



Biochar increases pakchoi yield by regulating soil bacterial communities but reduces it through soil fungi in vegetable soil

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Abstract

Purpose Biochar has been proven to mitigate the detrimental impacts on the high use of nitrogen (N) fertilizer on vegetable yield by influencing the microbial community, but the impacts on the microbial pathway of biochar remain unclear.

Methods A pot experiment of pakchoi was conducted with eight treatments, including normal level (100 mg N kg⁻¹: N1) and high level (200 mg N kg⁻¹: N2) of N fertilizer treatments, as well as four biochar levels (0%: B0, 0.5% w/w: B05, 1% w/w: B1, and 2% w/w: B2) to determine its practical effects.

Results High nitrogen fertilizer significantly reduced pakchoi yield. Biochar increased the pakchoi yield by 13.46–55.76% at N1 and 53.3–75.56% at N2, but the effect of the application of biochar on pakchoi yield rates was not significant. The bacterial and fungal diversity was significantly increased by biochar but was decreased by high N in vegetable soil. Soil bacterial communities were dominated by *Proteobacteria*, *Actinobacteriota*, *Gemmatimonadota*, *Acidobacteriota*, and *Chloroflexi*, while soil fungal communities were dominated by *Ascomycota*, *Chytridiomycota*, *Mortierellomycota*, and *Basidiomycota*. Biochar and nitrogen fertilizer all changed the bacterial and fungal community composition.

Conclusions Biochar enhanced the pakchoi yield through regulated bacterial communities, but the regulatory pathways vary under different N levels. It enhanced the prevalence of beneficial species involved in disease suppression and supported the soil organic matter degradation, thereby resulting in increased vegetable yield at N1. It increased the abundance of eutrophic species, which were related to the available nutrients in the soil and its retention capacity to improve the yield at N2. It was noteworthy that biochar could increase the risk of vegetable yield reduction by reduction of nutrient cycling phylum and enhancement of soil-borne plant pathogens at a higher nitrogen level.

Keywords High nitrogen fertilizer · Biochar · Soil microbial diversity · Yield · Agroecosystem

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1 Introduction

High nitrogen (N) fertilization reduced the N utilization efficiency (NUE) and crop yield, particularly in vegetable soil (Ma et al. 2019). The average annual nitrogen application to vegetable soils was 3–5 times higher than that of grain-producing soils in China (Sun et al. 2020b), and caused a series of environmental pollution and agricultural product safety problems (Ti et al. 2015). It is therefore urgent to decrease chemical fertilizer use and improve vegetable yields toward a sustainable agroecosystem (Ventura et al. 2019).

Biochar, based primarily on agricultural residues, such as straw and animal manure, has emerged for soil fertility improvement in agriculture. By the reason of extensive specific surface area, elevated pH, and abundant functional groups, biochar could improve the soil physical and chemical

properties while retaining soil-enriching nutrients (Novak et al. 2009; Ding et al. 2016); it can reduce the dependence on chemical fertilizers and enhance vegetable yields (Guo et al. 2020). The effectiveness of biochar in improving vegetable yields (Hossain et al. 2020) and reducing soil N loss (Gul et al. 2015) had been demonstrated in a number of studies. It suggested that soil N content might be increased by biochar through nutrient retention (Singh et al. 2022), while others had suggested that biochar exhibited no effect or even negative effects on it or crop yields due to the complex soil environments and microbial composition and functions (Ali et al. 2020). The conflicting observations were mostly attributable to biochar's influence on soil environments, while the effects on microbial processes remained unclear.

Microbial communities are crucial to various aspects of agroecosystems, including crop yield, soil carbon, the mobilization of nutrients, and structural enhancement (Rasul et al. 2022). It had been shown to be impacted by N in numerous studies, either directly or indirectly, by modifying the soil C/N content or other soil properties. Moreover, several studies had suggested a decline in soil microbial diversity as a consequence of N fertilizer, attributable to the heightened prevalence of nitrophilous species (Hu et al. 2017). Nonetheless, the precise microbial mechanisms underlying the impact of N fertilizer on vegetable yields remain undisclosed. The microbial community composition is largely regulated by N levels (Ramirez et al. 2010). With low N levels favoring increased microbial diversity, high N levels have the opposite effect (Zeng et al. 2016). Nonetheless, the relationship between bacterial diversity and crop yield did not always exhibit a positive correlation (Ren et al. 2020). Thus, focusing solely on bacterial diversity might not fully elucidate how biochar affected crop yield. Recently, it had been observed that crop yield was significantly affected by fungi. Nevertheless, the influence of biochar on plant growth via fungal interactions under varying levels of N fertilizer was yet to be comprehensively understood (Ullah et al. 2019). Furthermore, compared with the bacterial influences, the fungal communities exhibit a higher level of complexity and comprise numerous pathogenic fungi. Consequently, alterations in the fungal communities could lead to greater uncertainty regarding crop yield (Gao et al. 2021). Also, few studies had focused on uncovering the microbial pathway underlying the yield impacts of biochar, specifically regarding bacterial and fungal processes under varying levels of N. Therefore, further investigation is necessary to elucidate the interconnected relationships among biochar, soil bacteria and fungi, and crop yield. Considering the issue of high N fertilizer in the facility's vegetable field (Sun et al. 2020b), pot experiments were conducted using pig manure biochar as a treatment, as it is commonly employed in agricultural settings. In our pot experiments, two treatments of N fertilizer were designed, namely, normal level of 100 mg N kg⁻¹ and

high level of 200 mg N kg⁻¹, and four gradients of biochar (0%, 0.5%, 1%, and 2% w/w). The effect of biochar on vegetable yield, the communities' structure, and diversities of soil bacteria and fungi were investigated under varying levels of N fertilizer. In addition, we explored the primary microbial pathways of biochar that influenced pakchoi yield. We hypothesized that (1) microbial regulation of biochar on pakchoi yield mainly relies on bacteria; (2) biochar can also affect pakchoi yield by affecting the soil fungal community, but this effect may be negative, as a result of an increase in pathogenic fungi.

2 Materials and methods

2.1 Soil collection and properties

Soil was collected in a vegetable plantation of Shandong Province of China (121°69' N, 37°37' E), air-dried, cleaned, crushed, and sieved (2 mm) for the pot experiments. Table S1 shows the soil properties. A pH meter (OHAUS STARTER3100M) was used to measure the soil pH. Soil moisture content (SMC) was dried at 105 °C until the weight was fixed using the gravimetric method after 24 h. Combustion elemental analyzer was used to measure total C (TC) and N (TN). Fresh soil (10 g) was shaken with 1 M KCl (50 ml) for 1 h and filtered with Whatman No. 42 ashless filter paper, and then the soil ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) were determined by use of automated ion analyzer (Foss 5027, Denmark).

2.2 Biochar preparation and characterization

Biochar was made from pyrolyzed pig manure by N₂ purification under O₂-limitation (Sanli Company, Shandong Province). Pig manure was carbonized at a pace of 30 °C min⁻¹ to 500 °C and maintained for 2 h. Air-cooled biochar was milled and sieved (2 mm) before characterization and application to the pots. The properties of biochar are also illustrated in Table S1.

2.3 Experiment design

The pot experiment included eight treatments, each with three repetitions. The treatments consisted of two levels of N fertilizer (urea): N1 with 100 mg N kg⁻¹ and N2 with 200 mg N kg⁻¹. In addition, there were four levels of biochar: B0 with 0% biochar, B05 with 0.5% biochar, B1 with 1% biochar, and B2 with 2% biochar (all percentages are w/w).

Five hundred grams of dry-weight soil was placed in each box, and for a duration of 42 days, three pakchoi plants grew in one plot, specifically from July to August 2019. The specifications of each box were 15 cm in length, 10 cm in width,

and 6 cm in height. Prior to sowing, the soil was incubated for 1 week, and to keep the water holding capacity at 60%, deionized water was added every day.

All treatments received the same fertilizer rates, consisting of 0.17 g potassium (K) and 0.07 g phosphorus (P) (Zhong et al. 2022).

2.4 Soil properties and plant yield determination

Deionized water was used to wash harvested plants, and then roots and shoots were categorized accordingly. After that, plant samples were oven-dried to determine the N concentration. Pakchoi biomass was dried at 80 °C for 48 h and subsequently weighed. Soil and plant TC and TN were determined by using an element analyzer. As plants were harvested, the soil was also collected. Part of the soil samples were air-dried for subsequent analyses, while the remaining fraction was kept at –20 °C for molecular analysis.

2.5 Soil DNA extraction and Illumina HiSeq sequencing

The DNA was extracted by using a soil DNA kit (Omega Bio-Tek) following the instructions. The V3–V4 regions of the 16S rRNA gene of bacteria were amplified by primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). In order to amplify the internal transcribed spacer (ITS) regions of the fungal genome, primers for ITS1F and ITS2R were used (Bellemain et al. 2010). PCR amplicons were generated, purified, and sequenced using the primers and procedures outlined in Table S3. Sequencing procedure followed standard protocols (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China).

