PLASTIC FILM ALTERS SOIL PHYSICOCHEMICAL PROPERTIES AND MICROORGANISMS, AFFECTING THE GROWTH OF CIGAR TOBACCO

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Abstract. In recent years, frequent extreme drought and rainfall have seriously affected crop production. Film mulching has been applied to dry farming, but little is known about its influence on cigar tobacco growth in high-temperature, high-humidity environments. Here, in our study, plastic film was used as a soil mulching materials to investigate its influence on soil physicochemical properties, tobacco growth and rhizosphere soil microorganisms. The un-mulched soil served as the control. The results showed that the film mulching significantly increased soil organic matter (OM), available phosphorus (AP), available potassium (AK), chlorophyll and nicotine in tobacco leaf, while decreasing total sugar, reducing sugar and starch. Redundancy analysis further showed that OM, AP and AK were significantly positively correlated with chlorophyll a, lutein and carotenoid ($p < 0.05$). Principal component analysis (PCA) indicated that film mulching significantly influenced the community structure of rhizosphere soil microorganisms, and decreased the relative abundance of Proteobacteria, but increased the relative abundance of Chytridiomycota, Firmicutes and Chloroflexi. These increased species were significantly positively correlated with AP and AK ($p < 0.05$). Besides, the film mulching significantly increased the abundance of beneficial microorganisms (*Bacillus*), and decreased the abundance of pathogens (*Fusarium*), thereby controlling the occurrence of bacterial wilt. Our findings indicated that film mulching promotes cigar tobacco growth by improving soil physicochemical properties and microorganisms in high temperature and humidity environment.
Keywords: *film mulching, cigar tobacco, soil ecology, rhizosphere, microbial community*

Introduction

Presently, the rising trend of extreme droughts and heavy rainstorms has severely challenged global agricultural production. These climatic fluctuations directly and

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indirectly affect soil health and crop productivity, often reducing yield and deteriorating crop quality (Zittel et al., 2020; Beutel et al., 2021). Among adaptive strategies in dry farming, plastic film mulching is widely used to reduce water loss and stabilize soil temperature. Protecting against environmental stresses, plastic film mulching showed prospects of improving the soil for various crops: grain, vegetable, and fruit trees. Meanwhile, its behavior with more special crops, like cigar tobacco, in highly warm and humid areas, has rarely been explored (Bonanomi et al., 2015; Zhu et al., 2024).

Cigar tobacco (*Nicotiana tabacum*) needs definite soil and climatic conditions for the leaves to have optimum chemical composition for flavor and burn characteristics. In high-temperature, high-humidity environments, plastic film mulching could have a dual impact by promoting soil moisture retention, while both the soil physicochemical properties and microbial communities are altered (Briški et al., 2012; Zhang et al., 2024). Soil microorganisms make contributions to nutrient cycling, organic matter decomposition, and pathogen suppression, which in turn influence plant growth and resilience. To determine the overall effect of plastic mulching on crop health and yield quality, one has to understand how it impacts soil chemistry and alters microbial dynamics (Wang et al., 2023).

With the drastic changes in the global climate environment, frequent climate change leads to increased agricultural losses and threatens global food security (Butler and Kefford, 2018; Orlinka-Woniak et al., 2021; Sanjuán et al., 2022). Climate change has created considerable uncertainty about future water availability in many regions, with extreme droughts and rainfall in recent years having serious implications for human survival and crop yield (Hardelin and Lankoski, 2015; Saadi et al., 2015). Drought is considered to be the most damaging environmental stress, which directly affects the entire growing phase of plant seeds from germination to final fruit (Fu et al., 2021; Rabbi, 2021). Floods not only harm the growth and yield of crops, but also cause the loss of soil nutrients and the spread of diseases and pests (Rumanti et al., 2018; Bofana et al., 2022).

With the change in climatic trend, extreme weather conditions such as drought and heavy rainfall have almost become a routine and hence have adversely affected the yields all over the world. One of the most common methods for dry land farming involves retaining soil moisture and controlling soil temperature through a motion of a layer of plastic over the soil (Hou et al., 2020; Shah et al., 2023). Benefits arising under arid conditions by film mulching in various crops have been documented. However, it is barley known what effect it has on cigar tobacco under high-temperature and high-humidity conditions. Cigar tobacco is a high-quality leaf-producing type, and its yield and desirable chemical composition depend upon special soil properties and nutrient availability (Piotrowska-Cyplik et al., 2009; Luo et al., 2023). This current study attempts to investigate responses of some key soil properties and tobacco plant growth factors, such as soil organic matter, available phosphorus, available potassium, and plant factors of chlorophyll content and nicotine concentration, to plastic film mulch.

