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Biochar-based organic fertilizer application promotes the alleviation of tobacco (*Nicotiana tabacum* L.) continuous cropping obstacles by improving soil chemical properties and microbial community structure

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Abstract

Background Intensive monoculture poses a serious threat to agricultural sustainable development due to the phenomenon of continuous cropping obstacles. Although organic amendment has been considered an efficient and environmentally friendly solution to mitigate this tough issue, the associated mechanisms remain poorly understood. Here, a two-year field experiment was conducted with the application of four fertilizers, wood, rice straw, compound biochar-based organic fertilizers (WBF, RBF, CBF) and chemical fertilizer (CF) under tobacco rotation with broad bean and oilseed rape, respectively. This work aims to determine how BFs application alleviates tobacco CCO and to further reveal the underlying action mechanisms primarily focusing on the change of soil micro-ecology environments.

Results The results depicted that BFs addition decreased tobacco morbidity (by 15.7–85.0%), heavy metals (Cd, V, Cu, Zn) contents in tobacco, and improved tobacco leaf production yield (by 4.5–20.5%), economic value (by 14.6–34.4%) and chemical quality compared with CF. Rhizosphere soil chemical properties and the structure and diversity of microbial communities were enhanced under BFs treatments, reflecting in the growth of bacterial OTUs number, microbial alpha-diversity, the abundances of some beneficial genera (*Arthrobacter*, *Pseudomonas*, *Gemmatimonas*, *Trichoderma*, *Mortierella*, *Penicillium*, *Chaetomium*, etc.), and the reduction of the numbers of detrimental microbes (*Alternaria*, *Phytophthora nicotianae* and *Fusarium oxysporum*). Moreover, CBF amendment improved the stability and complexity of microbial co-occurrence networks. Soil total carbon, microbial structure, and diversity were the most important explanatory factors for the increase of tobacco leaf yield and economic value.

Conclusions Collectively, BFs application under rotation regime showed the great potential as a practical and environmentally friendly strategy to alleviate tobacco CCO by providing an optimized soil environment.

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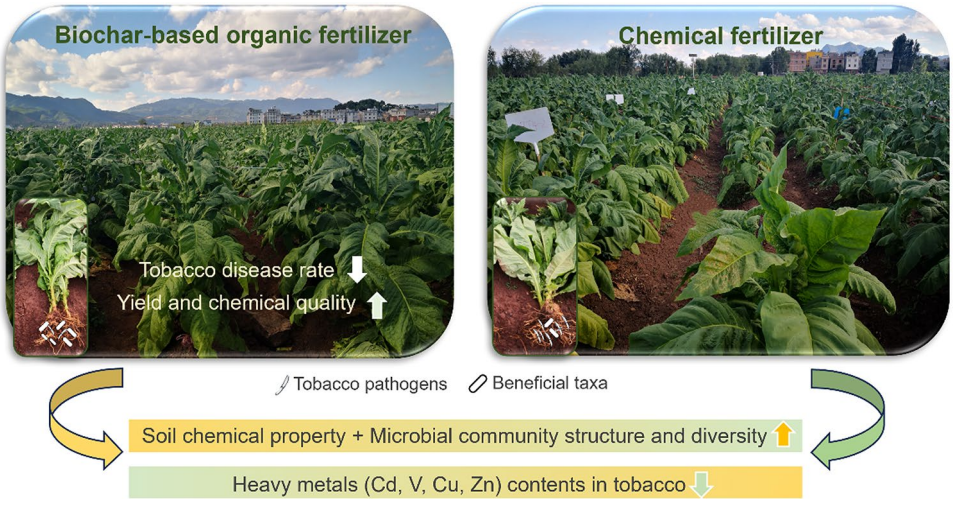
Graphical abstract



Keywords Biochar-based organic fertilizers, Crop rotation, Continuous cropping obstacles, Soil chemical properties, Rhizosphere microbial community

Introduction

Crop intensive monoculture has become a widespread cropping regime in agricultural production activities due to a global shortage of arable land, the drive of commercial interests and a lack of rational cropping concept [1, 2]. However, a variety of crops, such as cotton, soybean, *Panax notoginseng*, tobacco (*Nicotiana tabacum* L.), peanut (*Arachis hypogaea* L.), cucumber (*Cucumis sativus* L.), are sensitive and inadaptable to the continuous cropping (CC) pattern. That means, their yield and quality will drastically decline accompanied with the eruption of soil-borne diseases, sometimes even to disastrous levels after a few years of CC [2, 3, 4, 5, 6, 7]. This phenomenon is also known as "continuous cropping obstacles" (CCO) or "replant diseases" [3, 6], and the issues associated with CCO have aroused extensive attention as it seriously jeopardizes the soil ecosystem's health and agricultural sustainability. It is well documented that over 20% of arable land resources in China have been adversely influenced by crop CCO, leading to low agricultural yields, heavy economic losses, and poor soil quality [8].

Deterioration of rhizosphere soil microecology is considered the dominant reason for crop CCO, including the degradation of soil physicochemical properties, the deficiency and imbalance of nutrient elements, the aggravation of plant allelopathic autotoxicity, and the destruction of rhizosphere microbial community composition and structure [3, 6, 8]. The diversity and complexity of the causes of crop CCO bring us great challenges to thoroughly solve the problem when taking different crop varieties and their variable habitats into consideration. Although a majority of plant diseases can be quickly and effectively controlled by using chemical fumigants and pesticides, which are still popular in recent agriculture activities, their excess use has led to a series of environmental problems [2]. Therefore, environmentally friendly and sustainable measures are urgently needed, for instance, the adoption of crop rotation regime and the applications of biochar, biocontrol agents, (bio-) organic fertilizers, among others [2, 9, 10, 11, 12].

China ranks first in producing and consuming tobacco products, accounting for one-third of the global total tobacco leaf yield (over 2.2 million tons per year). Additionally, Yunnan province occupies around 45% of tobacco leaf production in China, and has historically been one of the core tobacco production regions [5, 10]. The tobacco business contributes a lot to the national total revenue and millions of tobacco growers in China, with the sale of flue-cured tobacco leaves becoming their main income source [10]. As a member of the plant family Solanaceae, tobacco is sensitive to intensive monoculture, and long-term tobacco CC results in considerable yield and economic losses every year [3, 5]. The rotation regime is the most direct and extraordinary approach to manage tobacco CCO currently, such as tobacco rice (*Oryza sativa* L.) rotation every two years, one year for tobacco cultivation and the next year for rice. However, this way is only applicable in large-scale intensive production regions with convenient irrigation systems, and usually requires more economic input when the field tillage pattern undergoes a major shift. Therefore, there is a dire need to explore sustainable and environmentally friendly

solutions to alleviate tobacco CCO within the limitation of chemical pesticides usage due to environmental concerns. Organic fertilizers (OFs) are favored by researchers and tobacco peasants, because they are characterized by excellent plant growth-promotion functions, low environmental risk and the realization of resource recycle on farmland. It has been well reported across many studies that OFs amendment promotes the alleviation of crop CCO through improving soil fertility, enzyme activities, organic matter (OM) content, carbon (C) and nitrogen (N) stocks, macro-aggregates' formation, and microbial community structure [9, 10, 11, 13].

Specifically, our previous study has demonstrated the benefits of biochar and vermicompost application in ameliorating tobacco CCO, and it could be at least partly explained by the improvement of soil physicochemical properties and bacterial community structure [14]. Biochar-based organic fertilizers (BFs) application with biochar and vermicompost as the main components is scarce in the tobacco cultivation field. Although many studies have preliminary revealed its associated action mechanisms, most of them merely focus on the changes of soil characteristics, elemental concentration, and the composition and diversities of microbial communities. The relationships between soil properties, tobacco yield and economic value, and the key factors triggering the alleviation of tobacco COO remain poorly understood. Based on this, we explored the effects of different novel types of BFs on tobacco CCO, elucidated more detained and targeted potential mechanisms, and further discovered the key contributors from the correlation and explanatory power analyses. Meanwhile, two local common rotation regimes involving tobacco-broad bean (*Vicia faba* L.) and tobacco-oilseed rape (*Brassica napus* L.) were designed accompanied with fertilizer treatments in a two-year field experiment, which can better verify the effects of BFs application on tobacco CCO under different study years and crop regimes. Compared to tobacco-rice rotation, tobacco rotation with broad bean and oilseed rape was executed in one year. The objectives of the current study were to disentangle the changes in soil chemical properties, and the composition and structure of rhizosphere microbial communities under the impact of CF and BFs application. As the change patterns were assessed, the key factors contributing to the alleviation of tobacco CCO were discovered. We hypothesize that BFs addition can efficiently improve tobacco yield and economic value under CC circumstances, and the positive functions can be largely ascribed to the amelioration of soil chemical characteristics and the shifts in rhizospheric microbial communities.