2.6 Bioinformatics and statistical analysis

With Trimmomatic, raw fastq files were filtered for quality. Subsequently, FLASH was used to merge the paired-end reads. The clustering of operational taxonomic units (OTUs) with 97% similarity cutoff was done using UPARSE (version 7.1 <http://drive5.com/uparse/>) with a novel “greedy” algorithm that performs chimera filtering and OTU clustering simultaneously. The Silva (SSU138) 16S rRNA database (bacteria) and UNITE8.0 database (fungi) were used to analyze the taxonomy of OTUs by RDP Classifier algorithm (<http://rdp.cme.msu.edu/>), with a confidence threshold of 70%. Alpha diversity indices (Shannon and ACE) were analyzed through the use of Mothur (V1.30.2 https://www.mothur.org/wiki/Download_mothur/).

2.7 Statistical analysis

Soil physiochemical properties, plant yield, and microbial diversity index were analyzed by two-way ANOVA with Duncan test ($p < 0.05$) by SPSS 18.0.

Principal co-ordinates analysis (PCoA) was performed based on Bray–Curtis distances to assess microbial structure differences among the treatments by R (version 4.1.2). Partial Least Squares Path Modeling (PLS-PM) was employed to test possible indirect and direct effects of biochar dosages, soil pH and C/N, microbial diversity, abundance, and structure on pakchoi yields by using the “plsmp” package in R (version 4.1.2).

Random Forest (RF) analysis was used to identify the major factors influencing pakchoi yields by “randomForest” package in R (version 4.1.2). The interactions between the soil microbial community and environmental variables ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, pH, SMC, TN, and TC) were explored by redundancy analysis (RDA).

The heatmap of the Pearson correlations between the top 20 genera of microbial compositions and soil properties were created with the “heatmap” package. Pearson’s correlation was used to examine the interactions between the yields, soil condition, and the relative abundance of the abundant phyla. For predicting 16S gene function, abundances of the OTU table were standardized using PICRUST 1.1.0.

3 Result

3.1 Pakchoi yield and soil properties

Pakchoi yield was significantly promoted by biochar while reduced by high N fertilizer. Biochar improved the pakchoi yield at both N levels (Table 1). B05 significantly increased the yield compared to B0 by 55.76% at N1 and 75.56% at N2. Moreover, the significant interaction of B and N was found in yield ($p < 0.05$). Across all treatments, SMC varied between 7 and 12% (w/w) (Table 1). Biochar significantly decreased SMC at N2 level ($p < 0.01$). Biochar increased the soil pH ($p < 0.01$) while decreasing by N fertilizer ($p < 0.01$). Significant interaction between N and B treatments was found in soil pH ($p = 0.01$). TC was considerably improved by biochar ($p < 0.01$) across all treatments. Biochar significantly decreased $\text{NH}_4^+\text{-N}$ ($p = 0.03$) and $\text{NO}_3^-\text{-N}$ ($p = 0.04$) at N1.

3.2 Alpha diversity of soil bacterial and fungal community

Biochar significantly affected bacterial and fungal Shannon and ACE index (Table 2). Bacterial Shannon index increased with the dosage of biochar, but decreased at N2 compared

Table 1 Soil properties and yield under biochar and N fertilizer treatments

Treatment	Yield (g)	SMC (%)	pH	TC (%)	TN (%)	C/N	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	
N1	B0	0.52 ± 0.14 ^b	8.44 ± 0.32	5.97 ± 0.03 ^b	0.66 ± 0.02 ^c	0.10 ± 0.01 ^b	6.60	18.42 ± 0.54 ^a	94.33 ± 3.59 ^a
	B05	0.81 ± 0.11 ^a	8.31 ± 0.62	5.95 ± 0.01 ^b	0.80 ± 0.04 ^b	0.10 ± 0.01 ^b	8.00	16.33 ± 0.56 ^{ab}	76.02 ± 3.36 ^b
	B1	0.59 ± 0.06 ^{ab}	8.12 ± 0.29	6.07 ± 0.01 ^a	0.84 ± 0.03 ^b	0.10 ± 0.01 ^b	8.40	14.96 ± 0.43 ^b	69.94 ± 1.66 ^{bc}
	B2	0.70 ± 0.12 ^{ab}	7.87 ± 0.28	6.09 ± 0.01 ^a	1.23 ± 0.04 ^a	0.12 ± 0.02 ^a	10.25	13.99 ± 0.40 ^c	60.91 ± 2.85 ^c
N2	B0	0.45 ± 0.06 ^b	10.37 ± 0.17 ^a	5.85 ± 0.01 ^d	0.71 ± 0.02 ^c	0.12 ± 0.01	5.92	21.73 ± 2.61	126.06 ± 6.30
	B05	0.79 ± 0.14 ^a	8.03 ± 0.76 ^b	5.92 ± 0.01 ^c	0.79 ± 0.04 ^c	0.12 ± 0.01	6.58	21.18 ± 0.48	113.88 ± 3.34
	B1	0.69 ± 0.12 ^{ab}	7.79 ± 0.31 ^b	5.98 ± 0.01 ^b	1.00 ± 0.04 ^b	0.12 ± 0.01	8.33	21.16 ± 1.55	113.58 ± 1.92
	B2	0.70 ± 0.10 ^{ab}	7.58 ± 0.36 ^b	6.13 ± 0.01 ^a	1.22 ± 0.05 ^a	0.13 ± 0.01	9.38	18.06 ± 1.26	107.92 ± 5.44

The different letters indicate the significance differences between treatments ($p < 0.05$)

SMC soil moisture content; TC soil total carbon; TN soil total nitrogen; C/N soil C/N ratio; NH₄⁺-N soil ammonium; NO₃⁻-N soil nitrate

to N1. Fungal Shannon index in B1 was significantly higher than that in B0, B05, and B2. The bacterial and fungal ACE index all were significantly improved by biochar. Notably, bacterial ACE index with biochar amendment at concentration of 1% was higher than others at N1 level.

3.3 Composition of soil bacterial and fungal community

Bacterial communities were primarily composed of *Proteobacteria* (30.81–35.34%), *Actinobacteriota* (24.60–31.89%), *Gemmatimonadota* (8.04–11.76%), *Acidobacteriota* (7.81–10.24%), and *Chloroflexi* (8.01–9.24%). At N1 level, biochar significantly influenced the relative abundance of *Nitrospirota*, *Myxococcota*, *Armatimonadota*, WPS-2, *Bdellovbrionot*, and *Sumnerlaeota*, while high N influenced *Gemmatimonadota*,

Nitrospirota, *Myxococcota*, WPS-2, *Armatimonadota*, and *Dependente* (Fig. S2). The soil bacteria in the genera level was also changed by biochar at both N levels (Fig. 1c). The relative abundance of *Arthrobacter*, *Luteimonas*, *RB41*, and *Streptomyces* was increased by biochar at two N levels. In comparison, *Sphingomonas*, *Gemmatimonas*, *Lysobacter*, and *Nitrospira* showed opposite responses. Relative abundance of *Gemmatimonas* was significantly enhanced at B05, and *Lysobacter* surged at B2. The relative abundance of *Nitrospira* soared at B1 and then failed at B2 at N2 levels.

Soil fungal communities were dominated by *Ascomycota* (48.39–67.13%), *Chytridiomycota* (9.61–34.60%), *Mortierellomycota* (8.03–24.82%), and *Basidiomycota* (5.34–9.50%) (Fig. 2a). Biochar dramatically improved the relative abundance of *Mortierellomycota*, *Ascomycota*, and *Basidiomycota*, while it reduced *Chytridiomycota* at N1 level. Biochar reduced the relative abundance of *Ascomycota*, *Chytridiomycota*, and *Basidiomycota*, while it increased *Mortierellomycota* at N2 level. Nevertheless, no significant disparity in fungal abundance was observed among the treatments, except for *Kickxellomycota* and *Glomeromycota* at N2 level (Fig. S2c, d). Biochar increased the relative abundance of *Mortierella* in all N treatments. *Chaetomium*, *Solicoccozyma*, and *Penicillium* were increased at N1 level, while they decreased at N2 level. *Fusarium* were significantly increased by biochar and N fertilizer (Fig. 2c, Table S5).

Based on PCoA results, it was determined that biochar primarily influenced the clustering of bacterial communities. Principal components (PC) 1 and 2 separately explained 54.74% and 7.62% of variance. The N1B05, N1B1, N2B05, and N2B1 were clustered together, and the other treatment was separated into two groups at PC1. The clustering results of biochar in fungal community were like bacteria at N2 levels, but were not obvious at N1 levels (Fig. S3b).

