Biochar-based fertilizer enhanced tobacco yield and quality by improving soil quality and soil microbial community

Shen Yan, Peng Wang, Xianjie Cai, Chuliang Wang, Lukas Van Zwieten, Hailong Wang, Quanyu Yin, Guoshun Liu, Tianbao Ren

PII: S2352-1864(24)00440-1
DOI: https://doi.org/10.1016/j.eti.2024.103964
Reference: ETI103964

To appear in: *Environmental Technology & Innovation*

Received date: 15 August 2024
Revised date: 12 November 2024
Accepted date: 11 December 2024

Please cite this article as: Shen Yan, Peng Wang, Xianjie Cai, Chuliang Wang, Lukas Van Zwieten, Hailong Wang, Quanyu Yin, Guoshun Liu and Tianbao Ren, Biochar-based fertilizer enhanced tobacco yield and quality by improving soil quality and soil microbial community, *Environmental Technology & Innovation,* (2024) doi:https://doi.org/10.1016/j.eti.2024.103964

Biochar-based fertilizer enhanced tobacco yield and quality by improving soil quality and soil microbial community

Shen Yan[a,b,c,1], Peng Wang[d,1], Xianjie Cai[e], Chuliang Wang[f], Lukas Van Zwieten[g,h], Hailong Wang[h,i], Quanyu Yin[a,b], Guoshun Liu[a,b,*], Tianbao Ren[a,b,*]

[a] College of Tobacco Science, Henan Agricultural University, Zhengzhou, 450002, China
[b] Henan Biochar Engineering Technology Research Center, 450002, China
[c] Staff Development Institute of CNTC, Zhengzhou, 450008, China
[d] Fujian Tobacco Monopoly Bureau, Fuzhou 350001, China
[e] Shanghai Tobacco Group Co., Ltd, Shanghai, 200082, China
[f] Guangdong Tobacco Industrial Co., Ltd, Guangzhou, 510030, China
[g] NSW Department of Primary Industries, Wollongbar, NSW 2477, Australia
[h] Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, 528000, China
[i] Guangdong Provincial Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou, 510650, China

[1] These authors contributed equally to this work and should be considered co-first authors.

*Correspondence: biochar2018@henau.edu.cn (T. Ren); liugsh1851@163.com (G. Liu)

Abstract

Biochar-based fertilizers have the potential to improve soil quality by enhancing soil microbial communities, yet their direct effects on crop yield and quality are not well understood. To address this gap, we conducted a pot-based field study to evaluate the impact of traditional chemical fertilizers alone, traditional fertilizers supplemented with biochar, and biochar-based fertilizers on soil properties and bacterial communities. We also investigated how these treatments affected tobacco (*Nicotiana tabacum*) yield and leaf aroma quality. Biochar-based fertilizers increased soil carbon (C) and nitrogen (N) pools compared to the control, leading to greater bacterial diversity and richness. Consequently, tobacco biomass increased from 449 g/pot to 517 g/pot, and leaf aroma content rose from 625 µg/g to 832 µg/g. Linear discriminant analysis and partial least squares path modeling identified 13 key bacterial phylotypes, including plant growth-promoting bacteria (PGPB) such as Burkholderiaceae, Novosphingobium, Bacillus, Chitinophagaceae, Sphingomonas, Acidobacteriaceae, Acidobacteria, and Flavobacterium, that positively influenced tobacco leaf aroma constituents. Our findings suggest that biochar and biochar-based fertilizers enhance soil bacterial communities, which in turn improve crop yield and product quality. This study highlights the potential of biochar-based fertilizers as a sustainable agricultural practice to enhance soil health and crop quality through microbial community modulation.

Keywords: Aroma; MiSeq sequencing; phylotype; soil carbon, Shannon Beta-Diversity.

Abbreviation List

Abbreviation	**Paraphrase**
AN	available nitrogen
C	carbon
C/N	carbon-nitrogen Ratio
DOC	dissolved organic carbon
EOC	easily oxidizable carbon
MBC	microbial biomass carbon
PCA	principal component analysis
RDA	redundancy analysis
TN	total nitrogen
TOC	total organic carbon

1. Introduction

Biochar is characterized by its unique physical and chemical properties, such as a large specific surface area and high porosity. It can possess inherent nutrient properties or be used as a carrier in biochar-based fertilizers to enhance nutrient use efficiency (Dong et al., 2020). An expanding body of literature shows that both biochar used in conjunction with fertilizers and biochar-based fertilizers themselves can positivelyaffect agricultural yields (Khan et al., 2024). Biochar interacts with plant roots to: 1) modify localized Eh and pH (Alkharabsheh et al., 2021), which reduces the root membrane potential and makes nutrient uptake less energy-demanding (Chew et al., 2020), and 2) alter the bacterial and fungal communities within the soil (Chew et al., 2020; Dong et al., 2020; Lu et al., 2023). Biochar can also reduce soil bulk density, and improve chemical properties, including, soil C and N contents, cation exchange capacity (CEC), and mineral nutrient availability(Yu et al., 2019; Murtaza et al., 2023). Biochars derived from manures have also been shown to improve plant available P, not only due to P contained in biochar, but also through the increased nutrient retention and liming effects (Slavich et al., 2013), consequently increasing plant yield.

However, some examples of the negative impacts of biochar on plant growth have been reported. Xiang et al. (2021) have been reviewed the biochars with a potential risk to the environment were classified according to their harmful components, surface properties, structure, and particle size, and the potential negative environmental effects of these biochars and the mechanisms inducing these negative effects..

Therefore, it is important to understand the potential limitations of biochar application to improve crop yield and soil properties under regional and site-specific conditions.Plant growth-promoting bacteria (PGPB) are associated with many plant species and they are commonly present in various environments (Hossain et al., 2023). The rhizosphere microbiome can affect the cycling of soil nutrients to make them plant-available, can produce antibiotics that inhibit disease, and can produce plant growth hormones such as indole acetic acid (IAA) and gibberellin (GAs) (Guzmán-Guzmán et al., 2024). Through affecting these plant growth-promoting factors, the soil microbiome can directly and/or indirectly affect the composition, biomass, and functioning of plant communities in ecosystems(Reed and Glick, 2023; Jalal et al., 2024). The composition and metabolic activities of the rhizosphere microbiome are directly controlled by soil properties and plant species composition (Philippot et al., 2013; Pérez-Jaramillo et al., 2017). Biochar application may positively or negatively affect the soil microbial community structure and function through altering the habitat(Seyedsadr et al., 2022; Kumar et al., 2023;). For example, Yan et al. (2019) found that applying biochar to purple soil(Pup-Orthic-Entisol) and paddy soil (Typical Stagnic Anthrosols)significantly affected the community structure of N-cycling microbes and improved the growth of tobacco (*Nicotiana tabacum*) plants (Yan et al., 2019). Similarly, Nielson suggested that biochar amendment enhanced crop yield due to the increase in the genus *Rhizobium* (Nielsen et al., 2018).

Tobacco (*Nicotiana tabacum*) is a globally traded crop. Quality (aroma) is an important index of tobacco plants that determines the market price of tobacco leaves (Zou et al., 2018). Recent studies have proposed that biochar affects the structure of soil bacteria and in turn the growth of tobacco plants and the subsequent quality of the leaf (Yan et al., 2022). However, the mechanism underlying the change in the soil bacterial community and its role in controlling tobacco aroma remains unclear.

We hypothesize that compared to chemical fertilizers, biochar-based fertilizers will (i) improve the richness and diversity of the soil through altering the soil C content and habitat, and (ii) enhance the quality of the leaf by altering the structure of the soil bacterial community. To test the hypotheses, we examined the effect of chemical fertilizer with and without biochar, and biochar-based fertilizer on

(i) tobacco quality; and (ii) the soil microbial communities. The effect of fertilizer type on the soil microbial community was studied using high-throughput sequencing using the MiSeq platform. Linear discriminant analysis effect size (LEfSe) analysis was used to select 57 key phylotypes that responded to fertilizers, and partial least squares path modeling (PLS-PM) was used to identify the key phylotypes that most effectively improved tobacco aroma. The relationship between key phylotypes and different tobacco aromas was also analyzed through redundancy analysis (RDA).