Materials and methods

Field experiment design

A two-year field trial was conducted from 2021 to 2022 in Yuxi City, Yunnan Province of Southwest China (24°38′29″N, 102°52′23″E), with an altitude of 1740 m, an annual mean temperature of 16 ℃, and a precipitation of 890 mm. The study field was red soil, which is the predominant soil type for tobacco plantations in Yunnan. Tobacco cultivar "Zhusha No.2" had been continuously monocultured for three years in the study field before starting our experiment. Tobacco rotation with broad bean and oilseed rape were separately executed in the field. Each rotation regime had four fertilizer treatments with a split plot design, wood, rice straw, compound biochar-based organic fertilizers (WBF, RBF, CBF) and chemical fertilizer (CF). There were four repetitions for each treatment, and each plot had a size of 66 m^2. BFs and CF were applied into plantation holes as base manure at rates of 1650 and 225 kg ha^{-1}, respectively, before tobacco seedling transplantation at a distance of 1.20 × 0.55 m in April 2021 and 2022. Tobacco seeds of cultivar "Zhusha No.2" were provided by Yuxi Zhongyan Seed Co., Ltd, and then sowed and cultivated in tobacco floating-seedling system in Yunnan Yuxi Tobacco Science Institute. About four times of topdressing were employed during the entire tobacco growth duration based on the local optimal tobacco production standard. In October, we planted broad bean and oilseed rape in the corresponding rotation field after the tobacco harvest. Broad bean and oilseed rape seeds were provided from Yuxi Sannong Plateau-Characteristic Modern Agriculture Co., Ltd. The field soil and fertilizer properties are presented in Tables 1 and 2, respectively.

Tobacco plant and soil sampling

In July 2022, tobacco morbidity was recorded in each plot by counting the number of tobacco plants with typical disease symptoms among the total numbers of tobacco plants chosen for calculation, such as black shank, root black rot, potato Y virus, tobacco tomato spotted wilt virus, and weather fleck [15]. In order to reduce personal error, two individuals with disease investigation experience independently executed the disease observation in each plot, and the average disease rate was calculated.

Table 1 Soil basic chemical properties of the study field

pH	OM ($g\ kg^{-1}$)	CEC ($cmol^{+}kg^{-1}$)	AN ($mg\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	AK ($mg\ kg^{-1}$)	TC (%)	TN (%)	TP (%)	TK (%)
7.1	34.8	21.2	121.1	46.5	167.1	1.63	0.13	0.17	1.31

OM, AN, AP, AK, TC, TN, TP, and TK represent organic matter, available N, P, K, and total C, N, P, K, respectively

Table 2 The basic properties of biochar-based organic fertilizers

	WBF	RBF	CBF
Water content (%)	3.9	4.9	4.8
pH	7.01	7.45	7.51
OM (g kg^{-1})	21.6	21.1	21.4
TC (%)	16.6	7.8	10.4
TN (%)	1.8	1.5	1.8
TP (%)	0.8	1.1	1.0
TK (%)	3.4	3.7	3.6
Na (ppm)	777	923	906
Cr (ppm)	26.1	24.7	26.8
Pb (ppm)	8.8	8.2	9.7
As (ppm)	3.9	2.6	2.8
Cd (ppm)	0.5	0.4	0.5
Hg (ppm)	ND	ND	ND
Component1-Wood biochar (%)	25	0	12.5
Component2-Rice straw biochar (%)	0	25	12.5
Component3-Vermicompost (%)	75	75	75

WBF, RBF, and CBF indicate wood, rice, and compound biochar-based organic fertilizers, respectively. Ppm and ND mean mg kg^{-1} and below the detectable limit, respectively

In July 2021 and 2022, five representative tobacco plants under topping stage were selected in each plot for agricultural traits analysis with a tape, including stem girth, plant height, maximum leaf length and width, and the number of productive leaves [16]. The selected plants were dug up with roots for wet biomass determination and dried at 65 ℃ for dry biomass analysis. Dried tobacco roots, stems, and leaves were digested with HNO_3-$HClO_4$-H_2O_2 solutions before element (Ca, P, S, Cr, V, Cu, Zn) concentration measurement by an inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP 6000 series, Thermo Scientific, United States) [17].

Correspondingly, the rhizosphere soil of the five chosen plants was collected to form a composite sample in each replicate by the root shaking method [18]. In total, we obtained 64 rhizosphere soil samples (4 fertilization treatments × 4 replicates × 2 rotation regimes × 2 cropping years). A subset of the fresh soil samples was stored at 4 ℃ for soil NH_4^+-N, microbial biomass C and N (MBC and MBN) content measurement; a part was stored at − 80 ℃ until soil DNA extraction followed by microbial community analysis; and the remaining was air-dried, ground, and sieved for chemical property determination. Meanwhile, the tobacco leaves of another ten plants in each plot were labeled and collected at four separate times at 10-day intervals under the harvest stage from July to August based on the maturity of the leaves. All manure leaves were flue-cured in sequential batches with three steps of yellowing, fixed color, and dry gluten period according to "Rules for curing technique of flue-cured tobacco" [19]. The curing conditions in the yellowing period were temperature 32–45 ℃, humidity 70–98%, and duration 24–72 h, while they were 45–55 ℃, 30–70%, and 20–40 h for the period of fixed color, 55–75 ℃, 30%, and 16–36 h for the dry gluten period, respectively. According to the national standard "Flue-cured tobacco" [20], the flue-cured tobacco was classified into 10 grades based on the leaf maturity, structure, body, oil, color intensity, length, waste and injury with the help of professional graders. We separately recorded the dry weight of all the flue-cured tobacco of each grade for the calculation of economic parameters as follows.

$$\text{Trade yield}\ \left(\text{kg ha}^{-1}\right) = \sum \text{yield of different grades} \quad (1)$$

$$\begin{aligned}\text{Economic value}\ &\left(\text{CNY ha}^{-1}\right)\\ &= \sum \text{yield of different grades}\\ &\times \text{corresponding prices of}\\ &\text{different grades in one year}\end{aligned} \quad (2)$$

$$\text{Mean price}\ \left(\text{CNY kg}^{-1}\right) = \frac{\text{Economic value}}{\text{Trade yield}} \quad (3)$$

$$\text{Percent of high-class leaf}\ (\%) = \frac{\sum \text{yield of high-class grades}}{\sum \text{yield of all different grades}} \quad (4)$$

Six C3F-grade flue-cured tobacco leaves were randomly selected in each plot, smashed into small pieces and filtered through a 0.25-mm mesh screen. The routine chemical components (total sugar, reducing sugar, total N, nicotine, K_2O, and chlorine) were measured using the continuous flow method (SEAL AA3, Germany) [21].

Analysis of soil chemical properties

Soil pH (soil: water = 1: 2.5, w/v) was measured with the glass electrode method [17]. Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in KCl solution were assessed colorimetrically with an ultraviolet spectrophotometer (UV-1890, Daojin Instrument Co., Ltd., Jiangsu, China) [22]. After extraction with $NaHCO_3$ solution, we used an UV spectrophotometer to detect soil available P (AP) by the molybdenum blue method (UV-1890, Daojin Instrument Co., Ltd., China) [17, 23]. Using an atomic absorption spectrometer (AAS, Analytik Jena novAA 300, Germany), soil available K (AK) was evaluated with neutral NH_4OAC extraction [17, 23]. Soil OM and total organic carbon (TOC) were determined by the $K_2Cr_2O_7$–H_2SO_4 colorimetric method, and total carbon and nitrogen (TC and TN) were assayed by the combustion method using an elemental analyzer (Elemental Vario EL Cube, Germany) [17, 23]. The chloroform fumigation extraction procedure was used to measure soil MBC and MBN contents with 0.5 M K_2SO_4 solution [24].