Table 2 Responses of bacterial and fungal α -diversity to biochar and N fertilizer treatments

Treatment	Bacteria		Fungi		
	Shannon	ACE	Shannon	ACE	
N1	B0	5.68 ± 0.13 ^b	1015 ± 31 ^b	3.43 ± 0.07 ^b	339 ± 7 ^b
	B05	5.91 ± 0.02 ^a	1040 ± 2 ^{ab}	3.48 ± 0.17 ^b	360 ± 4 ^a
	B1	5.93 ± 0.05 ^a	1051 ± 3 ^a	3.78 ± 0.11 ^a	374 ± 12 ^a
	B2	5.94 ± 0.05 ^a	1042 ± 17 ^{ab}	3.59 ± 0.13 ^{ab}	358 ± 14 ^a
N2	B0	5.65 ± 0.03 ^b	1019 ± 21 ^b	3.51 ± 0.01 ^{ab}	332 ± 6 ^b
	B05	5.87 ± 0.02 ^a	1051 ± 6 ^a	3.53 ± 0.03 ^{ab}	355 ± 19 ^a
	B1	5.84 ± 0.05 ^a	1051 ± 9 ^a	3.57 ± 0.08 ^a	363 ± 3 ^a
	B2	5.88 ± 0.03 ^a	1043 ± 6 ^a	3.41 ± 0.10 ^b	355 ± 1 ^a
p value	N	0.041 [*]	0.437	0.131	0.122
	B	0.000 ^{***}	0.005 ^{**}	0.010 ^{**}	0.000 ^{***}
	N*B	0.794	0.943	0.045 [*]	0.941

The different letters indicate the significance differences between treatments ($p < 0.05$)

*0.01 < p ≤ 0.05; **0.001 < p ≤ 0.01; *** p ≤ 0.001

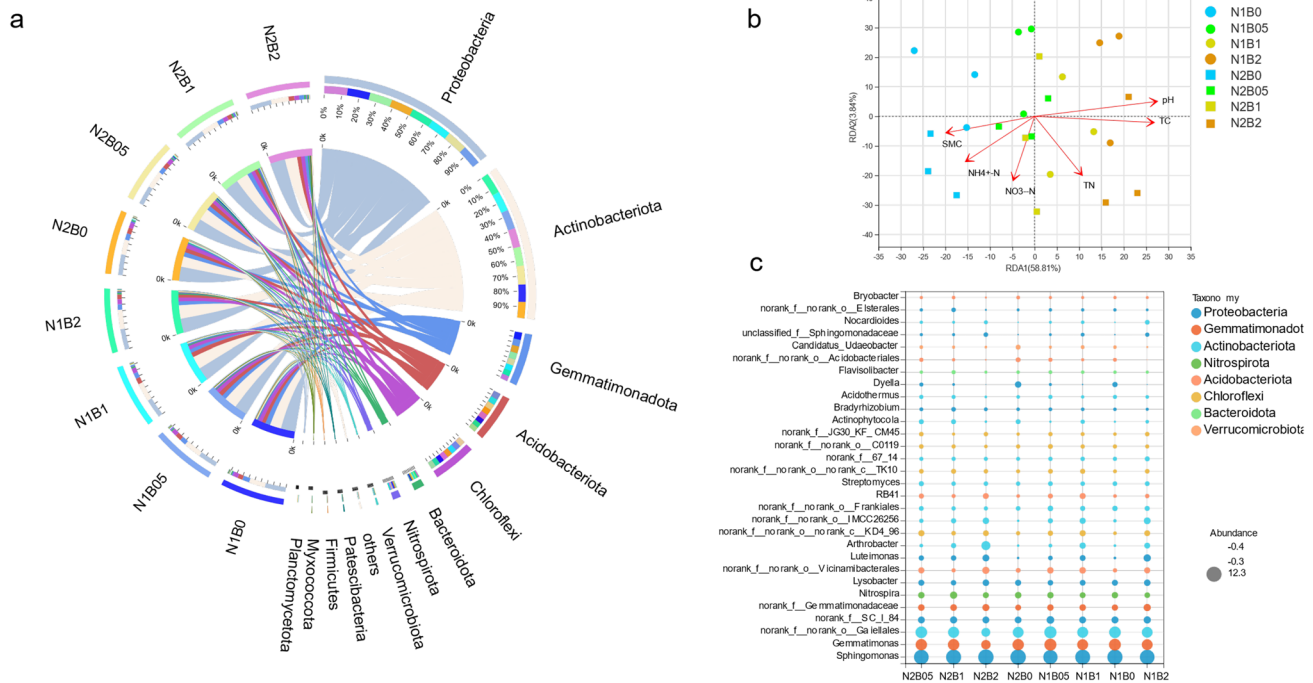


Fig. 1 Effect of biochar on different bacterial taxa at different N levels. **a** Circos plot of bacterial phyla abundances. The direction of the string is from the classification of the phylum to the grouping of the fertilization, and the thickness of the string indicates the level of the abundance of the corresponding group. **b** Redundancy analysis

3.4 Relationship between soil microbial communities, soil properties, and pakchoi yield

RDA assessed how soil properties affected the bacterial (Fig. 1b) and fungal (Fig. 2b) structure. The two axes respectively explained 58.81% and 12.13% of the total variation in bacterial and fungal communities; soil bacterial community structure was highly connected to the TC and pH, followed by SMC, $\text{NH}_4^+\text{-N}$, TN, and $\text{NO}_3^-\text{-N}$. Similarly, the structure of the fungal community was closely related to TC, pH, and SMC ($p < 0.05$). The Mantel test further certified that pH and TC were highly connected to the bacterial community ($p < 0.001$) (Table 3). The heat map analyzed the Spearman connection between soil properties and microbial composition (Fig. 3). Concerning bacteria community, the soil TC and pH were significantly relevant, which were found to be positively associated to *Luteimonas*, *Arthrobacter*, and *IMCC26256*, and were negatively correlated with *Sphingomonas*, *Gemmatimonas*, *Gaiellales*, *C0119*, and *Nitrospira*. For the fungal community, TN was negatively correlated with *Penicillium* and *Ascomycota*; TC and pH were correlated positively with *Mortierella*, *Metarhizium*, and *Aspergillus*, while

they correlated negatively with *Fusarium*, *Penicillium*, and *Chaetothyriales*.

RF predictions were employed to explore the importance of environmental parameters and bacterial and fungal diversity index affected pakchoi yields (Fig. 4). RF indicated that bacterial community diversity and biochar exhibited substantial effects on the pakchoi yields. In addition, soil pH, SMC, and C/N also affected the yield at two N application levels. The results of PLS-PM indicated that the variation of pakchoi yields were mainly driven by microbial abundance and diversity (Fig. 5). Significantly, the yield was positively correlated with bacterial diversity, while a negative association was observed between fungal diversity and structure with yield. *Firmicutes*, *Verrucomicrobiota*, *Armatimonadota*, and *Planctomycetota* were positively correlated to pakchoi yields; *Methylomirabilota* and *Actinobacteriota* were negatively correlated to pakchoi yields (Fig. 6). At bacterial genus level, *KF-CM45*, *TK10*, and *KD4-96* were positively correlated to pakchoi yields; *Lysobacter* and *Sphingomonas* were negatively correlated to yields. For fungal community, only *Rhizophlyctis* and *Chytridiomycota* were negatively correlated to pakchoi yield (Fig. 6). All treatments shared similar metabolic pathways (Fig. 7), however, excessive N application reduced the total abundance of main gene types.