2 Materials and methods

2.1 Study site

The experiment was carried out in Xinfeng, Jiangxi Province, China (25°27′11. 71″ N, 114°51′54. 25″ E) in 2017, which has a subtropical humid monsoon climate. The site features an average annual sunshine duration of 1,473–2,077 h, an average annual temperature of 18.0–19.7°C, a frost-free period of 250 d, and an average annual rainfall of 1410– 1762 mm. The soil type is classified as a hydroagric Stagnic Anthrosol(Gong, 2001). The soil total C was 16.6 mg/g and N was 2.1 mg/g, and the soil pH was 5.59.

2.2. Design of the experiment

The pot experiment was carried out in the tobacco open field. Paddy soil (tobacco-rice rotation soil, 25 kg) from the 0–20 cm topsoil layer was placed in plastic pots (40 × 30 × 22 cm; diameter at top × depth × diameter at bottom). Four treatments were implemented: (a) control with no fertilizer (**CK**); (b) chemical fertilizer (**NPK**) (conventional dosage for this region: nitrogen 7 g and N:P: K = 1:0.9:2.8, which is similar to the ratio of local farmingplan); (c) biochar + chemical fertilizer (**BC**); (d) biochar-based fertilizer (biochar as the fertilizer carrier; carbon content ≥30%, N:P:K =2:3:1.5) + chemical fertilizer (**BBF**). The application ratio of N:P:K (1:0.9:2.8) was the same as per regional conventional management, and was normalized across NPK, BC and BBF; consequently, any changes observed among treatments could not be attributed to differences in the total N,P, and K dose (Table S1). Seedlings of tobacco cultivar Yunyan 87 were transplanted into pots at a density of one seedling per pot; 25 replicates of each treatment were used (100 pots in total), and pots were placed at the same plant/row space as those in local fields (50 cm × 120 cm). All pots were arranged using a randomized design receiving the same water input from either irrigation or rainfall. Biochar was provided by Sanli New Energy Co., Ltd. (Shangqiu, China), and was made from rice straw heated at 350°C for 30 min under oxygen-limited conditions in a continuous carbonization furnace. The properties of biochar are provided in Table S2.

2.3 Sample selection and collection

Five representative tobacco plants were collected from each treatment 60 days after planting, and for each pot, rhizosphere soil samples (i.e., surface soil was shaken off the plants, and the soil that adhered to the surface of the roots was considered the rhizosphere soil) were collected (Yan et al., 2020). At the same time, tobacco leaves were harvested for curing (Smalla et al., 2001). Each soil sample (5 g) was frozen (-20 °C) prior to 16S rDNA analysis. Another aliquot of soil (200 g) was screened at field moisture using a 10-mesh sieve (2 mm), and subsequently refrigerated at 4°C (without air drying), for microbial biomass carbon (MBC) analysis (Jenkinson and Powlson, 1976). The remaining soil (200 g) was screened using a 20-mesh sieve (0.85 mm) (air-dried) and used for the chemical analyses. Tobacco samples (including leaves, stems, and roots) were heated at 105 °C for 15 min to inactivate the enzymes, then oven-dried at 65 °Cto a constant weight.Cured middle leaves (12th leaf from bottom to top, 75 days, commercially harvested) were collected for the assessment of aroma characteristics (Voisard et al., 1989).

2.4 Samples analyses

2.4.1 Chemical analysis of soil samples

Total soil C and N were quantified using a C/N elemental analyzer (Vario MAX; Elementar, Langenselbold, Germany). Dissolved organic C (DOC)(Jenkinson and Powlson, 1976) was determined using a Vario TOC Cube (Elementar, Langenselbold, Germany). Easily oxidized C (EOC) was quantified using the $KMnO_4$ (333 mM) oxidation method as previously described (Blair et al., 1995). Soil available N (AN) was quantified after Lu(Lu, 2000). Soil NH_4^+ and NO_3^- concentrations were determined using a continuous-flow auto-analyzer (AutoAnalyser3: Seal Analytical, Norderstedt, Germany).

2.4.2 Metagenomic analysis

2.4.2.1 DNA Extraction and PCR Amplification

Based on the manufacturer's protocols, microbial DNA of frozen soil samples (0.5g)was extracted using the E.Z.N.A. Soil DNA Kit (Omega Biotek, GA, USA). The V3–V4 region of the bacterial 16S ribosomal RNA gene was amplified with PCR amplification and yielded approximately 465 bp, using primers 806R (5′-GGACTACNNGGGTATCTAAT-3′) and341F (5′-CCTAYGGGRBGCASCAG-3′).

2.4.2.2 Illumina MiSeq sequencing analyses

Based on the manufacturer's instructions, 2% agarose gel was used to extract amplicons and AxyPrep DNA Gel Extraction Kit was used for subsequent purification. Purified samples were then quantified using QuantiFluor-ST (Promega Corporation, USA). Operational biological taxonomic units (OTU) from tags were obtained using Cluster analysis. Subsequent annotation of tags and OTUs was conducted using the Ribosomal Database Project classifier.

2.4.2.3 Bioinformatics

Clean reads of the Paired ends were merged into the original tags with a minimum overlap (10 bp) and a mismatch error rate (2%) using the fast length adjustment of short reads (FLASH, v. 1.2.11) (Magoč& Salzberg, 2011). Background sequences from raw tags were ignored using the quantitative insights into microbial ecology (QIIME) II pipeline (Kuczynski et al., 2012) under specific filtration conditions to collect high-quality clean tags. A reference-based chimera checking of clean tags was used by the UCHIME algorithm v. 4.1 (Edgar et al., 2011). The effective tags were clustered into OTUs of ≥ 97% similarity using the UPARSE pipeline (Vania et al., 2017). Key and vital sequences were classified into organisms by a naive Bayesian model based on the Greengenes database (DeSantis et al., 2006). Functional groups of microbiological OTUs were speculated by PICRUSt (Langille et al., 2013).

2.4.3 Tobacco aroma

Tobacco aroma was analyzed using liquid chromatography–gas chromatography coupled with mass spectrometry (LC-GC-MS), as previously reported (Qi et al., 2014).

2.5 Statistical analysis

A heatmap was generated using R software (Version: 3. 5. 2), and principal component analysis (PCA), redundancy analysis (RDA), boxplot analysis, least squares path modeling (PLS-PM), and multiple comparisons (LSD) were performed. Furthermore, Galaxy (https://huttenhower.sph.harvard.edu/galaxy/) was used for linear discriminant analysis effect size (LEfSe) analysis using 3.0 distance as the threshold for the logarithmic LDA score of discriminative features.

3 Results

3. 1 Addition of biochar effectively increases the soil carbon and nitrogen content

We first explored the effects of biochar addition on soil carbon and nitrogen content. Compared to the control (CK; no fertilizer), the soil total organic C (TOC) were significantly increased in biochars (12.9% in BC and 16.3% in BBF) , but NPK did not change the TOC compared to CK (Table 1). The BBF resulted in significantly greater easily oxidizable carbon (EOC, 6%, P = 0.0037), compared to the other treatments; whereas both BC and BBF resulted in higher soil dissolved organic carbon (DOC, 12%, P = 0.0018) and microbial biomass carbon (MBC) compared to either NPK or CK. The total nitrogen (TN), available nitrogen (AN), and mineral N content of soil as in the order BC, BBF> NPK ≥ CK. These results indicate that the addition of biochar effectively increases soil carbon and nitrogen content compared with fertilizer.