Quantitative polymerase chain reaction (qPCR) assay for tobacco pathogens

The absolute abundances of two pathogens causing tobacco black shank and root rot diseases, *Phytophthora nicotianae* and *Fusarium oxysporum*, were determined in rhizosphere soil using a SYBR Green assay with the primers Pn3 (forward, 5'-GACAAACCAGTCGCCAA TTT-3; reverse, 5'-TGAACGCATATTGCACTTCC-3') and JBR (forward, 5'- CATACCACTTGTTGTCTCGG C-3; reverse, 5'-GAACGCGAATTAACGCGAGTC-3'), respectively [25]. Standard curves were constructed by 10-fold serial dilutions of a plasmid containing a fragment copy of the target gene from the two pathogens. A serial dilution from 10^8 to 10^5 gene copies μL^{-1} was used as a standard for *Phytophthora nicotianae*, with the amplification efficiency of 90.9%, while it was from 10^7 to 10^2 gene copies μL^{-1} for *Fusarium oxysporum* with an amplification efficiency of 90.4%. Briefly, the qPCR assay was performed with a T100 Thermal Cycler (Bio-Red, United States) in 20 μL reaction mixtures, which contained 1 μL of templates, 10 μL of the Taq Plus Master Mix (2×), 0.8 μL of each primer, and 7.4 μL of distilled water. The thermal cycling procedure was 1 cycle of initial denaturation (95 °C for 5 min), denaturation (95 °C for 30 s), 35 cycles of annealing (58 °C for 30 s), and a final extension (72 °C for 60 s). Each sample was replicated three times. The melt curve was run at the end of the PCR process to verify the specificity of the amplified fragments. Finally, the absolute abundances of the two pathogens were expressed as copies g^{-1} soil from their Ct value and the standard curves.

Soil DNA extraction, PCR amplification, and high-throughput sequencing

According to tobacco growth and yield detected above, rhizosphere bacterial and fungal community diversity in CBF and CF treatments were analyzed to elucidate the potential microbial mechanisms. Soil genomic DNA was extracted using the Fast DNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA), and the concentration and purity of DNA were evaluated using a micro-spectrophotometer (Nano-300, Allsheng, Hangzhou, China) and 1.0% agarose gel electrophoresis. The V3-V4 region of the bacterial 16S rRNA gene was amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and the ITS1 region of fungi was amplified using ITS1F (5'-CTTGGTC ATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTT CTTCATCGATGC-3') [18, 26].

We obtained a total of 733,616 and 738,898 high-quality bacterial and fungal sequences, respectively, with their average counts of 45,851 (ranging from 37145 to 53237) and 46,181 (ranging from 36245 to 64209) in all samples. The purified PCR products were pooled (equimolar) and sequenced on the Illumina MiSeq PE300 platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). We used FLASH v1.2.11 to merge pair-end sequences [27]. By using UPARSE v11, operational taxonomic units (OTUs) at a 97% similarity cutoff were constructed [28, 29], and chimeras, erroneous sequences, those matching the chloroplast and mitochondria were identified and discarded. To facilitate downstream analyses, we only remained the OTUs with sequence counts greater than 5 in at least three samples and total counts greater than 20 in all samples. With a confidence threshold of 0.7, RDP Classifier v2.13 was used to determine the taxonomy affiliation of each 16S rRNA gene and ITS sequence in comparison to the 16S rRNA Silva 138 (https://www.arb-silva.de/) and the fung al ITS UNITE 8.0 reference database (https://unite.ut.ee /) [30].

Statistical analysis

Significance test was conducted in SPSS 26.0 at the level of $P < 0.05$. Prior to bioinformatic analyses, we rarefied the sequencing depth to the smallest sample size across all samples, and then processed through QIIME v1.9.1 [31]. ACE and Chao indicators were utilized to obtain the bacterial and fungal alpha-diversity (α-diversity) using Mothur v1.30.2 [32]. Meanwhile, we used the Kruskal-Wallis H test for the significant difference analysis of microbial abundances between two groups. Shared and unique OTUs among various samples were displayed using a Venn diagram. Microbial co-occurrence network diagrams under CBF and CF treatments were investigated and visualized using Gephi v0.9.2 [33]. According to [34], "keystone species" refer to the network members with high degree and between centrality values. The correlations among tobacco leaf yield, economic value, soil chemical properties, and microbial communities were assessed by Spearman correlations and Mantel tests in R v4.3.0 [7]. Meanwhile, we analyzed the relative importance of soil chemical properties, bacterial and fungal community structure, and diversity for tobacco leaf yield and economic value based on a linear regression model in *Relaimpo* package.

Results

Tobacco plant responses to BFs and CF application under field condition

Figure 1a and b depicted tobacco disease rate and the abundance of two typical pathogens in 2022, *Phytophthora nicotianae* and *Fusarium oxysporum*. The incidence of tobacco diseases decreased dramatically from 16.7–17.8% in CF amendment treatment to 2.7–15.0% in BFs amendment treatments, with the lowest of 2.7% under CBF addition coupled with tobacco broad bean rotation. However, no significant difference was observed in

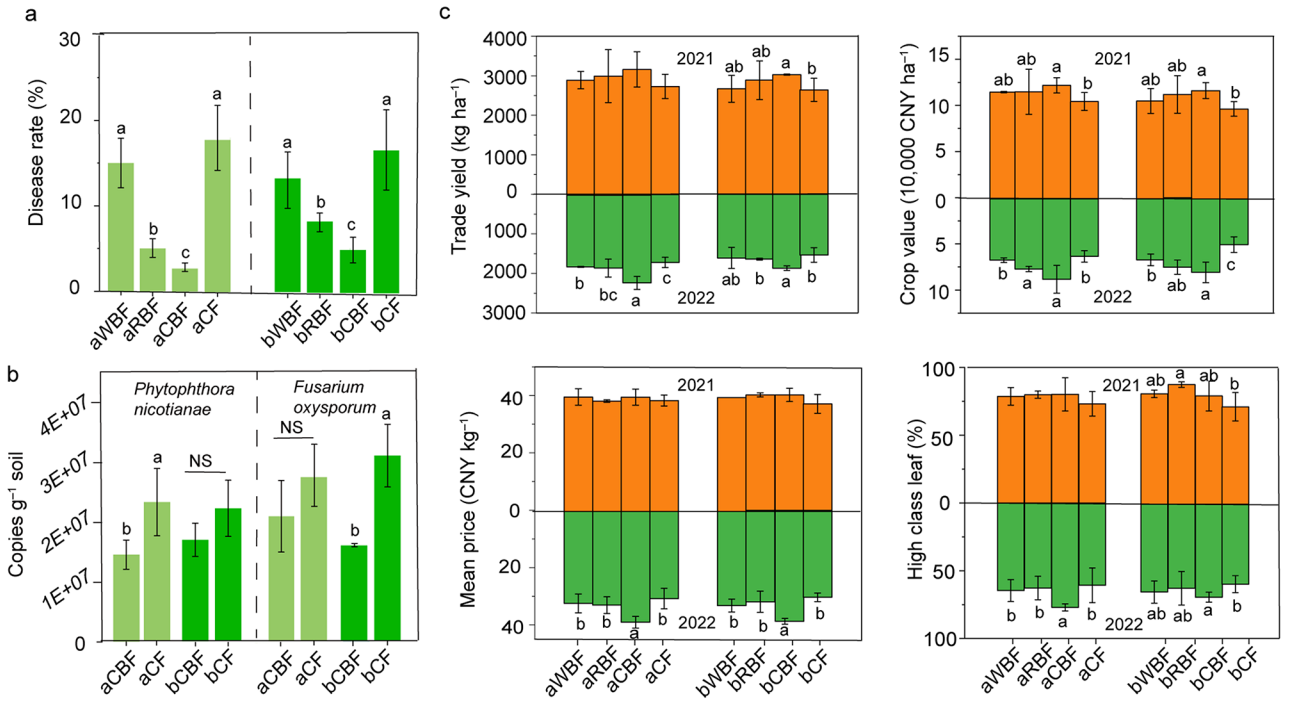


Fig. 1 Tobacco disease rate, economic parameters and the pathogen loads in rhizosphere soil under different fertilizer and rotation regime treatments for over 2 years. (**a**) Tobacco disease rate and (**b**) the absolute quantities of rhizosphere pathogens *Phytophthora nicotianae* and *Fusarium oxysporum* in 2022; (**c**) tobacco economic parameters in 2021 and 2022. (y)WBF, RBF, CBF, and CF represent wood, rice straw, compound biochar-based organic fertilizers, and chemical fertilizer, respectively (y = a or b, indicate tobacco rotation with broad bean or oilseed rape). Different letters above error bars show a significant difference among different fertilizer treatments (Duncan's test, $P < 0.05$, $n = 4$). No letter means no significant difference

disease incidence ($P \geq 0.05$) between WBF and CF treatments. Meanwhile, the numbers of pathogens decreased in CBF-treated rhizosphere soil relative to CF-treated soil under both rotation regimes (Fig. 1b). BFs addition in soil improved tobacco agricultural traits, wet and dry biomass under both rotation regimes and study years compared to CF, especially for CBF addition (Fig. S1).