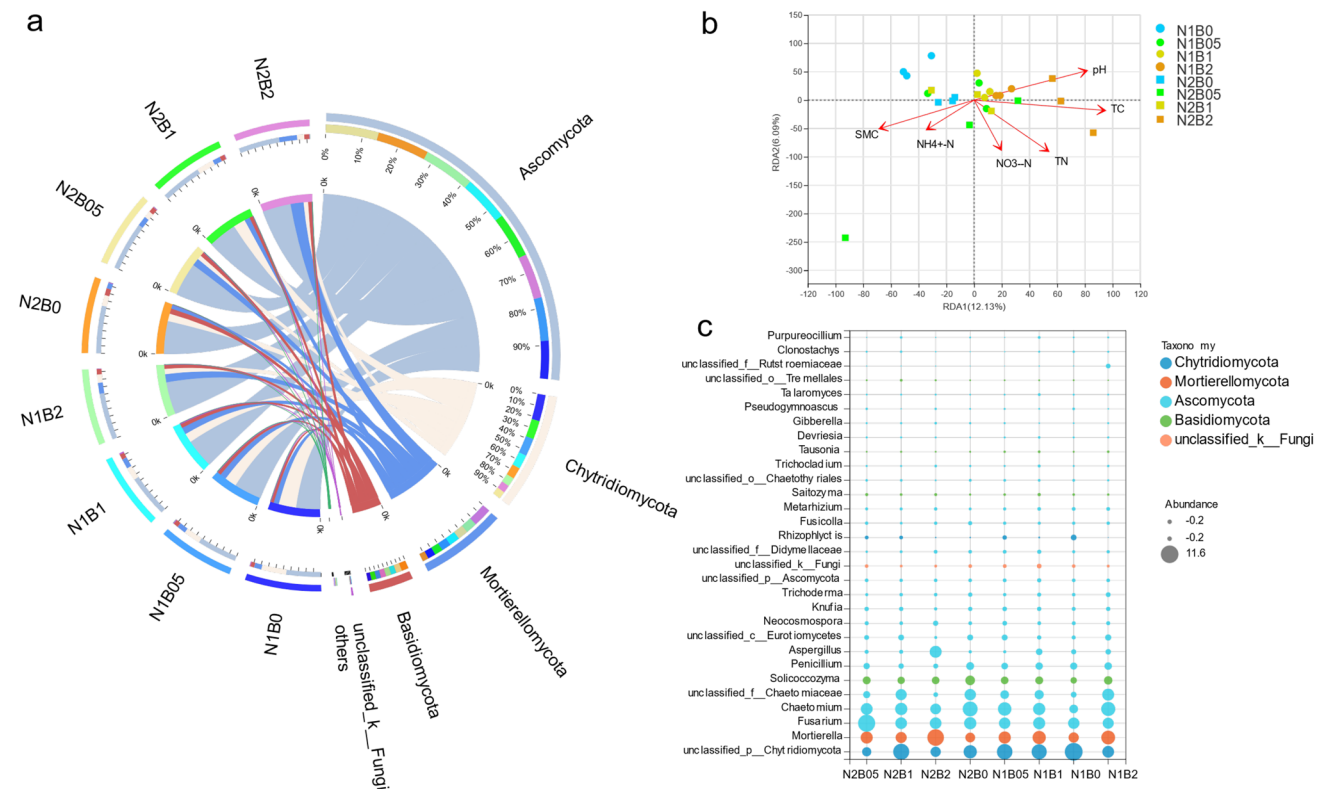


Fig. 2 Effect of biochar on different fungal taxa at different N levels. **a** Circos plot of fungal phyla abundances. The direction of the string is from the classification of the phylum to the grouping of the fertilization, and the thickness of the string indicates the level of the abundance of the corresponding group. **b** Redundancy analysis (RDA)

analysis of fungal community at the OTU level with soil variables. **c** Bubble chart shows the relative abundance of OTUs at genus-level taxonomy (y-axis) in different treatments (x-axis) and grouped with the phyla level corresponding to the genus

4 Discussion

4.1 Effects of biochar on pakchoi yields under different N levels

The application of biochar has widely been reported to increase crop yields (Wang et al. 2021; He et al. 2021). Our results showed that the improvement in pakchoi yields by biochar at normal N application level (13.46–55.76%) was lower than that of high N application levels (53.33–75.56%) (Table 1). Generally, high N fertilization led to soil acidification and C/N decline (Tables 1 and S4) that limit the growth of vegetables (Bai et al. 2020).

Table 3 Mantel-test analysis of soil bacteria and fungi with soil physicochemical properties

Project	Bacterial		Fungal	
	pH	TC	TC	TN
Mantel statistic r	0.606	0.561	0.223	0.263
p value	0.001	0.001	0.029	0.030

Biochar has specific properties that correct soil acidity due to its alkaline nature and high pH (9.17), and the functional groups of biochar such as $-\text{COOH}$ and $-\text{OH}$ also could contribute greatly to biochar alkalinity when pyrolyzed at lower temperatures ($< 500\text{ }^{\circ}\text{C}$). On the other hand, biochar could improve soil C/N ratio due to its high C contents (Xu, Cai et al. 2021; Xu, Ren et al. 2021) and increase soil nutrient retention capacity due to its developed pore structure (An et al. 2022). Then, it could promote inorganic N uptake by plants and improve pakchoi yield (Zheng et al. 2019; Xia et al. 2022).

4.2 Effects of biochar on the bacterial diversity and composition under different N levels

We found that bacterial diversity was significantly increased by biochar while decreased by excess N in vegetable soil (Table 2). It might be explained by biochar providing a suitable habitat and nutrition as well as expanding ecological niches for soil bacteria (Lehmann et al. 2011). Furthermore, biochar positively impacted bacterial diversity by indirectly influencing soil properties (Gao et al. 2021). For example,

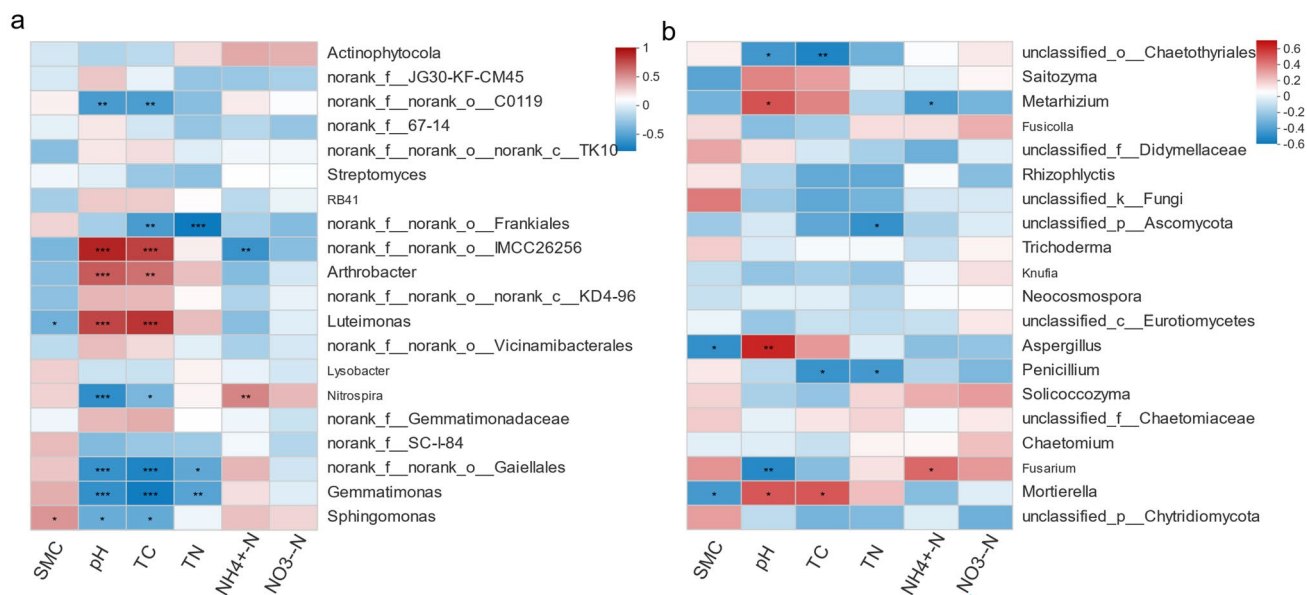


Fig. 3 Heatmap showing the Spearman connection between bacterial (a) and fungal (b) composition and soil variables of different treatments. Red indicates a positive correlation; blue, a negative correlation.

The darker the color, the greater the correlation. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

biochar significantly decreased soil moisture content, which indicated a lower bulk density and higher soil O_2 supply by the addition of biochar, then influencing the microbial communities and diversity (Zheng et al. 2019). High N fertilizer would decrease the soil pH and C/N (Table 1), then lead to soil acidification and increased toxicity of other pollutants (Geisseler and Scow 2014) and reduced carbon utilization efficiency by microorganisms (Lehmann et al. 2011). Thus, high N fertilizer application led to the reduction of microbial diversity in soil (Table 3).

In our study, the dominant bacterial phyla was *Proteobacteria*, *Actinobacteriota*, *Gemmatimonadota*, *Acidobacteriota*, and *Chloroflexi* in vegetable soil (Fig. 1 and Table S2). However, all the dominant bacterial phyla did not change with respect to biochar and N fertilizer, while in many non-dominant bacteria phyla such as *Actinobacteriota*, *Bacteroidota*, and *Myxococcota*, their relative abundance was increased by biochar at both N levels. It indicated that biochar mainly affects soil processes by affecting non-dominant bacterial phylum. For example, the change of bacterial genera was consistent with the phyla level; *Nitrospira*, *Frankiales*, *Gemmatimonas*, *Gaiellales*, and *Sphingomonas* were shown that they could reduce *Fusarium* wilt disease incidence and be involved in soil nitrification (Wang et al. 2021; Xu, Cai et al. 2021; Xu, Ren et al. 2021). These findings showed that biochar can increase N turnover rates and disease resistance of vegetables and therefore increase the vegetable yield through bacterial communities. However, our study also showed that biochar also had different effects on some bacterial species between N1 and N2 level soils. At

N1 level, biochar enhanced the relative abundance of species beneficial to disease suppression (*Acidobacteria*) and is involved in degradation of SOM (*Chloroflexi*) (Xu et al. 2016; Wang et al. 2018). At the N2 level, an increase in the relative abundance of eutrophic species, such as *Nitrospira*, *Patescibacteria*, and *Planctomycetota*, is closely linked to the rapid availability of soil nutrients.