3. 2 Addition of biochar effectively increases the dry weight and aroma of tobacco

Compared to the control, fertilizers (NPK, BC, and BBF) significantly increased dry weight of root and stem (Fig. 1a, b). For the leaf yield, BBF has the strongest yield increase effect 15.19 %, whereas NPK only increases the yield by 8.83 %. In addition to yield increasing, biochar addition also improved the aroma content of tobacco leaves. Compared to CK, the BC and BBF significantly increased the total aroma content of leaves by 40.81 % and 48.86 %.However, there was no significant difference between BC and NPK additions of the total aroma.

3. 3 Changes in soil bacterial community structure in response to fertilizer treatments

To explore the effects of fertilizers on soil microbial communities, we conducted metagenomic sequencing of tobacco rhizosphere soils with different treatment. High-throughput MiSeq sequencing detected 63, 301 to 87, 152 tags in 20 soil samples across the four treatments. A total of 6,059 to 8,274 operational taxonomic units (OTUs) were obtained through cluster analysis at a distance of 0.03. Tags and OTUs were accurately labeled.

The Venn plot shows the number of OTUs that are unique to each sample, or shared among samples, and displays overlaps in OTUs among samples (Fig. 2C). A total of 12,860 OTUs were detected under CK, 16,422 under NPK, 16,970 under BC, and 17,447 under BBF. Bacterial richness

(Chao1) and diversity (Shannon) changed in response to the different fertilizer treatments. For community richness, Chao1 increased by 17% (*P*= 0.0061) for NPK, 20% (*P*= 0.0013) for BC, and 22% (*P*= 0.0018) for BBF (Fig. 2A). The same trend was observed for bacterial diversity with the Shannon diversity being significantly improved under NPK (3. 3%, *P*= 0. 017), BC (4. 3%, *P*= 0. 0011), and BBF (4.0%, *P*= 0. 0031) (Fig. 2B) compared to the control. However, no difference was detected among the three fertilizer treatments for either bacterial richness or diversity. Thus, fertilizer addition rather than fertilizer type regulated bacterial richness and diversity. Beta diversity among groups was analyzed using PCoA (Fig. 2D) and showed three distinct groups belonging to BC and BBF, NPK and CK. Thus, the tobacco rhizosphere microbiota changed significantly in response to treatment. However, no significant difference was detected between BC and BBF.

3. 4 Key phylotypes in response to fertilizer treatments

To identify the specific bacterial taxa that responded to the treatments, LEfSe analysis was performed(Fig. 3A). A total of 57 OTUs were identified as the key phylotypes differing among treatments; specifically, 9 (CK), 16 (NPK), 16 (BC), and 16 (BBF) key phylotypes distinguished these treatment groups (Table S2). Taxonomic data on the 57 key phylotypes are shown in Fig. 3B. Specifically, there were six families, including Acidobacteriaceae, Micrococcaceae, Chitinophagaceae, Burkholderiaceae, Oxalobacteraceae, and Nitrosomonadaceae, and there were seven genera, including *Marmoricola*, *Bacillus*, *Novosphingobium*, *Sphingobium*, *Burkholderia*, *Paraburkholderia*, and *Ralstoni*a. *Pseudomonas* increased significantly after BBF, while six genera (including *Flavisolibacter*, *Chitinophaga*, *Flavobacterium*, *Nitrospira*, *H16*, and *Sphingomonas*) improved following the amendment with BC.

Based on the relative abundance of the 57 OTUs, PCA (Fig. 4A) and heatmap (Fig. 4B) analyses were performed. All samples were grouped into four discrete clusters. The heatmap cluster confirmed that BC and BBF treatments clustered together in one group, while CK and NPK treatments clustered into another group. In turn, the PCA plot showed that the four treatment groups formed four discrete clusters, and hierarchical clustering confirmed this result. Thus, the 57 phylotypes responded to treatments, and could be used as biomarkers for separating the soil microbial profiles.

3. 5 Microorganisms key phylotypes affected the aroma content and composition of tobacco

The correlation coefficients between tobacco aroma, tobacco yield, key phylotypes, and the soil C and N pool are described in Figure. 5A. Key phylotypes had no significant effect on tobacco growth, whereas the soil N pool was significantly and positively correlated with tobacco growth (*P*= 0. 0077). Thus, soil N content was the key factor affecting plant growth. No significant correlation was observed between tobacco aroma and tobacco growth. Key phylotypes exhibited a significant positive correlation with tobacco aroma (*P*= 0.00018). Soil C and N indices had a significant positive effect on key phylotypes (*P*= 0.0012; *P*= 0.0029, respectively), but showed no significant influence on tobacco aroma. Thus, the changes in soil bacterial community structure directly enhanced tobacco aroma, while soil C and N indirectly affected tobacco leaf aroma.

Redundancy analysis showed the correlation between key phylotypes and tobacco aroma (Fig. 5C). We examined the significance of the 57 OTUs, 13 of which significantly correlated with tobacco aroma (Table S3); OTU092 （Gaiellales） and OTU235 （Gemmatimonadaceae） were higher in CK and NPK, had the smallest effect on Megastigmatrienone, geranylacetone, and β-damascenone, and was negatively correlated with dihydroactinolide, solanone, and neophytadiene. In contrast, OTU414 (Burkholderiaceae), OTU158 (*Ralstonia*), OTU645 (*Novosphingobium*), OTU370 (*Bacillus*), and OTU079 (Chitinophagaceae) showed a positive correlation with Megastigmatrienone and geranylacetone; while OTU134 (*Sphingomonas*), OTU104 (*Bacillus*), OTU114 (Acidobacteriaceae), and OTU118 (Acidobacteria) showed a positive correlation with dihydroactinolide, solanone, and neophytadiene; in turn, OTU83 (*Flavobacterium*) and OTU333 (Acidobacteriaceae) showed a positive correlation with β-damascenone.

4 Discussion

4. 1 Biochar increased soil carbon content

This study evaluated the effects of inorganic fertilizer (NPK), biochar + NPK fertilizer, and a biochar-based compound fertilizer + NPK fertilizer on soil chemical properties and the soil bacterial community, and how these, in turn, influenced both the productivity and quality of tobacco. Chemical fertilizers have been shown to increase the total soil C content over the longer term, mainly resulting from the processing of C from plant material (Tang et al., 2020). In contrast, rapid changes to the soil C content have been observed with biochar amendment, where both the stable C content from the biochar directly increases soil C content, as well as further stabilizing rhizodeposits increases the soil C content (Dong et al., 2020; Weng et al., 2020; Ding et al., 2023). While biochar and BBF increased soil C content, BBF also significantly increased the labile organic-carbon content, as assessed using EOC and DOC, which represent more sensitive indicators of agricultural management-induced changes, compared to TOC (Liu et al., 2018). The EOC and DOC have also been shown to besensitive indicators of the effects of land-use changes on soil quality (Yang et al., 2005). The increase in labile C content supported the findings of Zhou et al. (Zhou et al., 2019b) who reported that wood biochar-based fertilizer increased labile C by 60%, compared with chemical fertilizer, in a pot experiment using black locust plants.

4. 2 Biochar improve the yield and total aroma of tobacco

Treatment influenced tobacco biomass production where plant dry weight of stem, leaf, and root were significantly higher under BC and BBF than under NPK, despite the matched N rate. This result supported a previous study that showed that straw biochar application at 7.2 t/ha increased tobacco yield and N use efficiency in paddy soils under aerobic conditions (Yan et al., 2019). This finding was attributed to the enhancement of the N pool and the increase in the pH of acidic soil. Importantly, results have shown that BC and BBF improved tobacco quality by increasing aroma-related constituents. Previous reports have also demonstrated similar findings that aroma-related content increased by nearly 17% (Xiao et al., 2015). Similarly, Huanhuan et al., (2017) showed that rice straw biochar application at 1.8 t/ha to black soil caused a 2-fold increase in solanone content in tobacco leaves.

