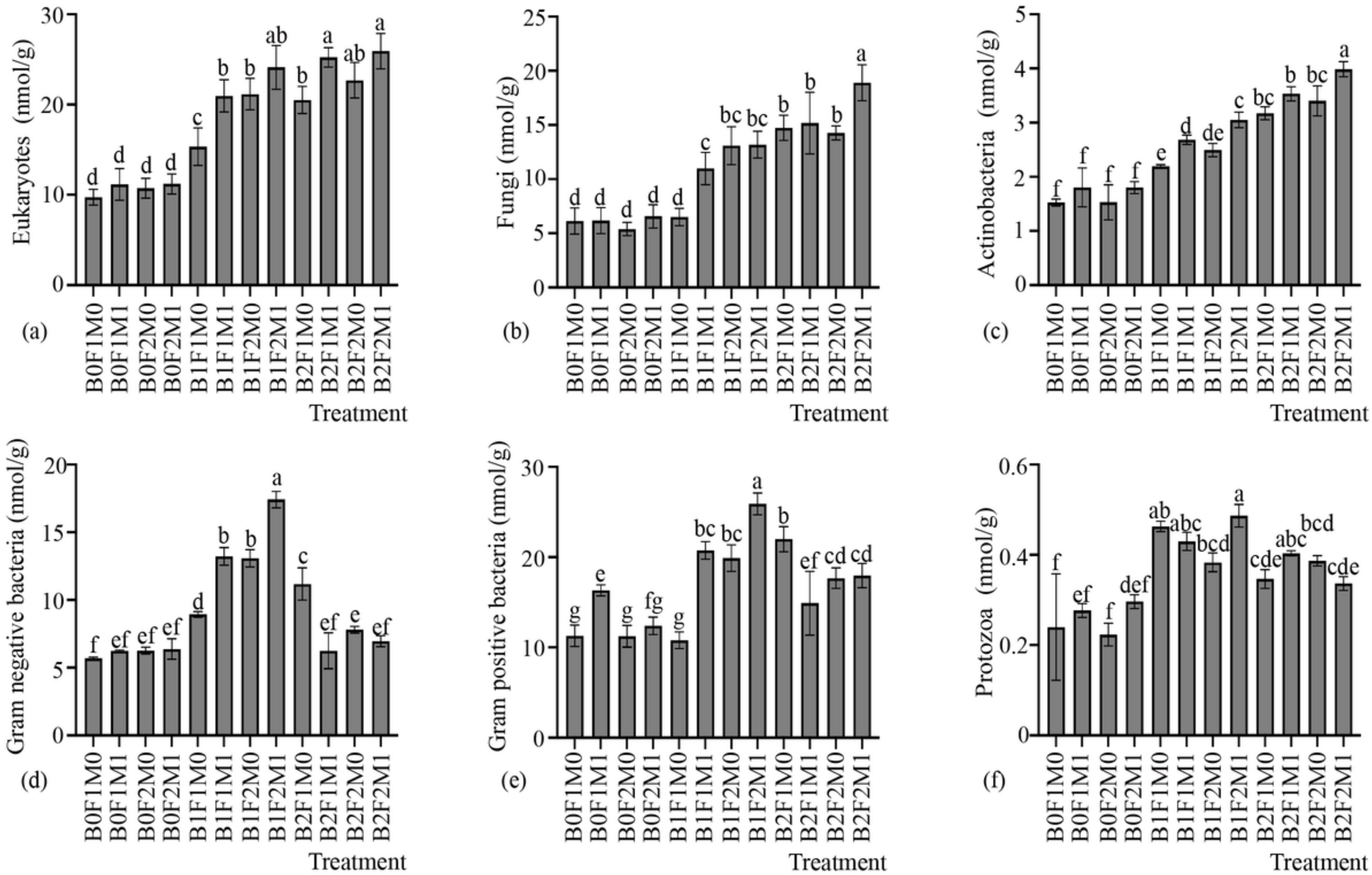



**Figure 9**

Effects of biochar, inorganic fertilizers and organic fertilizers on soil microbial communities at flowering stage. Bars followed by the same letter are not significantly different according to LSD-test (P≤ 0.05). Bars represent ± standard errors of the means.

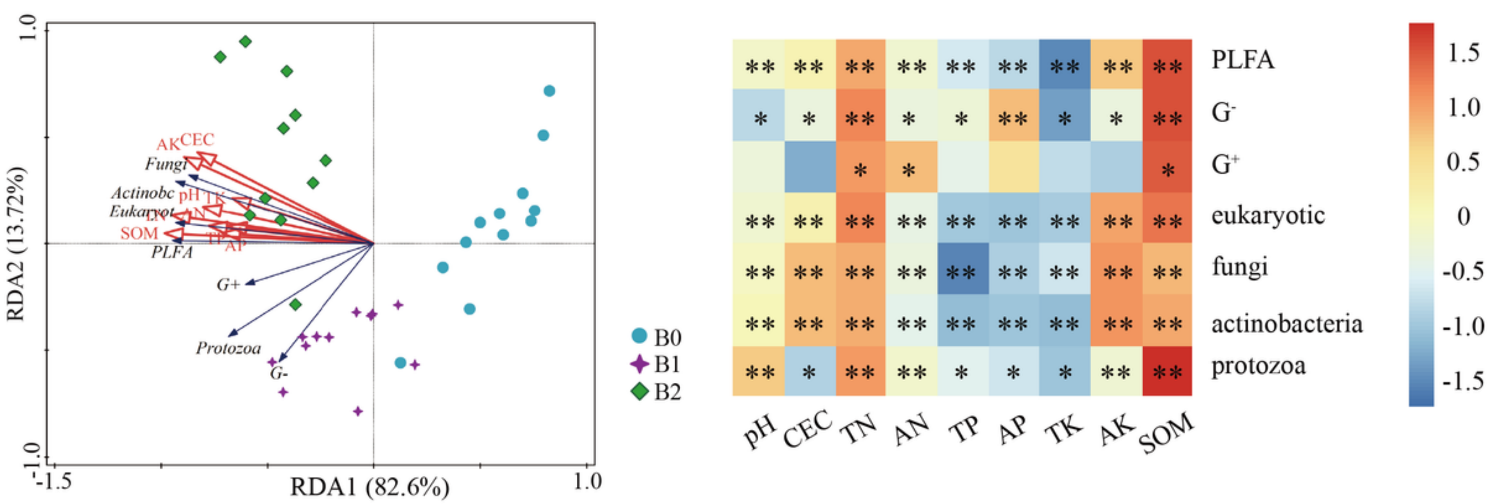



**Figure 10**

The redundancy analysis (RDA) and heatmap based on different treatments and microbial community compositions and soil fertility properties.