4.3 Effects of biochar on the fungal diversity and composition under different N levels

Previous studies had been conducted to study the effects of biochar on the structure of bacterial communities (Xu et al. 2016), but few studies focused on how biochar affects vegetable yields through fungal communities, especially under high N fertilizer (Yao et al. 2017). In our results, biochar increased fungal diversity by 10.20% and 4.58% at N1 and N2 levels, respectively (Table 2); previous studies have shown similar results that have demonstrated the positive impact of biochar on fungal diversity, albeit with greater sensitivity when compared to bacterial diversity (Bai et al. 2019; Xi et al. 2022; Dong et al. 2022). The heightened sensitivity may be attributed to the rapid changes in soil TC and TN induced by biochar, which is a major contributing factor, particularly in enhancing fungal diversity through the increased soil C content (Singh et al. 2022). Furthermore, studies have indicated that increased N input could potentially decrease fine root production, enhancing recalcitrant compounds, thereby limiting C availability for soil microorganisms and adversely affecting fungal diversity

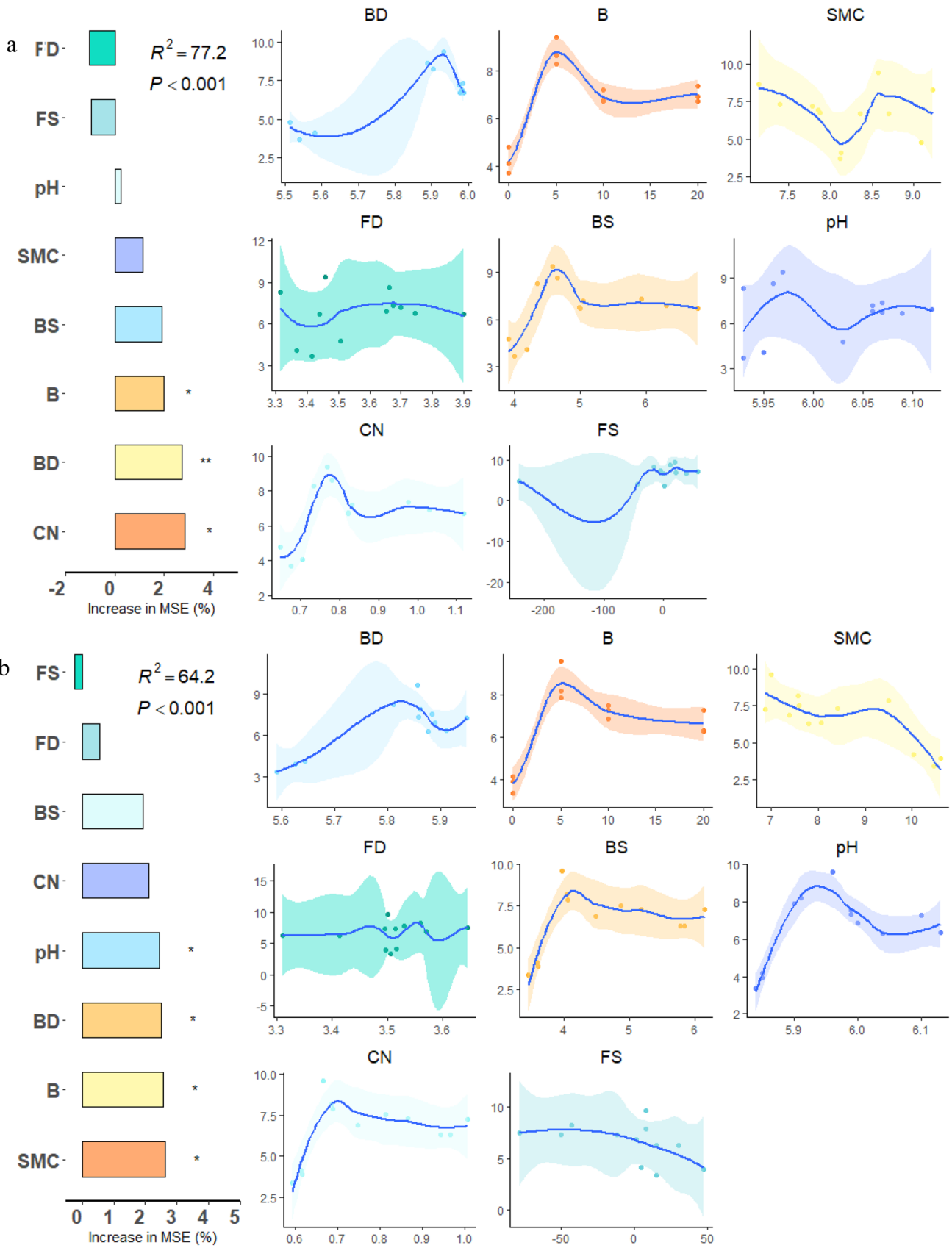


Fig. 4 Random Forest (RF) predictions of the most influential environmental parameters and bacterial and fungal diversity index on the pakchoi yield at N1 (a) and N2 (b) level. The left panel shows the relative importance of the parameters; the right panel shows the relationship between the parameters and pakchoi yield. The blue lines indicate the predicted trajectories of the pakchoi yield in response to the variables, respectively. BD, bacterial diversity; B, biochar application amount; SMC, soil moisture content; FD, fungal diversity; BS, bacterial community structure; pH, soil pH; CN, soil C/N ratio; FS, fungal community structure

(Ullah et al. 2019). In our results, biochar also increased soil C/N and complex organic substrates, and fungi prefer these changes (Iqbal et al. 2022), and so caused the increased soil fungal diversity by biochar, especially at N1 application level (Table 3).

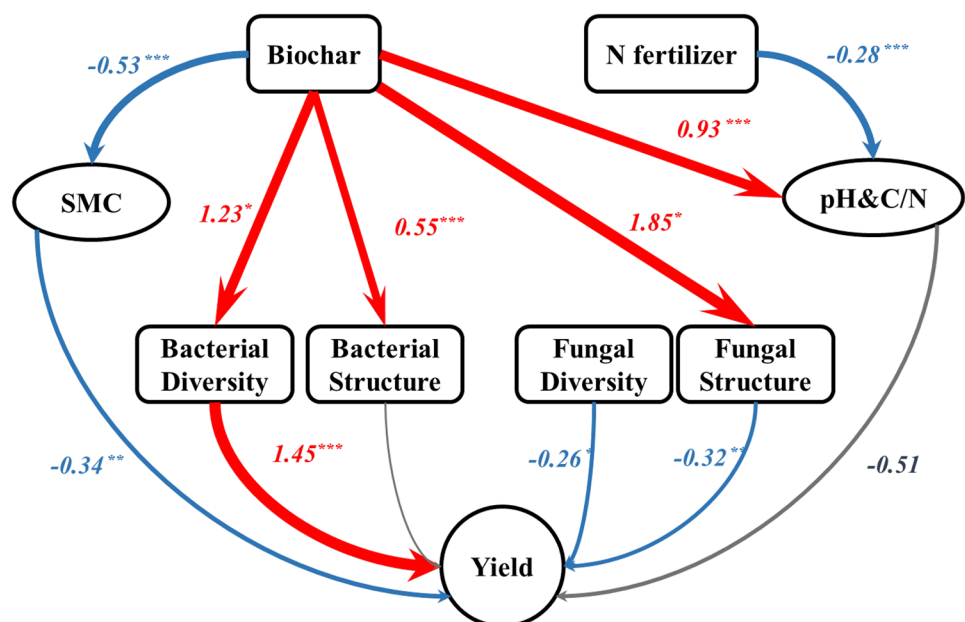
Several major phyla of soil fungal species have been found, including *Ascomycota*, *Chytridiomycota*, *Mortierellomycota*, and *Basidiomycota*. We found that N application level affected the effects of biochar on soil-dominant fungal species. At N1, biochar increased the relative abundance of *Mortierellomycota*, *Basidiomycota*, and *Ascomycota*, which was reported as an effective organic matter transformer and consuming SOM (Devi et al. 2020; Sun et al. 2020a; Santillán et al. 2021). At the genus level, biochar also increased the *Mortierella*, *Fusicolla*, *Chaetomium*, *Penicillium*, and *Aspergillus* abundance which were able to stimulate production of plant and vegetable growth hormones (Lay et al. 2018) and dissolve organic phosphorus, produce antibiotics and other compounds, induce plant defense, and reduce soil pathogenic bacteria (Wang et al. 2020). In contrast, at excess N levels, the relative abundance of those relative abundances were reduced, especially the *Chytridiomycota*, whereas the

decrease in C/N and higher DOC and DON might be the major factors for the opposite results at N2 levels (Yang et al. 2018; Yun et al. 2021). Notably, *Fusarium*, as one of potential soil-borne plant pathogens, was enhanced at B0.5 in N2 levels (Fig. 2c) (Dai et al. 2021). Overall, biochar might enhance the functionality of fungal communities in organic matter transport at normal N levels; however, biochar might enhance the ability of fungal communities to reduced soil available nutrients, and it also increased the abundance of pathogenic fungal species.