Moreover, all tobacco economic parameters were higher under BFs treatments relative to CF (Fig. 1c). Tobacco grown in CBF amendment soil owned the greatest trade yield (average 3105 kg ha^{-1} in 2021 and 2042 kg ha^{-1} in 2022) and crop value (average 12.0 ten thousand CNY ha^{-1} in 2021 and 8.3 ten thousand CNY ha^{-1} in 2022) in all treatments, presenting a 20.5% and 34.4% increment for average trade yield and crop value, respectively, as compared to CF. Simultaneously, a significant decline in tobacco trade yield and value was observed in 2022 than those in 2021, indicating the occurrence of an intensified tobacco CCO phenomenon with the prolongation of CC years.

The chemical components of flue-cured tobacco leaves are listed in Table S1. Overall, CBF application contributed to higher values in the total sugar, reducing sugar, K_2O contents, and the total sugar/nicotine, K_2O/Cl, reducing sugar/total sugar ratios in flue-cured tobacco. In comparison to CF, three types of BFs amendment reduced the Cl content in flue-cured tobacco leaves, validating the functions of BFs in the enhancement of chemical quality, with CBF posing the most favorable impacts in all treatments. A three-way ANOVA was employed to analyze the influences of different fertilization applications, rotation regimes, CC years, and their interactions on tobacco leaf yield and chemical quality. The results showed that tobacco leaf yield and chemical quality were strongly steered by different fertilizer additions and CC years ($F = 3.3–149.4$, $P < 0.05$) except the total sugar/nicotine ratio (Table 3).

Variation of soil chemical properties and element contents in tobacco

The results of soil chemical properties showed that BFs addition increased rhizosphere soil pH, with WBF presented the most pronounced effect in all BFs types (Fig. 2). There were improvements in soil OM, AP, AK, MBC, MBN, TC, and TN contents under BFs treatments, particularly for CBF addition (Fig. 2). However, this was not the case for soil NH_4^+-N content (Fig. 2). With respect to element contents in tobacco organs, BFs amendment resulted in higher Ca content, lower Cd, V, Cu, Zn contents in all tobacco organs, higher S and P contents in tobacco stems and roots, and lower S and P

Table 3 The three-way ANOVA test for the effects of different fertilizers, rotation regimes, and continuous cropping years on tobacco leaf yield and chemical quality (F)

	Yield	Total sugar/ nicotine	Total *N*/ nicotine	K_2O/Cl	Total sugar/ reducing sugar
Ferti	3.3 *	17.6 **	4.9 **	5.0 **	4.0 *
CC	149.4 **	3.5	18.3 **	4.4 *	26.5 **
Rota	0.8	1.9	0.0	50.5 **	0.0
Ferti × CC	0.4	8.6 **	0.2	2.1	2.9 *
Ferti × Rota	0.0	6.7 **	1.3	1.9	6.5 **
CC × Rota	2.3	0.7	0.0	25.2 **	13.3 **
Ferti × CC × Rota	0.2	2.4	2.93	0.7	4.0 *

Ferti = Fertilizers, CC = Continuous cropping years, Rota = Rotation regimes. A significant difference was marked with "*" ($P < 0.05$) or "**" ($P < 0.01$)

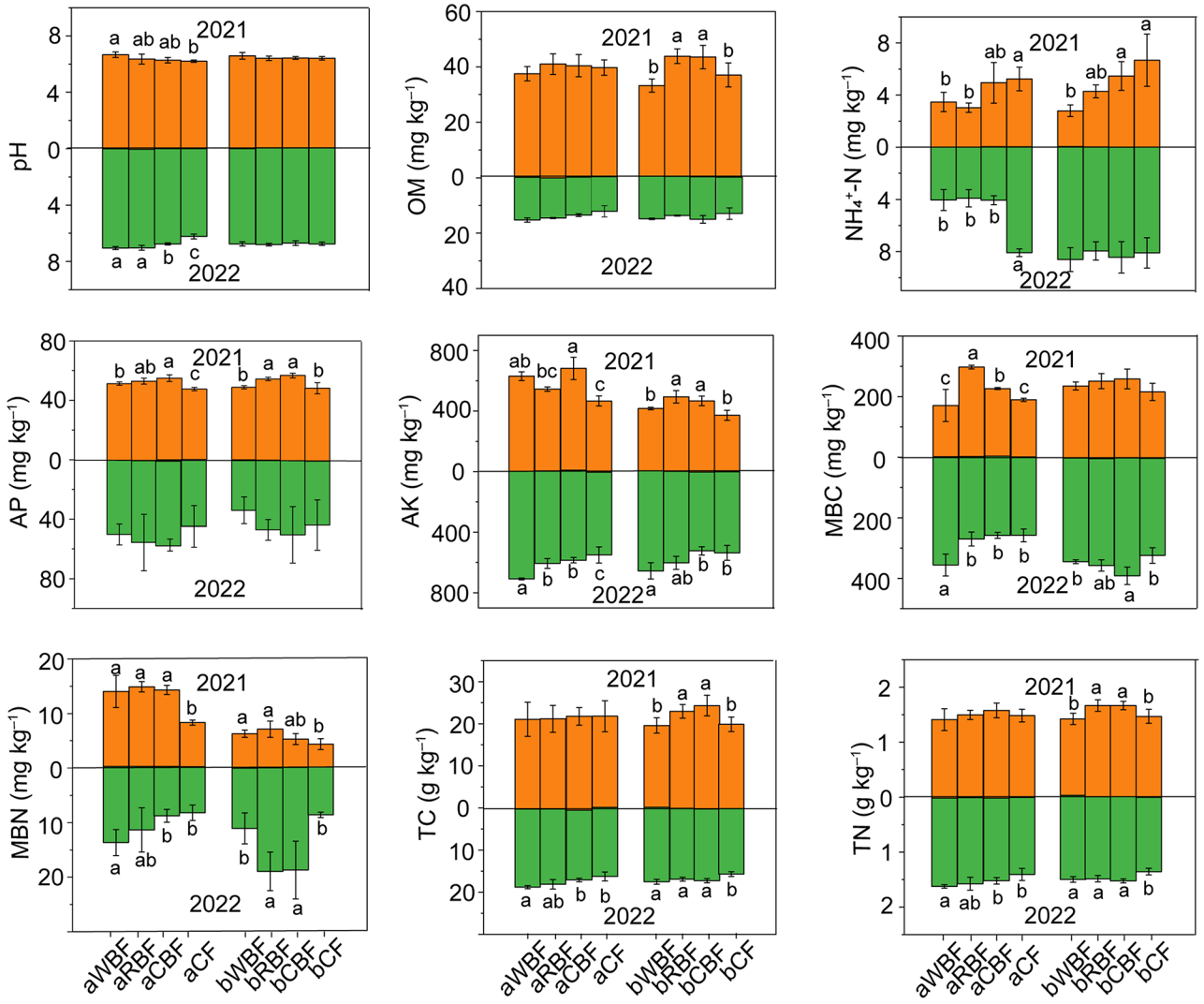


Fig. 2 Rhizosphere soil chemical properties under different fertilizer and rotation regime treatments for over 2 years. OM, AP, AK, MBC, MBN, TC, and TN indicate organic matter, available P and K, microbial biomass C and N, and total C and N, respectively. (y)WBF, RBF, CBF, and CF represent wood, rice straw, compound biochar-based organic fertilizers, and chemical fertilizer, respectively (y = a or b, indicate tobacco rotation with broad bean or oilseed rape). Different letters show a significant difference among different fertilizer treatments (Duncan's test, $P < 0.05$, $n = 4$). No letter means no significant difference

contents in tobacco leaves under both rotation regimes (Fig. S2).