4. 3 Biochar increased the abundance of soil bacterial community

All treatments significantly improved bacterial richness and diversity, and led to changes in the structure of the soil bacterial community compared to the control. However, BC/BBF and NPK fertilizers had different effects on the community structure of bacteria. We showed that NPK improved the richness and diversity of the bacterial community. This is in contrast to the data presented by Zhang et al.(2017) that nutrient sources did not affect the bacterial Operational Taxonomic Unit (OTU) richness or Shannon diversity index in alkaline soil. We are not sure if the difference in this result is related to the slight increase in soil pH with the addition of BC and BBF. Compared to chemical fertilizer alone, BC and BBF addition caused changes in the abundance and community structure, consistent with findings from increased soil pH, which can be induced by biochar or BBF (Zhang et al., 2017; Zhou et al., 2019a). The application of NPK plus BC and NPK plus BBF also had positive impacts on tobacco yield, possibly due tothe additional C and nutrient cycling as a result of greater DOC or EOC in soils. Previous studies have shown a positive correlation between plant biomass and soil organic matter increase, which is consistent with our results of increasing plant biomass after adding biochar. Therefore, it may also be due to changes in DOC and EOC(Tian et al., 2013). Treatments were clustered into three groups when using β-diversity, and the community structure of microbes was altered by the treatments. Interestingly, bacteria exhibited a similar response to soil amendment under BC and BBF, with biochar being the main factor influencing the abundance and community structure of soil microbes.

4. 4 Key phylotypes of soil in different fertilizer treatments

To evaluate microbial response to different fertilizer treatments, we performed LEfSe analysis. As a result, 57 key phylotypes were identified. Plant growth-promoting bacteria (PGPB) was significantly enhanced by 1) BBF, including *Bacillus, Novosphingobium, Pseudomonas*, Acidobacteriaceae, Micrococcaceae, Chitinophagaceae, and Burkholderiaceae. and 2) BC alone enhanced *Flavobacterium* and Sphingomonas. *Bacillus* and *Pseudomonas* have been reported to promote the growth of tobacco plants; indeed, these two species are used as biofertilizers, directly enhancing plant growth by synthesizing plant growth hormones and metabolites (Sivasakthi et al., 2014). *Sphingomonas* (Khan et al., 2014), Micrococcaceae (Hong et al., 2016), *Flavobacterium* (Soltani et al., 2010), Chitinophagaceae (Madhaiyan et al., 2015), and *Novosphingobium* (Zhang et al., 2016; Krishnan et al., 2017) are also PGPB that produce GAs and IAA, and promote root growth. The abundance of other bacteria (including *Acidobacteriaceae, Burkholderiaceae, Chitinophaga, Sphingobium, Nitrospira*, and *Nitrosomonadaceae*) also increased under BC and BBF treatments, thus contributing to soil nutrient cycling and the promotion of tobacco plant growth.

4. 5 Key phylotypes associated with specific aroma components

We found that some microorganisms are closely related to specific aroma components of tobacco. For example,the phyla Burkholderiaceae, *Novosphingobium*, *Bacillus*, and Chitinophagaceae showed a positive correlation with Megastigmatrienone and geranylacetone. *Sphingomonas*, *Bacillus*, Acidobacteriaceae, and Acidobacteria showed a positive correlation with dihydroactinolide, solanone, and neophytadiene. The phyla *Flavobacterium* and Acidobacteriaceae showed a positive correlation with β-damascenone. There is limited research showing that bacteria can alter the metabolic profiles of tobacco and in turn the quality of products. For example, Subhashini (Subhashini, 2016) found that potassium-mobilizing bacteria had a positive effect on tobacco quality as determined by the composition of reducing sugars of tobacco leaves. In our research, we provide evidence that PGPB helped to improve the aroma-related content of tobacco leaves.

4. 6 The model of biochar effect on yield and aroma quality of tobacco

As some factors (e.g., soil bacteria, soil C, and N) directly and indirectly affected the aroma of tobacco, a PLS-PM model was constructed to explore these effects. Although soil C and N are generally believed to be the main variables controlling tobacco aroma-related content, the PLS-PM model showed that soil bacteria were the most important factors. Improving the C and N content following BBF directly affected PGPB growth by adding C sources and nutrients. Thus, soil C and N had an indirect effect on tobacco aroma by affecting PGPB, which allowed plants to produce GAs and IAA. In addition, microbes induced root growth (Hong et al., 2016; Khan et al., 2014; Wanees et al., 2018), which promoted the secretion of the secondary metabolites of tobacco, such as nicotine and terpenoids. Soil bacteria and soil C content had no significant effect on plant dry weight; however, N was a key factor affecting plant growth, consistent with previous research (Delibacak et al., 2014). In tobacco production, equal emphasis is placed on yield and quality. Farmers should focus on enhancing soil fertility and soil bacterial community structure to improve tobacco yield and quality. Our results demonstrated that biochar-based fertilizer effectively improved tobacco aroma-related content by increasing the soil C and N content, and promoting the growth of beneficial microorganisms.

5 Conclusions

This study demonstrates that biochar or biochar-based fertilizers improved soil properties, the soil bacterial community structure, and the abundance of plant growth-promoting bacteria (PGPB), contributing to enhancement in crop quality and quantity. The enhancement of tobacco aroma components can be attributed to the promotion of some key microorganism phylotypes. The objective OF this study provided a promising methods for improving the quality of crops, and sustainable agricultural production

Acknowledgments: The authors thank Zhengyang Niu and Qingqing Guo for their technical assistance. The authors would also like to thank the Henan Province Science and Technology Research Project (2020GGJS047);Guangdong Zhongyan Industry Co., Ltd. Science and Technology Research Project (202244000340014014); the project Research on cultivation technology of soil carbon pool undertobacco-rice rotation mode in Fujian (2022350000240060). Shanghai Tobacco Group Technology Research and Development Project(2022310000140536) the Key Scientific and Technological Project of Hanzhong Tobacco Company (2021HZ05), and Guangdong Foundation for Program of Science and Technology Research (2023B1212060044).

Declarations of interest

The authors declare no conflict of interest.

References

Alkharabsheh HM, Seleiman MF, Battaglia ML, Shami A, Jalal RS, Alhammad BA, et al. Biochar and Its Broad Impacts in Soil Quality and Fertility, Nutrient Leaching and Crop Productivity: A Review. Agronomy-Basel 2021; 11.

Blair GJ, Lefroy RDB, Lise L. Soil Carbon Fractions Based on Their Degree of Oxidation, and the Development of a Carbon Management Index for Agricultural Systems. Australian Journal of Agricultural Research 1995; 46: 1459-1466.

Chew J, Zhu L, Nielsen S, Graber E, Mitchell DR, Horvat J, et al. Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice. Science of the Total Environment 2020; 713: 136431.

Delibacak S, Ongun AR, Ekren S. Influence of soil properties on yield and quality of tobacco plant in Akhisar region of Turkey. Eurasian Journal of Soil Science 2014; 3: 286-292.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 2006; 72: 5069-5072.

Ding X, Li G, Zhao X, Lin Q, Wang X. Biochar application significantly increases soil organic carbon under conservation tillage: an 11-year field experiment. Biochar 2023; 5: 1-14.

Dong D, Wang C, Van Zwieten L, Wang H, Jiang P, Zhou M, et al. An effective biochar-based slow-release fertilizer for reducing nitrogen loss in paddy fields. Journal of Soils and Sediments 2019: 1-14.

Dong D, Wang C, Van Zwieten L, Wang HL, Jiang PK, Zhou MM, et al. An effective biochar-based slow-release fertilizer for reducing nitrogen loss in paddy fields. Journal of Soils and Sediments 2020; 20: 3027-3040.

Edgar, Robert, C., Haas, Brian, J., et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011.

Gong Z. Chinese soil taxonomy: Science press, 2001.

Hallama M, Pekrun C, Lambers H, Kandeler E. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant and Soil 2019; 434: 7-45.