4.4 The microbial pathways of biochar to regulate pakchoi yield under different N levels

Biochar has been demonstrated to possess the ability to enhance soil microbial diversity through direct and indirect mechanisms. By modulating soil properties across various fertilization levels, biochar promotes plant growth and facilitates nutrient uptake (He et al. 2021). Biochar also is shown to boost crop growth by increasing bacterial diversity and dominant species abundance (Yu et al. 2019). Biochar significantly increased pakchoi yield majorly through the bacterial diversity compared with fungal diversity; the RF and PLS-PM analysis all showed that bacterial diversity was more important than fungal diversity (Figs. 4 and 5). It was not surprising since many previous studies showed that the changed bacterial diversity and the relative abundance of beneficial bacteria such as *Acidobacteria*, *Actinobacteriota*, *Chloroflexi*, and *Gemmatimonadota* (Yu et al. 2019; Yang et al. 2021; Qiu et al. 2022). They had also been proven to promote the effectiveness improvement of nutrients, especially nitrogen (Franklin and Mills 2006; Ye et al. 2021). In our results, although the soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents were

Fig. 5 The effects of biochar on pakchoi yield were tested by Partial Least Squares Path Modeling (PLS-PM). Note: The solid red and blue arrows imply significant ($p < 0.05$) positive and negative effects, respectively; gray dashed lines represent insignificant pathways. The numbers near the lines are standardized path coefficients or the significance of the variables in the model. *, **, and *** represent significant effects at 0.05, 0.01, and 0.001 probability levels, respectively



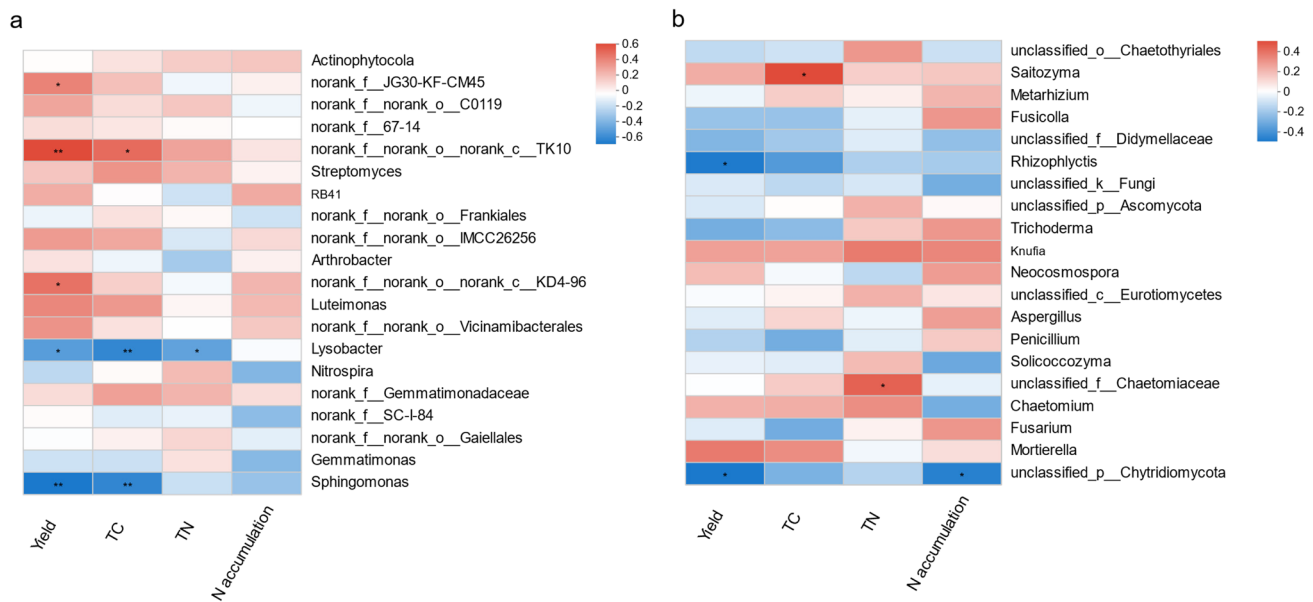


Fig. 6 Heatmap showing the Spearman connection between bacterial (a) and fungal (b) composition and pakchoi agronomic character of different treatments. Red indicates a positive correlation, and blue

indicates a negative correlation. The darker the color, the greater the correlation. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

reduced or were not changed by biochar at N1 and N2 levels, respectively, it might be due to the increase in pakchoi yield leading to its absorption of nutrients by biochar (Table 1). On the other hand, we also found that the strategy of biochar promoting vegetable yield is different between N1 and N2 levels. In the case of relatively insufficient nutrients, plant growth was limited and resulted in poor resistance or stress resistance (Trivedi et al. 2020). Biochar mainly increased the relative abundance of species beneficial to disease suppression and involved in SOM degradation, then improves the stress resistance of crops through conditional microbial community to the increase of vegetable yields. It leads to an increased nutrient absorption capacity of pakchoi from soil, and caused the soil NH_4^+ -N and NO_3^- -N contents to decrease in N1 level in our result (Table 1). At the same time, biochar could also increase the relative abundance of eutrophic species, closely related to soil fast-acting nutrients, and help in regulating the retention capacity of available nutrients through microbial community to improve the yields when nutrients were sufficient or excessive. Although biochar increased pakchoi yield and nutrient uptake capacity at both N levels (Table 1), then caused a reduction of soil inorganic nitrogen nutrients at N1 but no changes of it at N2, it proved that biochar could regulate the retention capacity of available nutrients through microbial community when nutrients were sufficient or excessive. It indicated that biochar and bacterial communities can adjust the promotion strategies for plant growth based on the changes of soil nutrient environments, thereby more effectively improving pakchoi yield.

On the other hand, we found that fungal diversity also played an important role in yields, but the increase in fungal diversity has a negative effect on yields, which was inconsistent with the positive correlation between yield and fungal diversity in previous studies (Rong et al. 2018). In our results, the reduction of nutrient cycling phylum (*Chytridiomycota*) and enhancement of soil-borne plant pathogens (e.g., *Fusarium* and *Ustilago*) by biochar might be the main pathway on vegetable yield (Fig. 6) (Dai et al. 2021; Hanrahan-Tan et al. 2022). However, the pakchoi yield had not been reduced but increased by biochar especially at N2 level (Table 1); this was because the fungal communities had less impact on yield compared with bacterial communities (Fig. 5). The positive effect of biochar on yield through bacterial communities was much greater than the negative effect of the fungal communities. Moreover, we also found that the strategy of biochar in regulating vegetable yield through fungi is also different between N1 and N2. At N1, biochar increased fungal abundance which related to an effective organic matter transformer and consuming, or was able to stimulate vegetable growth hormones and plant resistance (Lay et al. 2018) to increase the vegetable yields. However, biochar could decrease the abundance of effective organic matter transformers and consuming and increase the potential of soil-borne plant pathogens at N2 level, which suggested that biochar might have a negative impact on vegetable yield by regulating the composition of fungal communities. This was different from the strategy of biochar regulating bacterial communities' structure (Figs. 1 and 2);

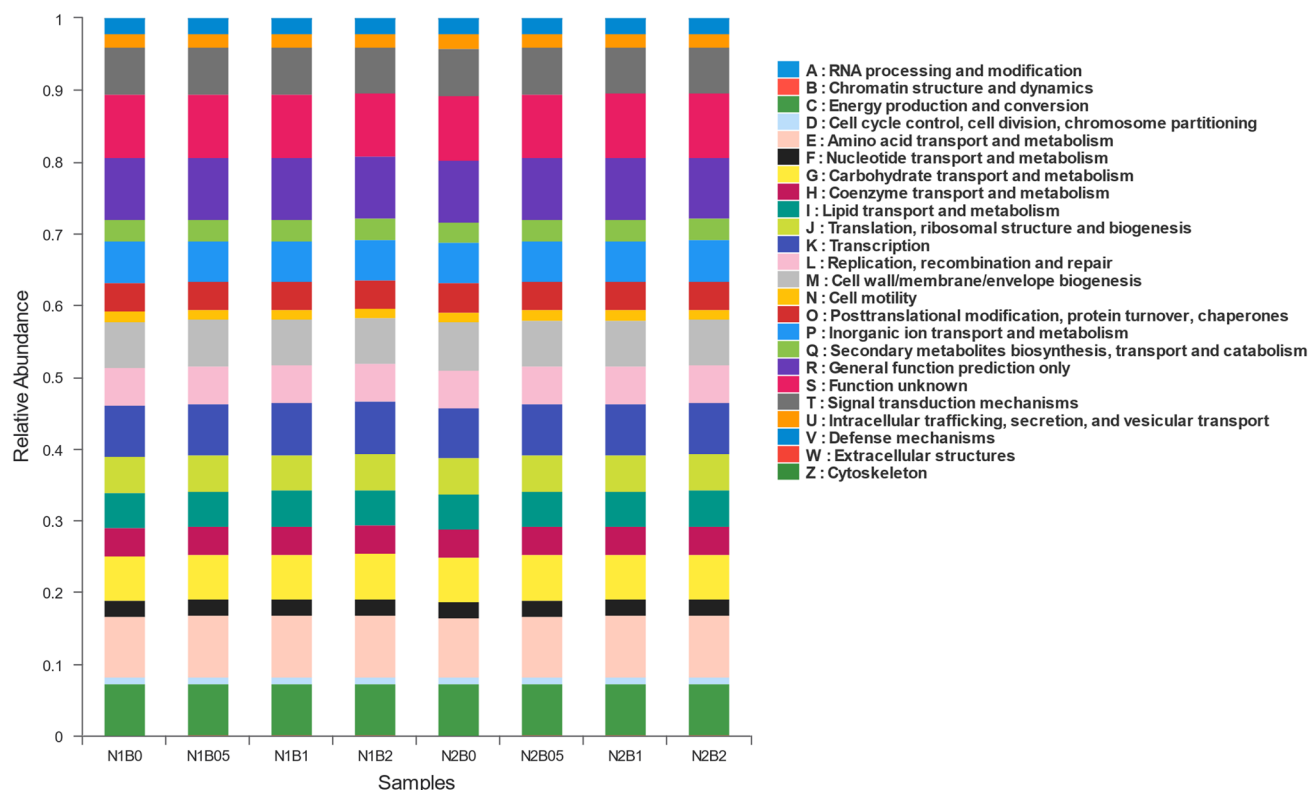


Fig. 7 Relative abundance of PICRUST inferred function of bacteria from different treatments