Soil mulch has been used globally as an effective strategy to improve soil properties and promote crop growth. Plastic film is widely used in crop production because it serves to conserve soil moisture, suppress weeds, control pests, regulate soil temperature (Lela et al., 2024). Especially in arid and semi-arid regions, the film maintains soil temperature and moisture, preventing weed growth and ultimately helping to improve agricultural production (Akpor et al., 2023). Previous studies have showed that mulching improves tuber yield, water productivity, and nitrogen use efficiency, increasing soil nutrient availability in arid fields by increasing soil temperature and moisture (Li et al., 2012; Tian

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et al., 2023). In rainy areas, ridging and mulching can also reduce soil erosion by rain and avoid soil erosion. However, soil high temperature and humidity caused by mulching inhibit root growth, which is usually harmful to crop growth (Akpor et al., 2023).

Plastic film mulching tends to show not only its effects on soil moisture retention but may further have an impact on nutrient dynamics and microbial community composition, at the very root of plant health. Such increased levels of OM, AP, and AK can enhance chlorophyll synthesis and nicotine accumulation in leaves, improving plant vigor and increasing leaf quality (Piotrowska-Cyplik et al., 2009; Mandić et al., 2023). Different mulching practices might influence the soil microorganisms regarding their composition and functioning, having significant importance for nutrient cycling, decomposition, and the suppression of pathogens. The population growth of some of the beneficial *Bacillus*, while reducing some pathogenic *Fusarium* species, culminates into overall increased plant resistance against bacterial wilt and other soil-borne diseases, hence resulting in improved crop resilience (Akpor et al., 2023).

In recent years, China's Hubei, Sichuan, Hainan, Yunnan and other production areas actively carry out pilot planting of cigar tobacco. However, due to the late start of cigar tobacco development in China, cigar tobacco is of low quality and small scale, and the ability to guarantee high-quality raw materials is obviously insufficient, which is difficult to meet the needs of cigar product formulation, and seriously affects and restricts the development of cigar (Zhang et al., 2012; Bibi et al., 2024; Rehman et al., 2024; Ullah et al., 2024). In Yunnan, where rainfall is unevenly distributed, tobacco faces drought and high humidity stress during the transplanting and growth stages, respectively. Flue-cured tobacco is mostly planted by ridging and mulching to promote tobacco growth and ensure tobacco quality. However, there are few studies on whether the ridging and mulching method is suitable for cigar tobacco cultivation in high temperature and humidity environment. Therefore, the aim of this study was to investigate the effects of plastic film mulching on changes in (1) soil chemical properties; (2) tobacco growth and quality; (3) diversity and composition of rhizosphere soil fungal and bacterial communities.

Materials and methods

Experimental design

The experiment was set in Longyang (N:25.13º E:99.17º, H:810m) with the monsoon climate type of South subtropical region. The field has a sandy loam texture. At the start of the trial, the main chemical properties of the surface soil (0–20 cm) were measured (pH 5.84, organic matter (OM) 18.00 g/kg, available nitrogen (AN) 86.00 mg/kg, available phosphorus (AP) 78.00 mg/kg, available potassium (AK) 140.00 mg/kg). The experiment treatment was divided into ridging with film mulching (Treatment) and ridging without film mulching (CK), and each plot sizes was 300 m^2. First, organic fertilizer (3000 kg/ha) and chemical fertilizer (3000 kg/ha) were mixed into soil before ridge, and then tobacco seedlings were transplanted, the row spacing was 110 cm and the plant spacing was 35 cm, and finally covered with black film (July 1st, 2023). The disease incidence of bacterial wilt was observed and recorded at the flourishing-growth period (August 1st, 2023), and the number of plants recorded and observed was not less than 50. Soil and plant samplings were conducted at the maturing period of cigar tobacco (October 1st, 2023). The disease incidence was calculated as follows:

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$$Disease\ Incidence\ (\%) = 100 \times \frac{Diseased\ Seedling}{Total\ Seedling} \quad \text{(Eq.1)}$$

Sampling

One composite rhizosphere soil sample was taken from each plot consisting of roots of 3 randomly selected tobacco plants. The roots were uprooted by shaking the roots, removing the loose soil at the roots, and collecting the soil at the roots with a sterile brush. Then, the fresh soil was passed through a 2 mm sieve and divided into two fractions. One of them were used for the analysis of soil microbial community. The other part was air dried for the determination of soil physicochemical properties. In addition, chlorophyll a, chlorophyll b, lutein, carotenoid, total sugar, reducing sugar, chlorine starch, total nitrogen and total plant alkaloids were measured for 30 tobacco plants, with one replicate per 10 plants and a total of three replicates and all plant parts were air dried.