Soil microbial community shift under different fertilizer treatments

Soil microbial community composition

There were at least 3800 bacterial and 1140 fungal OTUs existing in each sample from the Venn diagram (Fig. 3a). Specifically, 38.4% of soil bacterial OTUs (number of 2265) and 16.2% of fungal OTUs (number of 420) were shared across all treatments, while only 5.8–7.6% (344–447) and 9.0–17.9% (233–463) OTUs were unique in each treatment for bacteria and fungi, respectively. Application of CBF tended to increase soil bacterial OTUs numbers in both CC years, and different fertilization patterns shaped a more distinct fungal OTUs distribution than bacteria.

The top five most abundant rhizobacteria were phylum Actinobacteriota (39.1% of the total reads), Proteobacteria (27.9%), Chloroflexi (9.8%), Acidobacteriota (7.9%) and Bacteroidota (3.6%), while on the genus level were Sphingomonas (5.8%), Intrasporangium (5.7%), Arthrobacter (5.7%), Nocardioides (3.3%) and norank_f__norank_o__Vicinamibacterales (2.4%), which collectively accounted for 88.4% and 22.9% of all bacterial communities, respectively (Fig. 3b, S3a). Moreover, rhizosphere fungal communities were predominantly composed of phylum Ascomycota (80.9%), Mortierellomycota (9.6%), Basidiomycota (3.7%), unclassified_k__Fungi (3.0%) and Chytridiomycota (1.8%), while on the genus level were Fusarium (14.5%), Mortierella (10.6%), Plectosphaerella (9.3%), Gibberella (8.4%) and Talaromyces (4.4%), owning a total relative abundance of 99.0% and 47.2%, respectively (Fig. 3b, S3b). Remarkably, Ascomycota occupied the overwhelming majority of soil fungi (> 80%).

Soil microbial diversity and difference analysis

Higher bacterial and fungal richness and diversity were observed in the rhizosphere soil under CBF treatment as compared to CF (Fig. 3c). Bacterial Ace and Chao indices showed a significant difference between CBF and CF groups (Wilcoxon test, $P_{FDR} < 0.01$), however, there was no significant difference in all fungal α-diversity indices (Fig. 3c). Interestingly, rhizosphere microbial community diversity decreased with the prolongation of tobacco CC years, as corroborated by the reduced OTUs numbers, Ace and Chao index from 2021 to 2022. Rhizobacterial and fungal community structure showed significant differences between CBF and CF treatments in two CC years (PERMANOVA and ANOSIM tests, $P < 0.01$), and this phenomenon was more obvious in different CC years than those in fertilizer treatments, which was primarily presented along the first axis (Fig. 3d). Similar to microbial α-diversity, there was a more obvious difference in bacterial β-diversity than that of fungi under different groups.

To further explore the distinct taxonomy under CBF and CF treatments, we analyzed the heatmap and fold change of the relative abundance of the top 30 bacterial and fungal genera with a significant difference test. The results showed that CBF amendment soil enriched bacteria *Arthrobacter*, *Pseudomonas* in 2021, *Gemmatimonas*

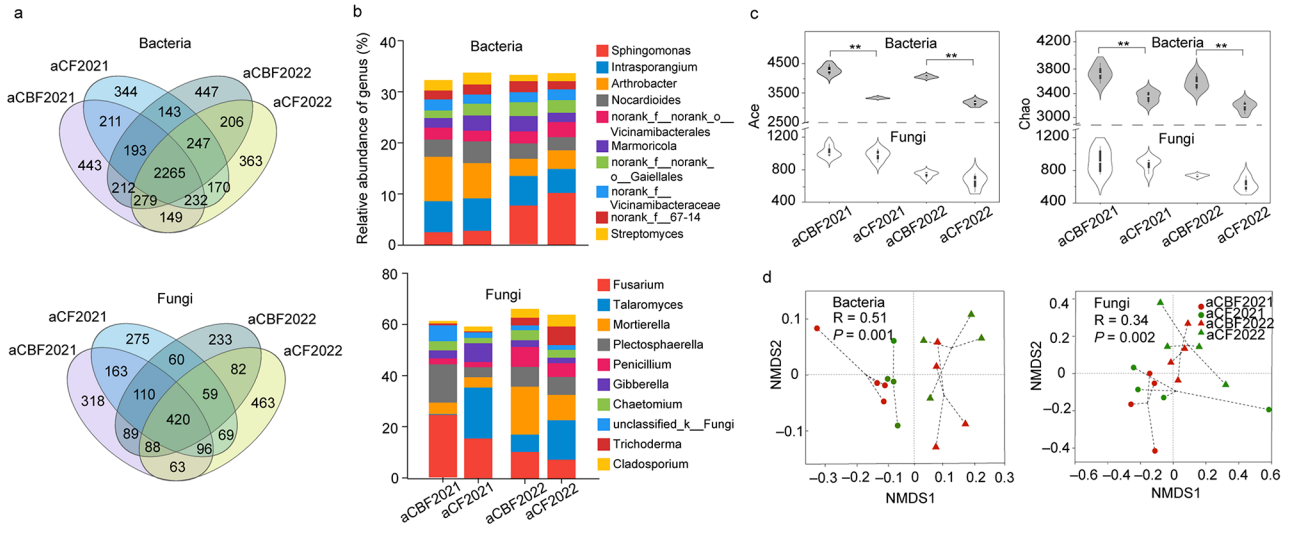


Fig. 3 The composition and diversity of rhizosphere microbial communities in tobacco-broad bean rotation regime under CBF and CF treatments for over 2 years. (**a**) Venn diagrams for shared and unique numbers of bacterial and fungal OTUs; (**b**) Ten most-abundant bacterial and fungal genus; (**c**) The ACE and Chao index of bacterial and fungal communities. "**" indicates a significant difference for bacterial ACE and Chao index between CBF and CF treatments (Wilcoxon test, $P_{FDR} < 0.01$, $n = 4$); (**d**) Non-metric multidimensional scaling (nMDS) ordinations of bacterial and fungal community composition across all rhizosphere soil samples on OTUs level based on Bray-Curtis distance. aCBF and aCF represent compound biochar-based organic fertilizer and chemical fertilizer treatments in tobacco-broad been rotation regime, respectively

in 2022, and depleted *Mycobacterium* in both CC years (Fig. 4a). In the case of soil fungi, CBF addition increased *Trichoderma* in 2021, increased *Mortierella*, *Penicillium*, *Chaetomium*, *Gibellulopsis*, and decreased *Rhizophlyctis*, *Debaryomyces*, *Talaromyces*, and *Alternaria* in both CC years (Fig. 4b).

Soil microbial co-occurrence networks

As expected, soil bacterial and fungal co-occurrence networks under CBF treatment depicted higher nodes, positive link percent, average clustering index, and lower network diameter and average path length as compared to CF (Fig. 5). Higher edges in bacterial and higher modularity in fungal co-occurrence networks were also detected in CBF amendment soil (Fig. 5). The results suggested that a more stable, complicated, and closely-linked microbial co-occurrence system was produced in the rhizosphere soil of tobacco plants in response to CBF application. Furthermore, different keystone taxonomies were shaped in microbial co-occurrence networks under CBF and CF treatments. The most important keystone species in CBF were bacteria *s_Stenotrophomonas_sp._MYb57*, *Bradyrhizobium_elkanii_g__Bradyrhizobium*, *Rhodococcus_erythropolis*, *Novosphingobium_resinovorum*, *Bacillus_megaterium_NBRC_15308__ATCC_14581*, fungi *s_Penicillium_alogum*, *Microdochium_colombiense*, *Chaetomium_grande*, *Sampaiozyma_sp*, *Oidiodendron_truncatum*, and those in CF were bacteria s_*Lentzea_aerocolonigenes*, *Sphingobacterium_multivorum*, *Rhodococcus_wratislaviensis*, *uncultured_bacterium_5G12*, *Flexivirga_sp._g__Flexivirga*, and fungi *s_Olpidium_brassicae*, *Staphylotrichum_sp*, *Papulaspora_sepedonioides* and *Mortierellomycotina* (Fig. 5).

Correlations among tobacco leaf yield, value, soil chemical properties and microbial community

Lastly, correlation and explanatory power analyses were applied to disentangle the relationships among tobacco leaf yield, value, and soil chemical and microbial properties. Soil pH, OM, and TN contents were identified as the most important chemical factors influencing soil bacterial and fungal community composition (Mantel test, $P < 0.05$, Fig. 6b). Furthermore, tobacco leaf yield and value were positively associated with soil TC, OM contents, bacterial and fungal Ace and Chao index, and the relative abundances of *Arthrobacter*, *Streptomyces* and *Fusarium* ($P < 0.05$). However, they showed negative relationships with the relative abundances of *Sphingomonas*, *Lysobacter*, *norank_f__Gemmatimonadaceae* and *Cladosporium*($P < 0.05$, Fig. 6a). Soil chemical properties, bacterial and fungal community diversity explained 25.6, 21.4, and 32.5% of the total variance for tobacco yield, and they were 24.4, 29.1, and 23.0% for tobacco value, respectively. Soil TC, bacterial PCoA1, and the bacterial and fungal Chao index were the most important explanation factors in all the analyzed indicators (Fig. 6c).