it was also different from many studies which showed that biochar could reduce the abundance of *Fusarium* in crop soil (Yao et al. 2017; Shen et al. 2019; Bonanomi et al. 2022). Most of these studies have conducted experiments in neutral or slightly alkaline soils; biochar further increased the soil pH then reduced the abundance of *Fusarium* in the soil (Elmer and Pignatello 2011; Zhu et al. 2021; Asif et al. 2023). However, the experimental soil pH was in the range from 5.0 to 6.0 in all treatments (Table 1). In acidic soils, biochar increased the soil pH then provided a favorable habitat for *Fusarium*. In addition, biochar could promote the growth of *Fusarium* in the soil by providing refuge from predation, maintaining optimal air and water permeability, and adsorbing antimicrobial compounds secreted by plant roots (Lehmann et al. 2011). The results showed that biochar considerably boosted pakchoi yield in both N levels; it was mainly due to the dominant niche of bacterial community and biochar which could increase the relative abundance of beneficial bacteria. However, there was a large area of acidic soil in farmland especially in southern China (Yang et al. 2022), and many vegetable fields had high nitrogen fertilizer conditions (Liang et al. 2013). Therefore, it was paid attention to increase the risk of crop yield reduction of biochar by regulating the structure and composition of fungal communities under high nitrogen fertilizer condition.

5 Conclusion

Our findings revealed that high nitrogen fertilizer significantly reduced pakchoi yield. Biochar increased pakchoi yield at both N levels, and majorly through regulated bacterial rather than fungal community. Notably, in N1 and N2, biochar influenced soil microbial regulation of vegetable yield differently. Under normal N levels, biochar demonstrated the ability to augment the relative abundance of species known for their beneficial roles in disease suppression and participation in SOM degradation. This, in turn, enhanced the stress resistance of vegetables through the conditional microbial community, leading to increased vegetable yields. In contrast, under high N levels, biochar promoted the abundance of eutrophic species, associated with the rapid cycling and retention of soil nutrients. This improvement in nutrient availability was facilitated by the microbial community, ultimately enhancing vegetable yields. However, the regulation of fungal communities by biochar is important to note, particularly in conjunction with high N fertilizer, which can potentially elevate the risk of reduced vegetable yields. Furthermore, it should be emphasized that the influence of biochar on soil microorganisms and vegetable yield is a long-term effect that necessitates further investigation.

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Data availability Data from this study are included in the article and supplementary materials.

Declarations

Conflict of interest The authors declare no competing interests.

References

- Ali I, Ullah S, He L et al (2020) Combined application of biochar and nitrogen fertilizer improves rice yield, microbial activity and N-metabolism in a pot experiment. *PeerJ* 8:e10311. <https://doi.org/10.7717/peerj.10311>
- An N, Zhang L, Liu Y et al (2022) Biochar application with reduced chemical fertilizers improves soil pore structure and rice productivity. *Chemosphere* 298:134304. <https://doi.org/10.1016/j.chemosphere.2022.134304>
- Asif M, Haider MS, Akhter A (2023) Impact of biochar on Fusarium wilt of cotton and the dynamics of soil microbial community. *Sustainability* 15:12936
- Bai Y-C, Chang Y-Y, Hussain M et al (2020) Soil chemical and microbiological properties are changed by long-term chemical fertilizers that limit ecosystem functioning. *Microorganisms* 8:694. <https://doi.org/10.3390/microorganisms8050694>
- Bai Z, Wu X, Lin J-J et al (2019) Litter-, soil- and C:N-stoichiometry-associated shifts in fungal communities along a subtropical forest succession. *Catena* 178:350–358. <https://doi.org/10.1016/j.catena.2019.03.037>
- Bellemain E, Carlsen T, Brochmann C et al (2010) ITS as an environmental DNA barcode for fungi: An in silico approach reveals potential PCR biases. *BMC Microbiol* 10:189. <https://doi.org/10.1186/1471-2180-10-189>
- Bonanomi G, Zotti M, Idbella M et al (2022) Mixtures of organic amendments and biochar promote beneficial soil microbiota and affect Fusarium oxysporum f. sp. lactucae, Rhizoctonia solani and Sclerotinia minor disease suppression. *Plant Pathol* 71:818–829. <https://doi.org/10.1111/ppa.13514>
- Dai Z, Xiong X, Zhu H et al (2021) Association of biochar properties with changes in soil bacterial, fungal and fauna communities and nutrient cycling processes. *Biochar* 3:239–254. <https://doi.org/10.1007/s42773-021-00099-x>
- Devi R, Kaur T, Kour D et al (2020) Beneficial fungal communities from different habitats and their roles in plant growth promotion and soil health. *Microbial Biosystems* 5:21–47
- Ding Y, Liu Y, Liu S et al (2016) Biochar to improve soil fertility. A Review. *Agron Sustain Dev* 36:36. <https://doi.org/10.1007/s13593-016-0372-z>
- Dong Z, Li H, Xiao J et al (2022) Soil multifunctionality of paddy field is explained by soil pH rather than microbial diversity after 8-years of repeated applications of biochar and nitrogen fertilizer. *Sci Total Environ* 853:158620. <https://doi.org/10.1016/j.scitotenv.2022.158620>
- Elmer WH, Pignatello JJ (2011) Effect of biochar amendments on mycorrhizal associations and Fusarium crown and root rot of asparagus in replant soils. *Plant Dis* 95:960–966
- Franklin RB, Mills AL (2006) Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. *Microb Ecol* 52:280–288. <https://doi.org/10.1007/s00248-006-9033-0>
- Gao M, Yang J, Liu C et al (2021) Effects of long-term biochar and biochar-based fertilizer application on brown earth soil bacterial communities. *Agr Ecosyst Environ* 309:107285. <https://doi.org/10.1016/j.agee.2020.107285>
- Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biol Biochem* 75:54–63. <https://doi.org/10.1016/j.soilbio.2014.03.023>
- Gul S, Whalen JK, Thomas BW et al (2015) Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agr Ecosyst Environ* 206:46–59. <https://doi.org/10.1016/j.agee.2015.03.015>
- Guo X, Liu H, Zhang J (2020) The role of biochar in organic waste composting and soil improvement: a review. *Waste Manage* 102:884–899. <https://doi.org/10.1016/j.wasman.2019.12.003>
- Hanrahan-Tan DG, Henderson L, Kertesz MA, Lilje O (2022) The effects of nitrogen and phosphorus on colony growth and zoospore characteristics of soil chytridiomycota. *J Fungi* 8:341. <https://doi.org/10.3390/jof8040341>
- He X, Xie H, Gao D et al (2021) Biochar and intercropping with potato–onion enhanced the growth and yield advantages of tomato by regulating the soil properties, nutrient uptake, and soil microbial community. *Front Microbiol* 12:695447. <https://doi.org/10.3389/fmicb.2021.695447>
- Hossain MZ, Bahar MM, Sarkar B et al (2020) Biochar and its importance on nutrient dynamics in soil and plant. *Biochar* 2:379–420. <https://doi.org/10.1007/s42773-020-00065-z>
- Hu X, Liu J, Wei D et al (2017) Effects of over 30-year of different fertilization regimes on fungal community compositions in the black soils of northeast China. *Agr Ecosyst Environ* 248:113–122. <https://doi.org/10.1016/j.agee.2017.07.031>
- Iqbal A, Ali I, Yuan P et al (2022) Combined application of manure and chemical fertilizers alters soil environmental variables and improves soil fungal community composition and rice grain yield. *Front Microbiol* 13:856355. <https://doi.org/10.3389/fmicb.2022.856355>
- Lay C-Y, Bell TH, Hamel C et al (2018) Canola root-associated microbiomes in the Canadian prairies. *Front Microbiol* 9:1188. <https://doi.org/10.3389/fmicb.2018.01188>
- Lehmann J, Rillig MC, Thies J et al (2011) Biochar effects on soil biota – a review. *Soil Biol Biochem* 43:1812–1836. <https://doi.org/10.1016/j.soilbio.2011.04.022>
- Liang LZ, Zhao XQ, Yi XY et al (2013) Excessive application of nitrogen and phosphorus fertilizers induces soil acidification and phosphorus enrichment during vegetable production in Yangtze River Delta, China. *Soil Use Manag* 29:161–168. <https://doi.org/10.1111/sum.12035>
- Ma G, Liu W, Li S et al (2019) Determining the optimal N input to improve grain yield and quality in winter wheat with reduced apparent N loss in the North China Plain. *Front Plant Sci* 10:181. <https://doi.org/10.3389/fpls.2019.00181>
- Novak JM, Busscher WJ, Laird DL et al (2009) Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil Sci* 174:105–112. <https://doi.org/10.1097/SS.0b013e3181981d9a>
- Qiu L, Li D, Li Z et al (2022) Biochar-induced variations in crop yield are closely associated with the abundance and diversity of keystone species. *Sci Total Environ* 827:154340. <https://doi.org/10.1016/j.scitotenv.2022.154340>
- Ramirez KS, Craine JM, Fierer N (2010) Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biol Biochem* 42:2336–2338. <https://doi.org/10.1016/j.soilbio.2010.08.032>
- Rasul M, Cho J, Shin H-S, Hur J (2022) Biochar-induced priming effects in soil via modifying the status of soil organic matter and