Hong SH, Ham SY, Kim JS, Kim IS, Lee EY. Application of sodium polyacrylate and plant growth-promoting bacterium, Micrococcaceae HW-2, on the growth of plants cultivated in the rooftop. International Biodeterioration & Biodegradation 2016; 113: 297-303.

Jeffery S, Verheijen FG, van der Velde M, Bastos AC. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, ecosystems & environment 2011; 144: 175-187.

Jenkinson D, Powlson DS. The effects of biocidal treatments on metabolism in soil—V: A method for measuring soil biomass. Soil biology and Biochemistry 1976; 8: 209-213.

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, et al. Bacterial Endophyte Sphingomonas sp LK11 Produces Gibberellins and IAA and Promotes Tomato Plant Growth. Journal of Microbiology 2014; 52: 689-695.

Kookana RS, Sarmah AK, Van Zwieten L, Krull E, Singh B. Biochar application to soil: agronomic and environmental benefits and unintended consequences. Advances in agronomy. 112. Elsevier, 2011, pp. 103-143.

Krishnan R, Menon RR, Likhitha, Busse HJ, Tanaka N, Krishnamurthi S, et al. Novosphingobium pokkalii sp nov, a novel rhizosphere-associated bacterium with plant beneficial properties isolated from saline-tolerant pokkali rice. Research in Microbiology 2017; 168: 113-121.

Kuczynski J, Stombaugh J, Walters WA, Gonzǿlez A, Caporaso JG, Knight R. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Current protocols in microbiology 2012; 27: 1E. 5.1-1E. 5.20.

Kumar A, Bhattacharya T, Shaikh WA, Roy A, Chakraborty S, Vithanage M, et al. Multifaceted applications of biochar in environmental management: a bibliometric profile. Biochar 2023; 5: 11.

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology 2013; 31: 814.

Liu X, Rashti MR, Dougall A, Esfandbod M, Van Zwieten L, Chen C. Subsoil application of compost improved sugarcane yield through enhanced supply and cycling of soil labile organic carbon and nitrogen in an acidic soil at tropical Australia. Soil and Tillage Research 2018; 180: 73-81.

Lu R. Soil agro-chemical analyses. Chinese.). Agric. Tech. Press, Beijing 2000.

Ling Xiang, Shaoheng Liu, Shujing Ye, Hailan Yang, Biao Song, Fanzhi Qin, Maocai Shen, Chang Tan, Guangming Zeng, Xiaofei Tan. Potential hazards of biochar: The negative environmental impacts of biochar applications. Journal of Hazardous Materials,420,2021,126611.Madhaiyan M, Poonguzhali S, Senthilkumar M, Pragatheswari D, Lee JS, Lee KC. Arachidicoccus rhizosphaerae gen. nov., sp nov., a plant-growth-promoting bacterium in the family Chitinophagaceae isolated from rhizosphere soil. International Journal of Systematic and Evolutionary Microbiology 2015; 65: 578-586.

Md Amzad Hossain, Md Summon Hossain, Mahmuda Akter. Challenges faced by plant growth-promoting bacteria in field-level applications and suggestions to overcome the barriers. Physiological and Molecular Plant Pathology,126,2023,102029,

Nielsen S, Joseph S, Ye J, Chia C, Munroe P, van Zwieten L, et al. Crop-season and residual effects of sequentially applied mineral enhanced biochar and N fertiliser on crop yield, soil chemistry and microbial communities. Agriculture Ecosystems & Environment 2018; 255: 52-61.

Palansooriya KN, Wong JTF, Hashimoto Y, Huang L, Rinklebe J, Chang SX, et al. Response of microbial communities to biochar-amended soils: a critical review. Biochar 2019; 1: 3-22.

Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LF, de Hollander M, Garcia AA, et al. Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. The ISME journal 2017; 11: 2244-2257.

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 2013; 11: 789-799.

Qi D, Fei T, Sha Y, Wang L, Li G, Wu D, et al. A novel fully automated on-line coupled liquid chromatography – gas chromatography technique used for the determination of organochlorine pesticide residues in tobacco and tobacco products. Journal of Chromatography A 2014; 1374: 273-277.

Shi RY, Hong ZN, Li JY, Jiang J, Abdulaha-Al Baquy M, Xu RK, et al. Mechanisms for Increasing the pH Buffering Capacity of an Acidic Ultisol by Crop Residue-Derived Biochars. Journal of Agricultural and Food Chemistry 2017; 65: 8111-8119.

Sivasakthi S, Usharani G, Saranraj P. Biocontrol potentiality of plant growth promoting bacteria (PGPR)-Pseudomonas fluorescens and Bacillus subtilis: A review. African journal of agricultural research 2014; 9: 1265-1277.

Slavich P, Sinclair K, Morris S, Kimber S, Downie A, Van Zwieten L. Contrasting effects of manure and green waste biochars on the properties of an acidic ferralsol and productivity of a subtropical pasture. Plant and Soil 2013; 366: 213-227.

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, et al. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and environmental microbiology 2001; 67: 4742-4751.

Soltani A-A, Khavazi K, Asadi-Rahmani H, Omidvari M, Dahaji PA, Mirhoseyni H. Plant growth promoting characteristics in some Flavobacterium spp. isolated from soils of Iran. Journal of Agricultural Science 2010; 2: 106.

Subhashini D. Effect of NPK fertilizers and co-inoculation with phosphate-solubilizing arbuscular mycorrhizal fungus and potassium-mobilizing bacteria on growth, yield, nutrient acquisition, and quality of tobacco (Nicotiana tabacum L.). Communications in Soil Science and Plant Analysis 2016; 47: 328-337.

Tian J, Dippold M, Pausch J, Blagodatskaya E, Fan MS, Li XL, et al. Microbial response to rhizodeposition depending on water regimes in paddy soils. Soil Biology & Biochemistry 2013; 65: 195-203.

Vania C, Zhang X, Lopez A. UParse: the Edinburgh system for the CoNLL 2017 UD shared task. Proceedings of the CoNLL 2017 Shared Task: Multilingual Parsing from Raw Text to Universal Dependencies, 2017, pp. 100-110.

Voisard C, Keel C, Haas D, Dѐfago G. Cyanide production by Pseudomonas fluorescens helps suppress black root rot of tobacco under gnotobiotic conditions. The EMBO Journal 1989; 8: 351-358.

Wanees AE, Zaslow SJ, Potter SJ, Hsieh BP, Boss BL, Izquierdo JA. Draft Genome Sequence of the Plant Growth-Promoting Sphingobium sp. Strain AEW4, Isolated from the Rhizosphere of the Beachgrass Ammophila breviligulata. Microbiology Resource Announcements 2018; 6.

Weng Z, Liu X, Eldridge S, Wang H, Rose T, Rose M, et al. Priming of soil organic carbon induced by sugarcane residues and its biochar control the source of nitrogen for plant uptake: A dual 13C and 15N isotope three-source-partitioning study. Soil Biology and Biochemistry 2020: 107792.

Xiao Z, Xiao J, Li L, Zhang W, Li J, Lv P, et al. Effects of different biochar application rates on neutral aroma components and smoking quality of flue-cured tobacco. Acta Agriculturae Jiangxi 2015; 27: 69-73.

Yan S, Niu Z, Yan H, Zhang A, Liu G. Influence of Soil Organic Carbon on the Aroma of Tobacco Leaves and the Structure of Microbial Communities. Current Microbiology 2020: 1-12.

Yan S, Niu ZY, Yan HT, Yun F, Peng GX, Yang YF, et al. Biochar application significantly affects the N pool and microbial community structure in purple and paddy soils. Peerj 2019; 7.

Yang CM, Yang LZ, Zhu OY. Organic carbon and its fractions in paddy soil as affected by different nutrient and water regimes. Geoderma 2005; 124: 133-142.