Analysis of soil physicochemical properties

Soil pH was determined using an Inolab pH/Cond 720 in a water suspension (water:soil, 2.5:1, v-w); AN was determined by the alkali diffusion method; AP determined by $NaHCO_3$ leaching and the molybdenum-antimony colorimetric method (UV visible spectrophotometer HACH L5, USA); AK was determined by ammonium acetate extraction and flame photometry (Flame graphite furnace atomic absorption spectrometer ZEEnit700P, GER); OM was determined using the potassium dichromate-sulfuric acid heating method (Shi et al., 2022).

Statistical analyses

The data was analyzed using SPSS software (ver 17.0). Mean separation among treatments was analyzed by Student t-test. RDA analysis were performed using Canoco software (ver 5.0). PCA analysis were performed using Canoco software ver 5.0 (Shi-Wen et al., 2000).

Results and discussion

Results

The results of soil physicochemical properties showed that compared with Ck, soil OM, AP and AK were significantly improved by mulching treatment, soil pH and AN were increased, but the change was not significant ($p < 0.05$, *Table 1*). According to *Table 1*, plastic film mulching has an evident impact on the physico-chemical properties of the soil, including SOM, AN, AP, and AK. For a treatment group with the application of plastic film mulching, some characteristics of the soil appeared to be improved in comparison with CK without any mulching application.

Table 1. *Effect of plastic film mulching on soil physicochemical properties*

Treatments	pH	OM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
Ck	5.48±0.25	17.46±0.51	102.2±10.72	61.88±3.71	355.48±71.09
Mulching treatment	5.55±0.07	21.97±0.44*	106.12±3.64	102.36±1.14**	632.08±44.22**

The statistical significance is denoted by * $p < 0.05$; ** $p < 0.01$

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In particular, the OM increased to 21.97 g/kg in the treated group, while there is a significant difference with the control value of 17.46 g/kg at $p < 0.05$. The increase of OM shows that plastic film mulching possibly retains or accumulates organic matter within the soil. Besides, the values of AP and AK in the soil have increased significantly under the treatment of mulching. AP varied from 61.88 mg/kg in the control to 102.36 mg/kg in the mulched soil, its increase being highly significant ($p < 0.01$). Accordingly, AK reaches a value of 632.08 mg/kg in the mulched soil, which is approximately double the value recorded for the control group (355.48 mg/kg), with a similar high significance of the difference ($p < 0.01$).

There were slight changes in the soils' pH and and concentration. The soil pH remained quite stable, ranging between 5.48 and 5.55 for control and treatment, respectively, proving that the mulching treatment did not significantly change the acidity of the soil. Similarly, AN experienced a slight build-up in the treated samples, at 106.12 mg/kg for the treated samples and 102.2 mg/kg for the control samples; this was also not statistically significant. Plastic film mulching is inclined towards increasing the soil organic matter, available phosphorus, and available potassium, which are all the nutritional elements required by plants for growth. This, therefore, means that the mulching is going to provide a favorable nutrient environment for higher agricultural productivity. The content of chlorophyll in tobacco was significantly affected by film mulching. Compared with Ck, the contents of chlorophyll a, chlorophyll b, lutein and carotenoid in tobacco were significantly increased by 95.26%, 44.35%, 116.09% and 154.47%, respectively (*Figure 1a-d*). Film mulching also significantly reduced the incidence of tobacco bacterial wilt by 95.20% (1.1) (*Figure 1e*). At the same time, the film mulching has a certain impact on the quality of tobacco. Especially, the contents of total sugar, reducing sugar and chlorine starch were significantly decreased ($p < 0.05$), while the contents of total nitrogen and total plant alkaloids were increased, but the differences were not significant (*Figure 1f*).

The coverage index of each ecoregion was over 0.99, and no significant difference was found ($p > 0.05$, *Table 2*), indicating that the sequencing results were reasonable and could better reflect the actual situation of bacterial and fungal communities. The alpha diversity (including Shannon, Simpson, Ace and Chao diversity) could reflect richness and diversity of soil fungal and bacterial communities. For fungi, Ace and Chao index of film mulching treatment were lower than Ck, but Simpson index of treatment was significantly lower than Ck ($p < 0.05$). For bacteria, Ace, Chao and Shannon index of treatment was lower than Ck. The Coverage index of the bacterial community and fungal community in each treatment was higher than 0.99. No significant difference between treatments was found ($p > 0.05$), which proved that sequencing depth was sufficient and the results could reliably represent the actual structure of the bacterial and fungal communities.