Discussion

The primary objectives of this study were to determine how BFs application alleviate tobacco CCO and further disentangle the key factors that were potentially associated with this function. Our results revealed a positive effect of BFs addition in soil on the alleviation of tobacco CCO under two rotation regimes and two study years, which was reflected in reduced tobacco morbidity and improved tobacco agronomic traits, wet and dry biomass, economic parameters, and intrinsic chemical quality under CC conditions. CBF owned the optimal efficiency as compared with WBF and RBF, and this might be ascribed to synergistic cooperation between wood and rice straw biochar in the enhancement of tobacco growth. Nonetheless, the phenomenon of tobacco CCO was gradually aggravated with the prolongation of CC years. Therefore, it is suggested to combine BFs amendment with other practical agricultural measures for tobacco sustainable production.

A considerable number of previous studies have demonstrated the excellent ability of biochar and vermicompost as soil conditioners to regulate and recover soil properties and improve crop yield and quality [2, 35, 36, 37, 38, 39]. Co-application of biochar and vermicompost significantly alleviated cucumber (*Cucumis sativus* L.) CCO [37], and this is coherent with the results in our present study. [36] reported that the growth of *Radix pseudostellariae* was improved with biochar addition by stimulating the changes in abundance and metabolism processes of rhizobacteria and fungi, and by inhibiting the activities of pathogenic fungi on plants. As recently shown by [2], biochar addition in soil (15t ha^{-1}) significantly increased the survival rate of *Panax notoginseng* under ten-years CC circumstances through changing soil physicochemical properties and microbial diversities. Vermicompost function in soil-borne disease control and crop yield and quality enhancement is largely linked to its properties of porous structure, rich nutrients, good water-holding capacity, and abundant beneficial antagonistic microbes [38, 39]. Based on these previous reports, we hypothesized that the mechanisms of BFs-associated alleviation of tobacco CCO was related to one or more of the following factors: inhibition in pathogen loads, amelioration in soil chemical characteristics, or shifts in rhizospheric microbial communities. To address this hypothesis, we focused on analyzing the changes of these factors under different fertilizer treatments and seeking the closed correlations between the factors and tobacco indicators.

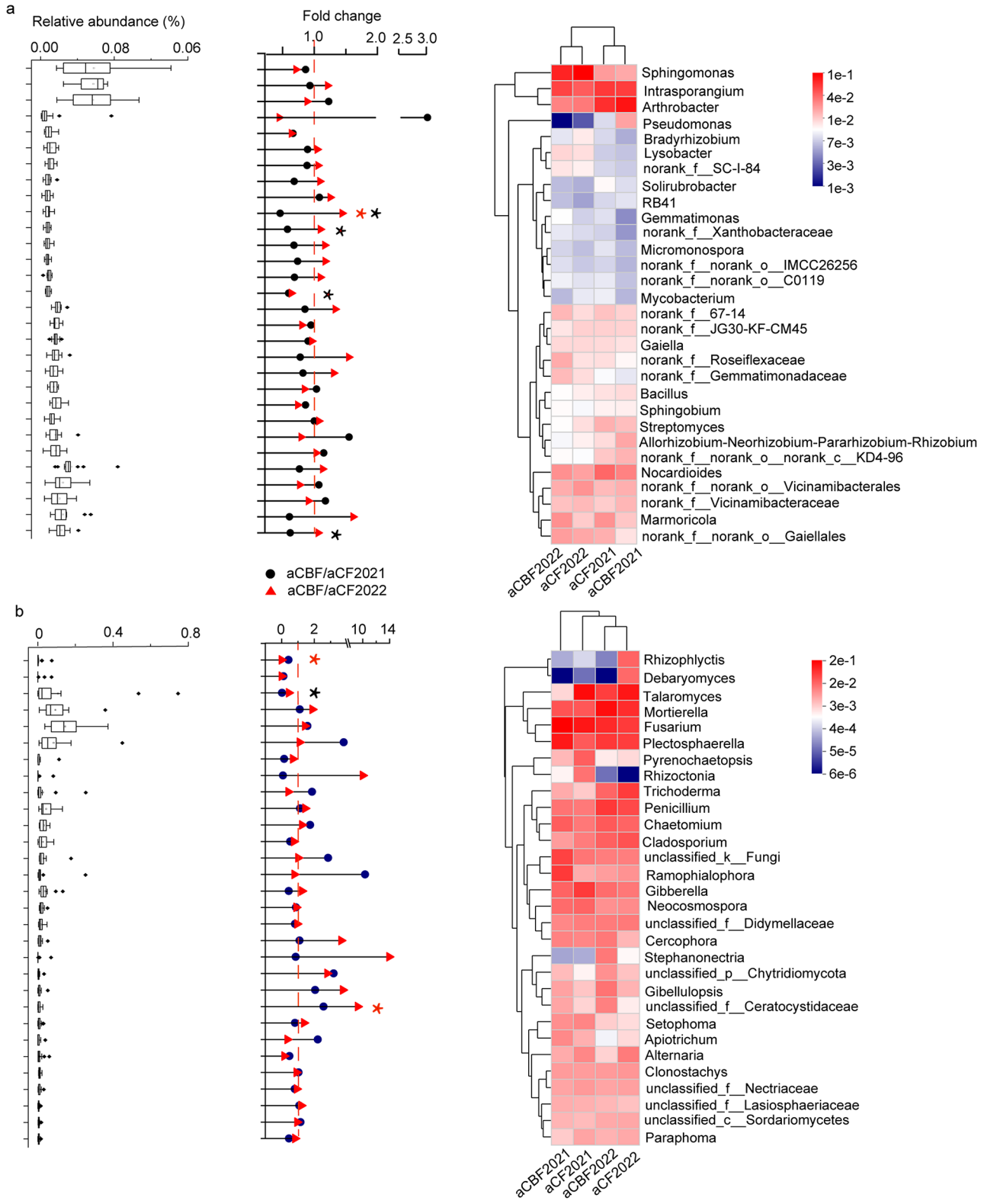


Fig. 4 The changes of the relative abundance of rhizosphere microbial communities in tobacco-broad bean rotation regime under CBF and CF treatments for over 2 years. (**a**) The abundances of the top 30 bacterial and (**b**) fungal genera: relative abundance over all samples; fold change in relation to CBF and CF treatments; and heatmap of the relative abundance for each genus. "*" indicates a significant difference for the relative abundance of genus between CBF and CF treatments (Wilcoxon test, $P_{FDR} < 0.05$, $n = 4$). aCBF and aCF represent compound biochar-based organic fertilizer and chemical fertilizer treatments in tobacco-broad been rotation regime, respectively