Yu HW, Zou WX, Chen JJ, Chen H, Yu ZB, Huang J, et al. Biochar amendment improves crop production in problem soils: A review. Journal of Environmental Management 2019; 232: 8-21.

Zhang L, Gao JS, Kim SG, Zhang CW, Jiang JQ, Ma XT, et al. Novosphingobium oryzae sp nov., a potential plant-promoting endophytic bacterium isolated from rice roots. International Journal of Systematic and Evolutionary Microbiology 2016; 66: 302-307.

Zhang Y, Shen H, He X, Thomas BW, Lupwayi NZ, Hao X, et al. Fertilization shapes bacterial community structure by alteration of soil pH. Frontiers in Microbiology 2017; 8: 1325.

Zhou Z, Gao T, Van Zwieten L, Zhu Q, Yan T, Xue J, et al. Soil Microbial Community Structure Shifts Induced by Biochar and Biochar‐Based Fertilizer Amendment to Karst Calcareous Soil. Soil Science Society of America Journal 2019a; 83: 398-408.

Zhou ZD, Gao T, Van Zwieten L, Zhu Q, Yan TT, Xue JH, et al. Soil Microbial Community Structure Shifts Induced by Biochar and Biochar-Based Fertilizer Amendment to Karst Calcareous Soil. Soil Science Society of America Journal 2019b; 83: 398-408.

Zou CM, Li Y, Huang W, Zhao GK, Pu GR, Su JE, et al. Rotation and manure amendment increase soil macro-aggregates and associated carbon and nitrogen stocks in flue-cured tobacco production. Geoderma 2018; 325: 49-58.

Tabassum B, Khan A, Tariq M, Ramzan M, Khan MSI, Shahid N, et al. Bottlenecks in commercialisation and future prospects of PGPR. Applied Soil Ecology 2017; 121: 102-117.

Tang HM, Li C, Xiao XP, Pan XC, Cheng KK, Shi LH, et al. Effects of long-term fertiliser regime on soil organic carbon and its labile fractions under double cropping rice system of southern China. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 2020; 70: 409-418.

Yan S, Ren TB, Mahari WAW, Feng HL, Xu CS, Yun F, et al. Soil carbon supplementation: Improvement of root-surrounding soil bacterial communities, sugar and starch content in tobacco (N. tabacum). Science of the Total Environment 2022; 802.

Tables

Table 1. Soil properties following amendment with fertilizers.

	TOC (%)	DOC (mg/kg)	EOC (g/kg)	MBC (mg/kg)	TN (%)	AN (mg/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	pH
CK	1. 47b	116 c	4. 06b	248b	0. 18c	120. 5b	6. 72c	82. 3b	5. 56b
NPK	1. 46b	127c	4. 23b	254b	0. 20b	125. 1b	8. 73b	84. 8b	5. 43b
BC	1. 66a	148b	4. 29b	273a	0. 21a	161. 7a	10. 5a	97. 0a	6. 01a
BBF	1. 71a	168a	4. 57a	268a	0. 21a	166.0a	10. 6a	100a	5. 87ab

Different lowercase letters within the same column indicate significant differences among treatments at the level of $p < 0.05$.

[b] TOC, total organic carbon; DOC, dissolved organic carbon; EOC, Easily oxidized carbon; MBC, microbial biomass carbon; TN, total nitrogen; AN, available nitrogen; CK, control with no fertilizer; NPK, chemical fertilizer; BC, biochar + chemical fertilizer; BBF, biochar-based fertilizers+ chemical fertilizer.

Figures

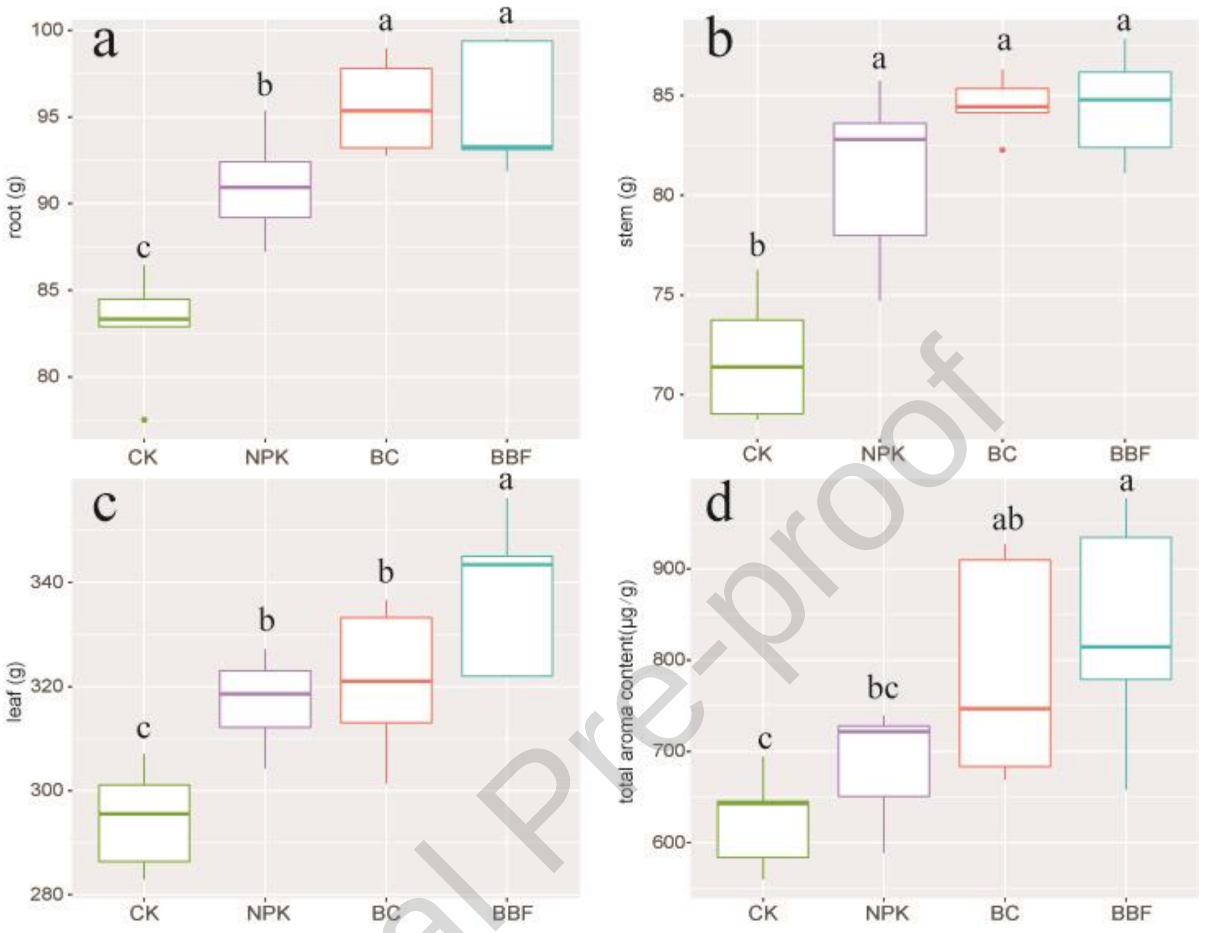


Fig. 1. *Addition of biochar effectively increases the dry weight and aroma of tobacco* (A)Dry weight of root; (B) Dry weight of stem; (C) Dry weight of leaf; (D) aroma related content of leaf. N=5. Different lowercase letters indicate significant differences among treatments at the level of $p < 0.05$,the lines in the box are the mean values. CK, control with no fertilizer; NPK, chemical fertilizer; BC, biochar + chemical fertilizer; BBF, biochar-based fertilizers+ chemical fertilizer.