For fungi, Ace and Chao indices were always lower in film mulching treatment than in the control, indicating that mulching slightly decreased the estimated fungal species richness. In contrast, the Simpson index showed significantly higher evenness in mulching than in the non-mulching plots, indicating that the mulching treatments significantly increased evenness in the fungal community in less than 0.05 probability. For bacteria communities, Ace, Chao, and Shannon indices were all lower in the treatment compared with the control, indicating that bacterial richness and diversity decreased under film mulching. However, no significant change of Simpson index reflected that bacteria evenness kept relatively stable among treatments.

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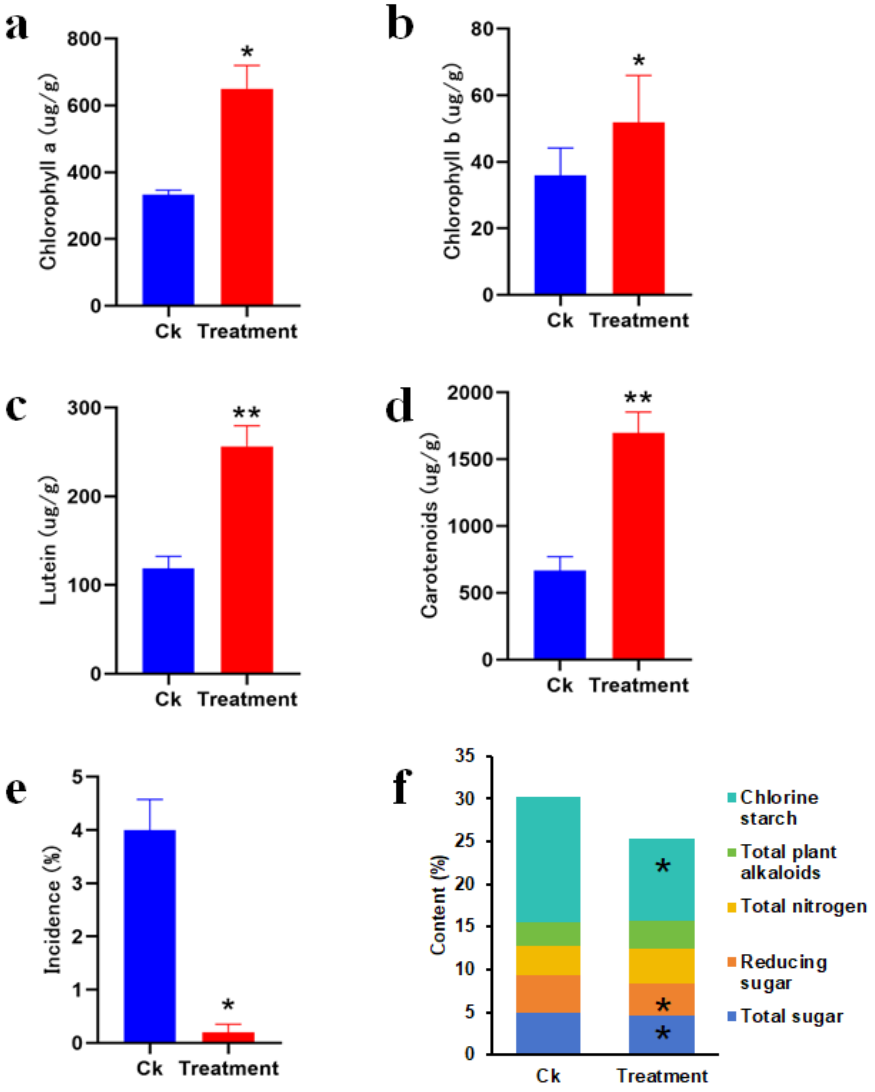

Figure 1. *The effect of film mulching on cigar tobacco growth. (a). Chlorophyll a; (b) Chlorophyll b; (c) Lutein; (d) Carotenoid; (e) Incidence rate of bacterial wilt; (f) Quality of tobacco leaves. The statistical significance is denoted by * $p < 0.05$; ** $p < 0.01$*

Table 2. *Alpha diversity of bacterial and fungal communities in rhizosphere soil*