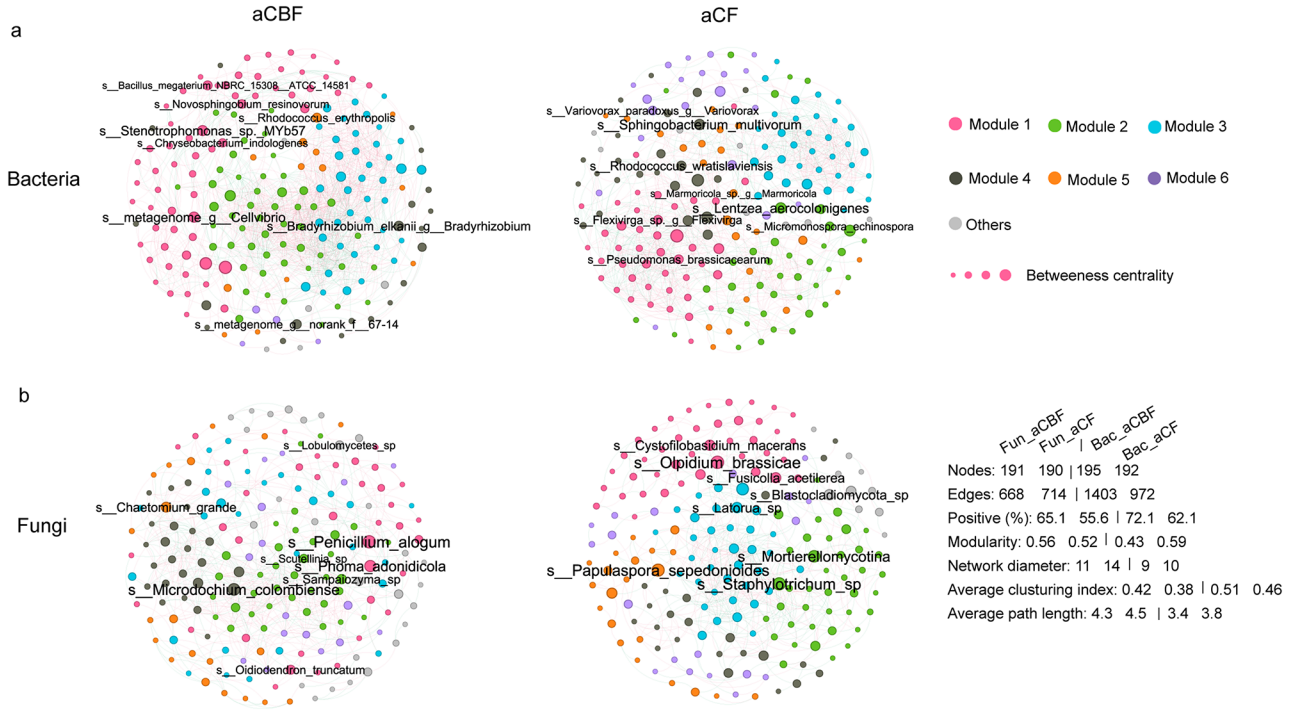


Fig. 5 Microbial community co-occurrence networks in tobacco rhizosphere soil under CBF and CF treatments. (**a**) Bacterial and (**b**) fungal co-occurrence networks based on correlation analysis (spearman's $\rho \geq 0.8$, $P < 0.05$). Only the first 200 dominant species were used to analyze. The nodes are colored by modularity class. The size of each node is proportional to the betweenness centrality, and of each edge is proportional to the ρ value. The top 8 species owing the highest betweenness centrality were shown in each network

Soil acidification has been identified as an important contributor to tobacco CCO, as it can result in phenolic acid accumulation and the alteration of bacterial community structures and diversities in soil [8]. Specifically, acidic soil conditions (pH 4.5–5.5) foster the growth of tobacco pathogen *Ralstonia solanacearum* and suppress the activities of antagonistic bacteria, e.g., *Pseudomonas fluorescens* and *Bacillus cereus* [40]. Biochar addition can increase soil pH due to its alkaline feature, thus contributing to the amelioration of soil acidification caused by long-term CF application and CC pattern [2], and this is in accordance with our present study. Simultaneously, soil OM, AP, AK, MBC, MBN, TC, and TN contents increased under BFs treatments in our study. Biochar and vermicompost additions directly supplement soil with organic C and OM, which are essential for nutrient retention, the formation of soil macroaggregates, and the improvement of soil fertility, quality, and ecosystem's balance [2, 39, 41, 42]. Noteworthily, soil C pool loss has aroused considerable concerns in the tobacco industry because it coincides with reduced tobacco chemical quality and desirable smoking characteristics, such as a strong caramel-sweet aroma [35]. Biochar application promotes C restoration and hormonal actions in soil, then changes the secondary metabolisms in tobacco plants, and ultimately stimulates aroma substances and taste production in tobacco leaves [43]. Biochar and vermicompost can improve soil nutrient levels through the direct release of rich nutrients in these two materials and the indirect changes in soil enzymic activities relating to soil C, N, P, K-cycling [18], which provide tobacco plants with more available nutrients. These results suggested that an amelioration in soil chemical properties plays an important role in alleviating tobacco CCO with BFs addition, particularly for soil TC and OM contents, as supported by the Spearman correlation and explanatory power analyses.

The availability of heavy metals in soil and their accumulation in tobacco have a great possibility to hinder the normal growth of tobacco and pose a severe underlying risk to the health of human beings and animals [44]. In addition to soil basic chemical properties, BFs application may reduce the accumulation of heavy metals in tobacco, which is a typical Cd accumulator [45]. In this study, a reduced trend was observed in Cd, V, Cu, and Zn contents in tobacco plants with BFs application, and this is in consensus with the previous studies, which reported lower Cr, Cu, Cd, Ni, Pb, Zn contents in soil and their accumulation in tobacco under biochar application alone or with vermicompost [44, 46, 47]. This phenomenon may be attributed to the immobilization processes of ion exchange, surface precipitation, electrostatic attraction in biochar, and adsorption processes of OM and humus in vermicompost, which synergistically declined heavy metal availability [44, 46, 47, 48, 49]. Besides, biochar

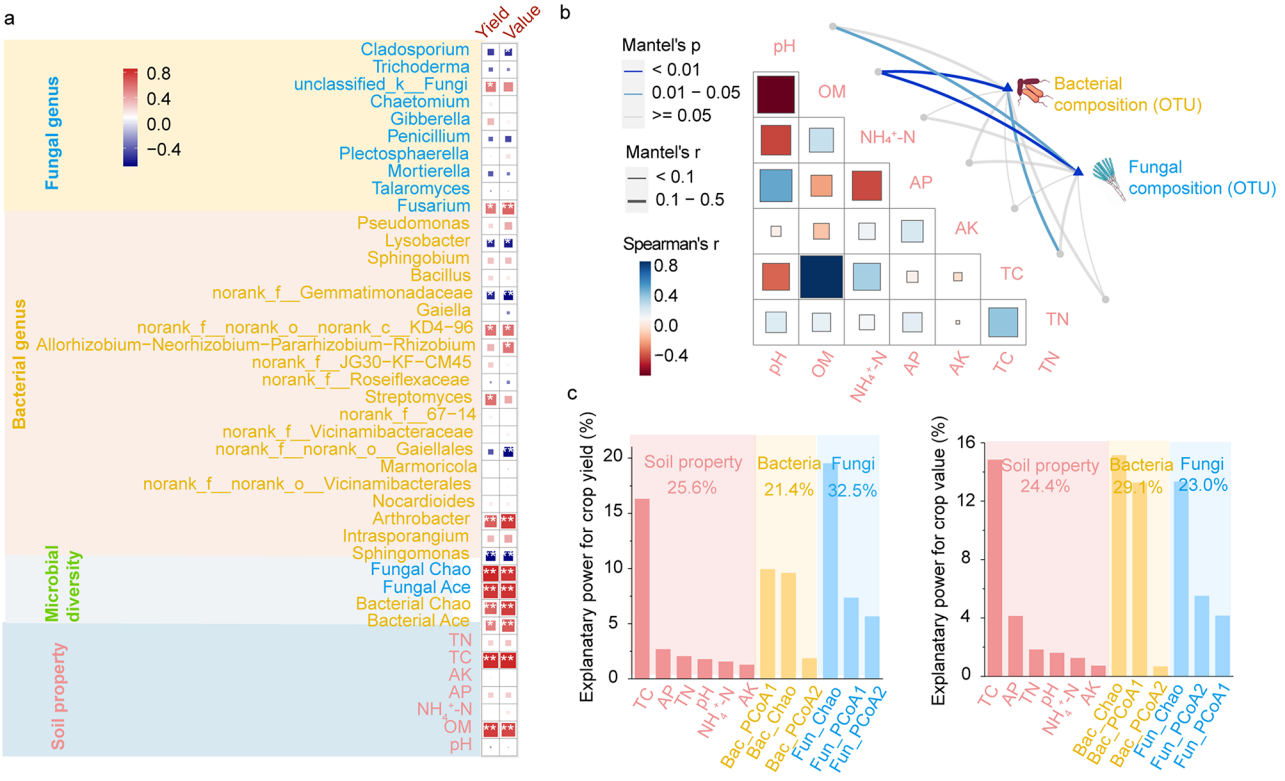


Fig. 6 Correlation and contribution analyses among soil properties, microbial communities and tobacco parameters. (**a**) Spearman correlation heatmap among tobacco leaf yield, economic value, soil properties and microbial communities. Only the first 20 dominant bacterial and 10 dominant fungal genera were shown. "*" and "**" indicate statistical significance at $P < 0.05$ and $P < 0.01$, respectively; (**b**) Mantel tests for soil properties, bacterial and fungal compositions at OUT level; (**c**) The explanatory power of soil properties, bacterial and fungal community structure and diversity for tobacco yield and economic value based on a linear regression model. AP, AK, TC, and TN represent soil available P, K, and total C and N contents, respectively

can increase soil pH value, and then indirectly reduce the availability of heavy metals in soil [50]. We speculate that BFs addition in soil weakens the negative influences of heavy metals on tobacco plants' growth, and this can at least partially explain the positive effects of BFs application on tobacco CCO alleviation. Although the heavy metal contents in tobacco plants decreased with BFs application for 2 years, this phenomenon need to be further studied over a longer experiment period, as the heavy metals in biochar may be released with the aging processes in soil.