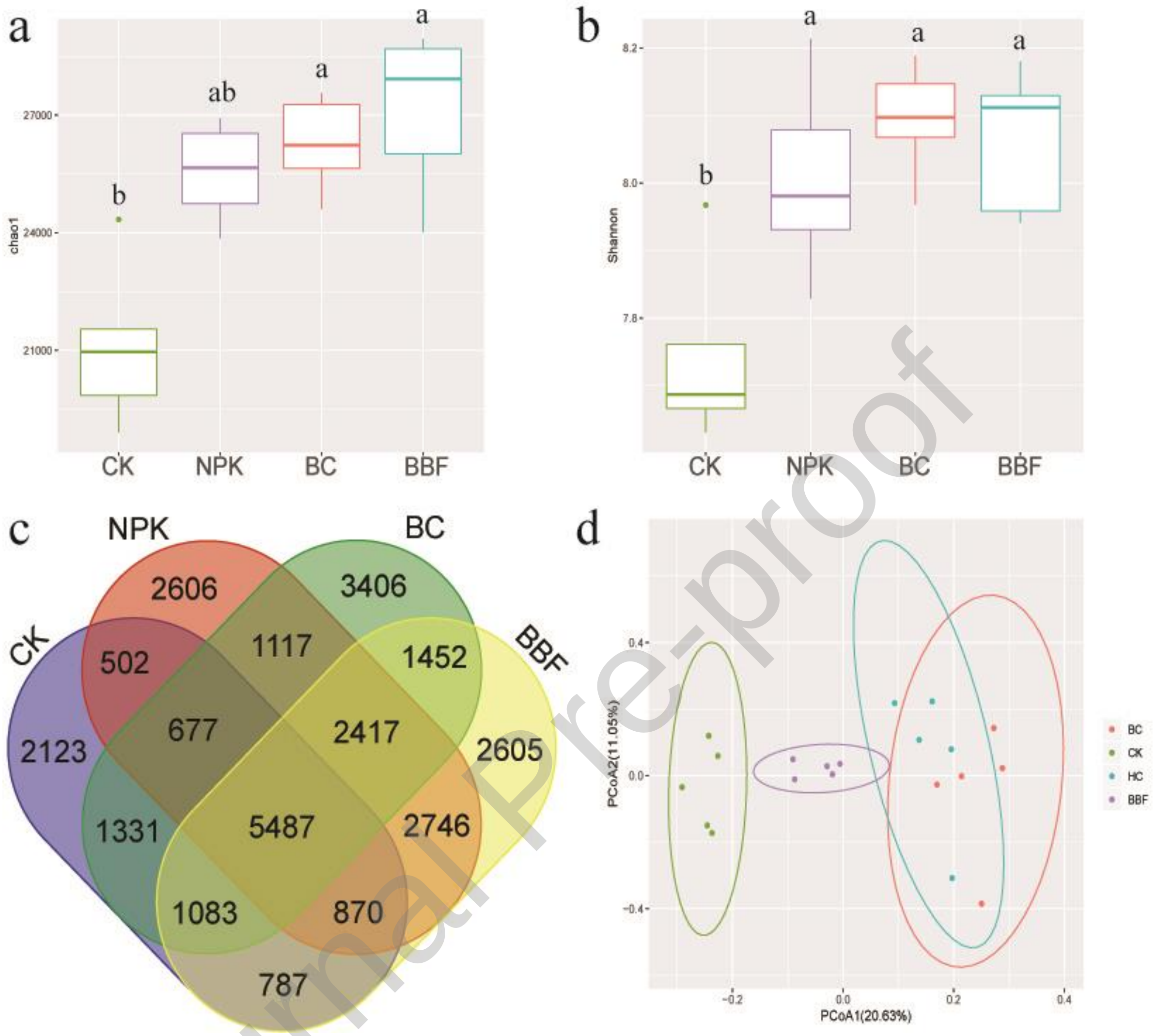


Fig. 2. Effect of fertilizer treatments on soil bacterial communities. (A) Chao1; (B) Shannon; (C) Venn for OTU; (D) Beta-Diversity. Different lowercase letters indicate significant differences among treatments at the level of $p < 0.05$,the lines in the box are the mean values. CK, control with no fertilizer; NPK, chemical fertilizer; BC, biochar + chemical fertilizer; BBF, biochar-based fertilizers+ chemical fertilizer.

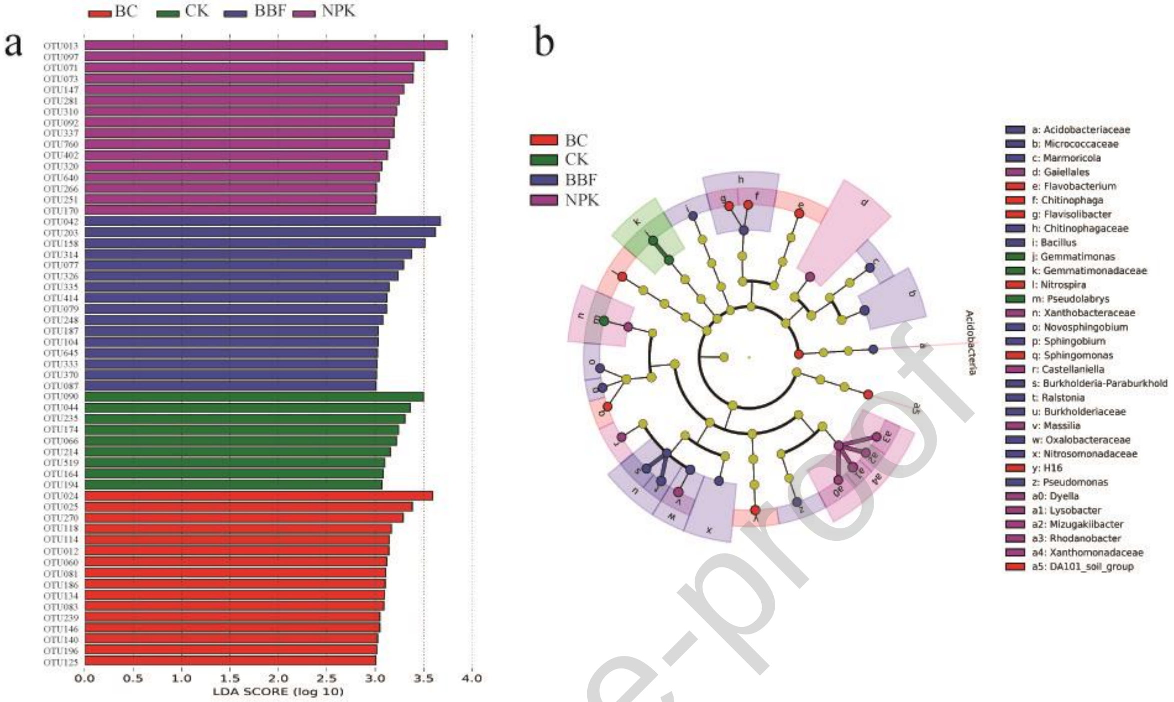


Fig. 3. Key phylotypes detected by LEfSe following fertilizer application. (A) Comparison of LDA scores based on Operational Taxonomic Units; (B) Cladogram plot based on 57 Key Phylotypes. CK, control with no fertilizer; NPK, chemical fertilizer; BC, biochar + chemical fertilizer; BBF, biochar-based fertilizers+ chemical fertilizer.