- microflora: A review. *Sci Total Environ* 805:150304. <https://doi.org/10.1016/j.scitotenv.2021.150304>
- Ren N, Wang Y, Ye Y et al (2020) Effects of continuous nitrogen fertilizer application on the diversity and composition of rhizosphere soil bacteria. *Front Microbiol* 11:1948. <https://doi.org/10.3389/fmicb.2020.01948>
- Rong Q, Li R, Huang S et al (2018) Soil microbial characteristics and yield response to partial substitution of chemical fertilizer with organic amendments in greenhouse vegetable production. *J Integr Agric* 17:1432–1444. [https://doi.org/10.1016/S2095-3119\(18\)61946-X](https://doi.org/10.1016/S2095-3119(18)61946-X)
- Santillán J, López-Martínez R, Aguilar-Rangel EJ et al (2021) Microbial diversity and physicochemical characteristics of tropical karst soils in the northeastern Yucatan peninsula Mexico. *Appl Soil Ecol* 165:103969. <https://doi.org/10.1016/j.apsoil.2021.103969>
- Shen Z, Xue C, Penton CR et al (2019) Suppression of banana Panama disease induced by soil microbiome reconstruction through an integrated agricultural strategy. *Soil Biol Biochem* 128:164–174. <https://doi.org/10.1016/j.soilbio.2018.10.016>
- Singh H, Northup BK, Rice CW, Prasad PVV (2022) Biochar applications influence soil physical and chemical properties, microbial diversity, and crop productivity: A meta-analysis. *Biochar* 4:8. <https://doi.org/10.1007/s42773-022-00138-1>
- Sun K, Chen H, Zhang Q et al (2020a) Influence of humic acids on fungal laccase-initiated 17 α -ethynylestradiol oligomerization: transformation kinetics and products distribution. *Chemosphere* 258:127371. <https://doi.org/10.1016/j.chemosphere.2020.127371>
- Sun X, Liang B, Wang J et al (2020b) Soil N transformation rates are not linked to fertilizer N losses in vegetable soils with high N input. *Soil Tillage Res* 202:104651
- Ti C, Luo Y, Yan X (2015) Characteristics of nitrogen balance in open-air and greenhouse vegetable cropping systems of China. *Environ Sci Pollut Res* 22:18508–18518. <https://doi.org/10.1007/s11356-015-5277-x>
- Trivedi P, Leach JE, Tringe SG et al (2020) Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* 18:607–621. <https://doi.org/10.1038/s41579-020-0412-1>
- Ullah S, Ai C, Ding W et al (2019) The response of soil fungal diversity and community composition to long-term fertilization. *Appl Soil Ecol* 140:35–41
- Ventura M, Alberti G, Panzacchi P et al (2019) Biochar mineralization and priming effect in a poplar short rotation coppice from a 3-year field experiment. *Biol Fertil Soils* 55:67–78. <https://doi.org/10.1007/s00374-018-1329-y>
- Wang C, Liu S, Zhang Y et al (2018) Bacterial communities and their predicted functions explain the sediment nitrogen changes along with submerged macrophyte restoration. *Microb Ecol* 76:625–636. <https://doi.org/10.1007/s00248-018-1166-4>
- Wang M, Xue J, Ma J et al (2020) *Streptomyces lydicus* M01 regulates soil microbial community and alleviates foliar disease caused by *Alternaria alternata* on cucumbers. *Front Microbiol* 11:942. <https://doi.org/10.3389/fmicb.2020.00942>
- Wang M, Wu J, Zhou T et al (2021) Effects of copper and florfenicol on nirS- and nirK-type denitrifier communities and related antibiotic resistance in vegetable soils. *Ecotoxicol Environ Saf* 213:112011. <https://doi.org/10.1016/j.ecoenv.2021.112011>
- Xi N, Chen D, Bahn M et al (2022) Drought soil legacy alters drivers of plant diversity-productivity relationships in oldfield systems. *Sci Adv* 8:eabn3368. <https://doi.org/10.1126/sciadv.abn3368>
- Xia H, Riaz M, Zhang M et al (2022) Biochar-N fertilizer interaction increases N utilization efficiency by modifying soil C/N component under N fertilizer deep placement modes. *Chemosphere* 286:131594. <https://doi.org/10.1016/j.chemosphere.2021.131594>
- Xu N, Tan G, Wang H, Gai X (2016) Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur J Soil Biol* 74:1–8. <https://doi.org/10.1016/j.ejsobi.2016.02.004>
- Xu H, Cai A, Wu D et al (2021) Effects of biochar application on crop productivity, soil carbon sequestration, and global warming potential controlled by biochar C: N ratio and soil pH: A global meta-analysis. *Soil Tillage Res* 213:105125. <https://doi.org/10.1016/j.still.2021.105125>
- Xu Y, Ren S, Liang Y et al (2021) Soil nutrient supply and tree species drive changes in soil microbial communities during the transformation of a multi-generation Eucalyptus plantation. *Appl Soil Ecol* 166:103991. <https://doi.org/10.1016/j.apsoil.2021.103991>
- Yang J, Wei H, Zhang J et al (2022) Land use and soil type exert strongly interactive impacts on the pH buffering capacity of acidic soils in south China. *Sustainability* 14:12891. <https://doi.org/10.3390/su141912891>
- Yang W, Li C, Wang S et al (2021) Influence of biochar and biochar-based fertilizer on yield, quality of tea and microbial community in an acid tea orchard soil. *Appl Soil Ecol* 166:104005. <https://doi.org/10.1016/j.apsoil.2021.104005>
- Yang W, Yang M, Wen H, Jiao Y (2018) Global warming potential of CH₄ uptake and N₂O emissions in saline-alkaline soils. *Atmos Environ* 191:172–180. <https://doi.org/10.1016/j.atmosenv.2018.08.017>
- Yao Q, Liu J, Yu Z et al (2017) Three years of biochar amendment alters soil physicochemical properties and fungal community composition in a black soil of northeast China. *Soil Biol Biochem* 110:56–67. <https://doi.org/10.1016/j.soilbio.2017.03.005>
- Ye Z, Li J, Wang J et al (2021) Diversity and co-occurrence network modularization of bacterial communities determine soil fertility and crop yields in arid fertigation agroecosystems. *Biol Fertil Soils* 57:809–824. <https://doi.org/10.1007/s00374-021-01571-3>
- Yu H, Ling N, Wang T et al (2019) Responses of soil biological traits and bacterial communities to nitrogen fertilization mediate maize yields across three soil types. *Soil Tillage Res* 185:61–69. <https://doi.org/10.1016/j.still.2018.08.017>
- Yun C, Yan C, Xue Y et al (2021) Effects of exogenous microbial agents on soil nutrient and microbial community composition in greenhouse-derived vegetable straw composts. *Sustainability* 13:2925. <https://doi.org/10.3390/su13052925>
- Zeng J, Liu X, Song L et al (2016) Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol Biochem* 92:41–49. <https://doi.org/10.1016/j.soilbio.2015.09.018>
- Zheng N, Yu Y, Shi W, Yao H (2019) Biochar suppresses N₂O emissions and alters microbial communities in an acidic tea soil. *Environ Sci Pollut Res* 26:35978–35987. <https://doi.org/10.1007/s11356-019-06704-8>
- Zhong L, Li G, Qing J et al (2022) Biochar can reduce N₂O production potential from rhizosphere of fertilized agricultural soils by suppressing bacterial denitrification. *Eur J Soil Biol* 109:103391. <https://doi.org/10.1016/j.ejsobi.2022.103391>
- Zhu J, Cao A, Wu J et al (2021) Effects of chloropicrin fumigation combined with biochar on soil bacterial and fungal communities and *Fusarium oxysporum*. *Ecotoxicol Environ Saf* 220:112414

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