Soil microbiome plays crucial roles in soil multifunctionality and is linked to stimulation of plant defense mechanisms [51, 52]. A majority of studies have broadly reported the excellent abilities of organic amendments to alleviate environmental stresses and shift soil microbial communities [9, 11, 34, 47, 52]. In our study, rhizobacterial OTUs numbers and α-diversity increased with CBF amendment under both tillage regimes, demonstrating that CBF can improve soil bacterial community diversity and richness, similar to earlier observations in organic manure-treated or biochar-treated soil [14, 37, 42]. Microbial diversity is considered an important indicator to evaluate soil health as it can control the invasion of crop pathogens by stimulating the functionality of terrestrial ecosystems [53, 54]. Thus, we hypothesize that a rise in rhizobacterial α-diversity facilitates the alleviation of tobacco CCO with CBF addition. However, rhizosphere fungal α-diversity did not show any apparent difference between CBF and CF treatments, demonstrating more significant effects of CBF addition on bacterial α-diversity than fungi. Furthermore, CBF amendment in soil also transformed bacterial and fungal community structures as compared to CF, and this was associated with the enhancements in tobacco yield and economic value according to the Spearman correlation and explanatory power analyses.

Agricultural practices and environmental variability possess a great influence on the complexity and stability of soil microbial community, which has been extensively studied and verified using network analysis [6, 55–56]. [57] demonstrated that a higher abundance of *Ralstonia solanacearum* in the tomato rhizosphere resulted in decreased microbial connections in co-occurrence networks. Here, we observed more complex and stable rhizosphere microbial co-occurrence networks with CBF amendment, and this revealed that CBF addition in soil improved positive collaborations and interactions

in rhizosphere microbes, which was consistent with the results from [34]. Besides, the keystone roles of some beneficial taxa were stimulated in the microbial networks, such as bacteria *Stenotrophomonas_sp._MYb57*, *Bradyrhizobium_elkanii_g__Bradyrhizobium*, *Novosphingobium_resinovorum*, *Bacillus_megaterium_NBRC_15308__ATCC_14581*, and fungi *Penicillium_alogum*, *Chaetomium_grande*. It is likely that the positive roles of these beneficial microorganisms are strengthened in managing and adjusting soil microbial networks.

Our results showed that CBF amendment in soil enriched some underlying pathogen-suppressive and plant growth-promoting microbes (PGPM), such as bacterial genus *Arthrobacter*, *Pseudomonas*, *Gemmatimonas*, and fungal genus *Trichoderma*, *Mortierella*, *Penicillium*, *Chaetomium* and *Gibellulopsis*. Stimulation in soil indigenous *Pseudomonas* populations can enhance the suppression of Fusarium wilt disease in banana [11]. [58] reported that Pseudomonadaceae members played a predominant role in the plant disease-suppressive microbial consortia by producing a putative chlorinated lipopeptide encoded by NRPS genes. Simultaneously, some other taxa, *Arthrobacter*, *Trichoderma*, *Mortierella*, *Penicillium*, *Chaetomium* and *Gibellulopsis*, have been recognized as disease suppression-associated microbes and produced into biocontrol agents with broad applications in fields [5, 58, 59, 60, 61, 62, 63]. Oppositely, there was a decline in the relative abundances of some potential plant pathogens and their synergetic microorganisms under CBF treatment, such as *Mycobacterium*, *Rhizophlyctis*, *Debaryomyces* and *Alternaria*. *Alternaria solani*, belonging to the *Alternaria* genus, is a notorious fungal pathogen causing pear black spot disease and blight disease in Tomato and *Solanum lycopersicum* [64, 65, 66]. In addition, based on the correlation analysis, tobacco leaf yield and economic value were negatively correlated with the relative abundances of *Sphingomonas*, *Lysobacter*, *norank_f__Gemmatimonadaceae* and *Cladosporium.* [6] reported that the relative abundance of *Sphingomonas* increased with the prolongation years of continuous cotton cultivation, which was in accordance with our results. *Cladosporium* is also identified as a type pf plant pathogens that can induce leaf lesions on *Vicia faba* seedlings [67]. Therefore, we consider that a healthier soil microecology environment was formed in response to CBF application, which contributed to the control of tobacco diseases and the alleviation of tobacco CCO. Moreover, the shifts in soil microbial community composition and structures were primarily steered by soil pH, OM, and TN contents. Lastly, our results indicated that BFs action in the alleviation of tobacco CCO can be appreciably ascribed to the improvements in soil chemical properties and microbial community structures and diversities, with soil TC, bacterial PCoA1, and bacterial and fungal Chao indexes showing the most important explanatory power.

Here we provide an effective and environmentally friendly scheme for the alleviation of tobacco CCO, and this can also provoke some thoughts and inspiration according to the results of current study. For instance, screening and isolating crucial beneficial microbes is suggested to further elucidate and validate their positive roles in tobacco growth in future studies. Bio-organic fertilizers production with isolated microbes is a fascinating strategy for targeted manipulation of rhizosphere microbial assemblages. Additionally, we need to explore the optimal application rate and frequency of BFs as well as combinations with other useful agricultural measures under a long-term field condition.

Conclusions

The present study confirmed that BFs addition in soil mitigated tobacco CCO and contributed to tobacco sustainable farming, and this positive function was triggered by changes in soil chemical properties, heavy metals (Cd, V, Cu, Zn) contents in tobacco plants, and rhizospheric microbial community composition and structures. Specifically, BFs amendment in tobacco CC soil improved microbial diversities, structures and their co-occurrence networks accompanied with a growth of some beneficial microbes and a decrease of tobacco pathogens (*Phytophthora nicotianae* and *Fusarium oxysporum*) in the rhizosphere. This study highlights a joint action mechanism of BFs application in the alleviation of tobacco CCO, and provides a useful guide for BFs practical application in fields and an important theoretical basis for future elucidation of another mechanism of crop CCO mitigation. BFs addition may be a promising approach or strategy for agriculture sustainable development.

Abbreviations

CCO	Continuous cropping obstacles
WBF	Wood biochar-based organic fertilizer
RBF	Rice straw biochar-based organic fertilizer
CBF	Compound biochar-based organic fertilizer
CF	Chemical fertilizer
OFs	Organic fertilizers
OM	Organic matter
ICP-OES	Inductively coupled plasma optical emission spectrometer
NH_4^+-N	Ammonium nitrogen
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
DNA	Deoxyribonucleic acid
pH	Power of hydrogen
AP	Available phosphorous
AAS	Atomic absorption spectrometer
AK	Available potassium
TOC	Total organic carbon
TC	Total carbon
TN	Total nitrogen
qPCR	Quantitative polymerase chain reaction
16S rRNA	16S ribosomal ribonucleic acid
ITS	Internal transcribed spacer
OTUs	Operational taxonomic units

NCBI	National Center for Biotechnology Information
SRA	Sequence Read Archive
PERMANOVA	Permutational multivariate analysis of variance
ANOSIM	Analysis of similarities
PCoA	Principal coordinate analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06266-7.

Supplementary Material 1

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Author contributions
All authors took part in the study conception and design. D.C., Y.Z., G.W., K.D., J.L., X.S., Y.X., Y.C. performed experimental work and collected data. D.C. and K.D. performed the data interpretation. D.C. wrote the manuscript, and X.Y. helped to edit the manuscript. All authors read and approved the final manuscript.

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Data availability
The raw sequence data were deposited in NCBI Sequence Read Archive (SRA) database with the accession number PRJNA944097. Data in this study will be made available on reasonable request.

Declarations

Ethics approval and consent to participate
The current field study including the collection of tobacco plants, is complying with relevant institutional, national and international guidelines. Tobacco sampling is under private land owner's permissions and only used for research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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