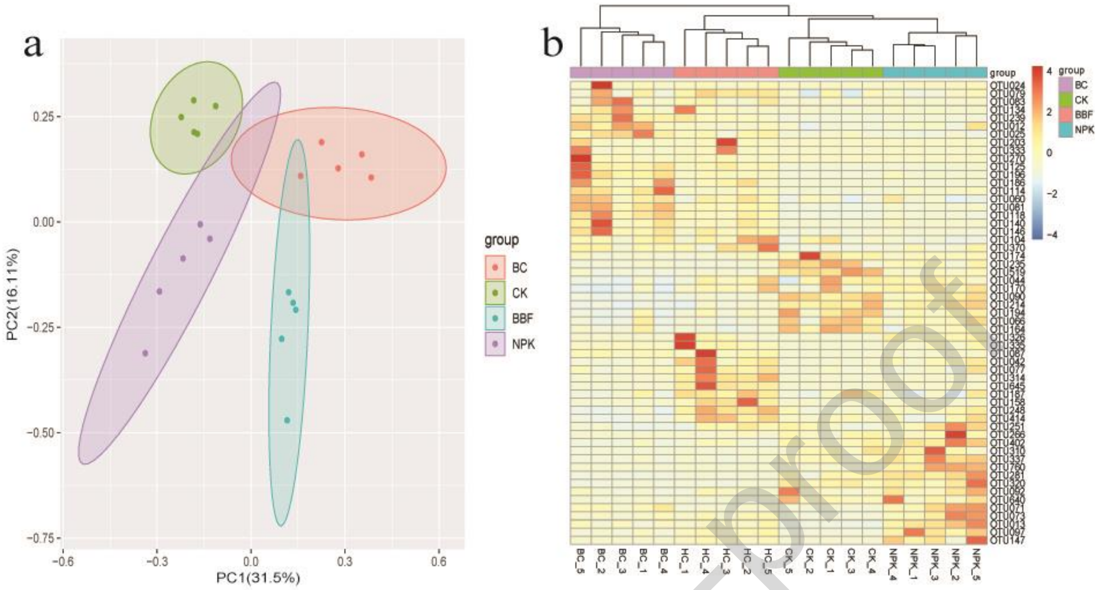


Fig. 4. The Principal component analysis and heapmap analysis of key phylotypes. (A) Principal component analysis and (B) Heatmap analysis based on the relative abundance of the 57 Key Phylotypes. CK, control with no fertilizer; NPK, chemical fertilizer; BC, biochar + chemical fertilizer; BBF, biochar-based fertilizers+ chemical fertilizer.

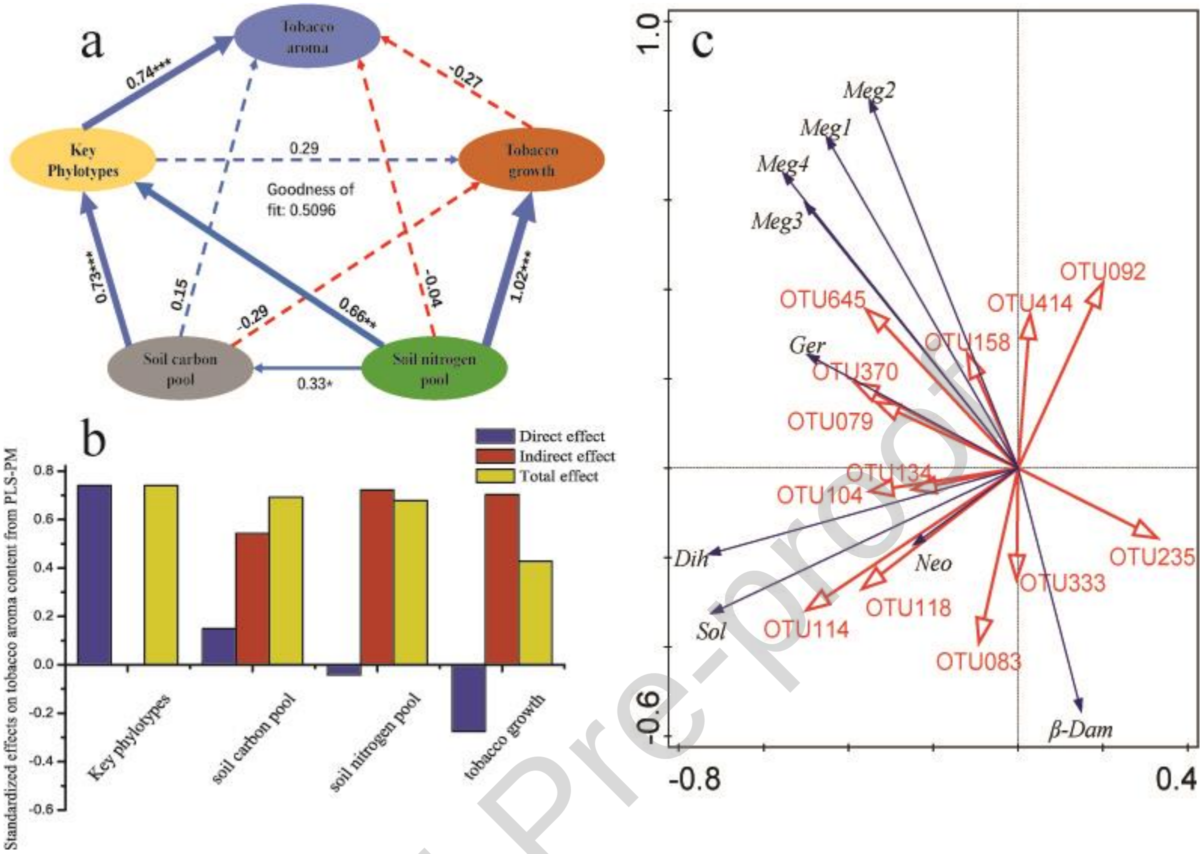
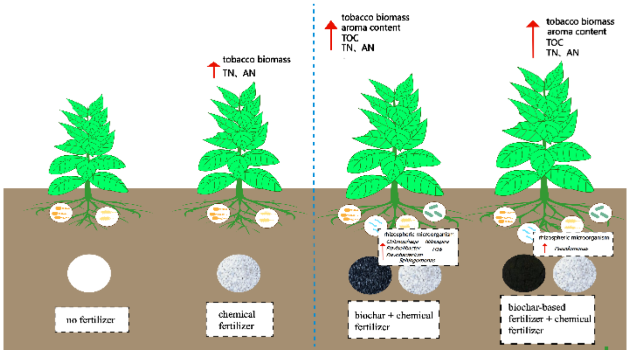


Fig. 5. Correlation between soil bacteria and tobacco quality indices. (A) PLS-PM describes the relationships among key phylotypes, tobacco growth, and C and N content concerning aroma content. Larger path coefficients have wider arrows, and blue and red colors represent positive and negative effects, respectively. Path coefficients and coefficients of determination (R^2) were calculated after 999 bootstraps. Significance levels are indicated by * (*P*< 0. 05), ** (*P*< 0. 01), and *** (*P*< 0. 001). (B) Standardized direct and indirect mean effects derived from PLS-PMs. (C) Redundancy analysis for microorganisms and tobacco aroma components. (Sol: solanone; β-Dam: β-damascenone; Ger: geranylacetone; Dih: dihydroactinolide; Neo: neophytadiene; Meg 1: Megastigmatrienone 1; Meg 2: Megastigmatrienone 2; Meg 3: Megastigmatrienone 3; Meg 4: Megastigmatrienone 4).

Graphical abstract



CRediT authorship contribution statement

Shen Yan: Data curation, Formal analysis, Investigation, software,Writing – original draft, Methodology, Writing – review & editing. **Peng Wang:** Data curation, Formal analysis,Funding acquisition, Investigation,Writing – review & editing. **Xianjie Cai:** Conceptualization, Project administration,Resources,Writing – review & editing. **Chuliang Wang:** Data curation,,Resources, Writing – review & editing. **Lukas Van Zwieten**: Formal analysis, Supervision, Writing – review & editing. **Hailong Wang:** Methodology, Resources, Writing – review & editing. **Quanyu Yin:** Methodology, Software, Writing – review & editing. **Guoshun Liu:** Conceptualization, Formal analysis, Funding, acquisition, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Tianbao Ren:**Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing

Declaration of interests

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

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Biochar-based fertilizer enhanced the soil C and nitrogen pools which is directly affected PGPB growth.
- Biochar-based fertilizer effectively improved tobacco aroma-related content.
- Tobacco aroma components can be affected by the promotion of key phylotypes belonging to PGPR.