Category	Treatments	Ace	Chao	Shannon	Simpson	Coverage
Fungi	Ck	768.72±28.88	769.60±22.54	3.90±0.15	0.07±0.01	0.997
	Mulching treatment	729.16±33.39	726.70±33.97	3.99±0.08	0.05±0.01*	0.997
Bacteria	Ck	4562.35±222.94	4376.27±221.42	6.60±0.08	0.005±0.0005	0.997
	Mulching treatment	4040.24±89.51	3822.51±75.88	6.49±0.04	0.006±0.0003	0.997

The statistical significance is denoted by * $p < 0.05$; ** $p < 0.01$

The results of PCA showed that the two treatments could be separated significantly on the PC1 axis (*Figure 2a,b*). The contribution rates of PC1 and PC2 to the species composition differences among different treatments were 18.34%/12.84% (fungi) and

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17.46%/13.33% (bacteria), respectively. In soil fungal and bacterial communities, Ck and Treatment could be significant distinguished on the PC1 axis. The results indicated that film mulching significantly affected the community structure of fungi and bacteria.

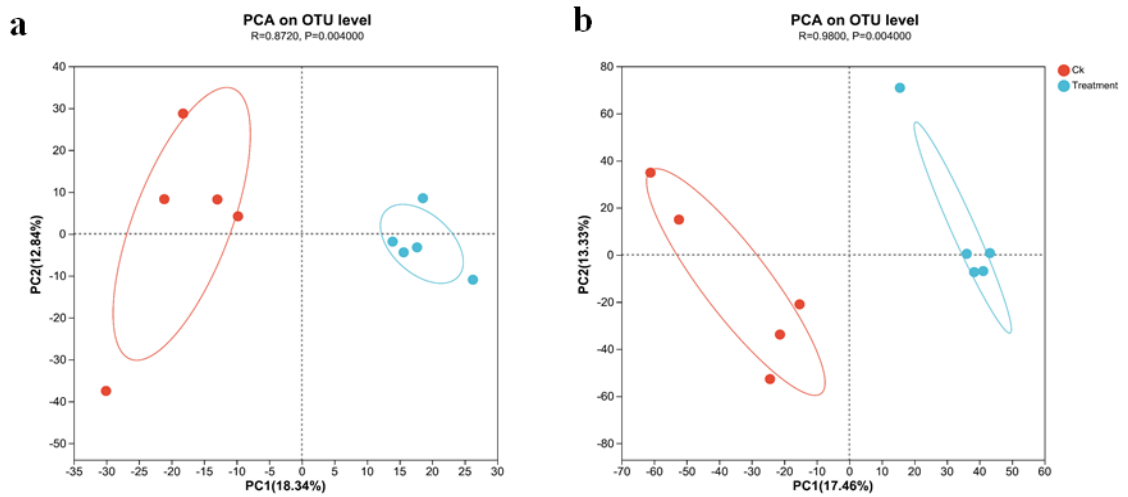

Figure 2. *Similarity and differentiation of the fungal and bacterial communit. (a) PCA plot of fungal communities at the OTU level; (b) PCA plot of bacterial communities at the OUT level*

The fungal phyla with high relative abundances were Ascomycota, Basidiomycota, Mortierellomy and Chytridiomycota. More than 90.00% of these fungi were detected in different groups, and film mulching treatment significantly increased the relative abundance of Chytridiomycota (*Figure 3a*). The results of the dominant fungal genera showed that the relative abundance of *Thielavia*, *Albifimbria*, *Curvularia*, *Acrophialophora* and *Sagenomella* were significantly increased by film mulching treatment, and the relative abundance of *Chaetomium*, *Fusarium*, *Saitozyma* and *Neocosmospora* were significantly decreased (*Figure 3c*). The bacterial phyla with high relative abundances were Actinobacteriota, Proteobacteria, Firmicutes, Chloroflexi, Acidobacteriota, Gemmatimonadota, Patescibacteria and Bacteroidota (*Figure 3b*). More than 91.00% of these bacteria were detected in different groups. The relative abundance of Firmicutes and Chloroflexi in film mulching treatment were significantly higher than Ck, and Proteobacteria in film mulching treatment was significantly lower than that Ck. The results of the dominant bacterial genera showed that the relative abundance of *Bacillus*, *Micromonospora* and *Bryobacter* were significantly increased by film mulching treatment, and the relative abundance of *Sphingomonas*, *Knoellia*, *Nitrospira*, *Haliangium*, *Flavisolibacter* and *Paenibacillus* were significantly decreased (*Figure 3d*).

The redundancy analysis was conducted with tobacco growth and microorganisms as response variables, and soil physicochemical properties as explanatory variables (*Figure 4*). The results showed that AP and AK were the main controlling factors affecting tobacco growth and rhizosphere microorganisms. Specifically, AP and AK were significantly positively correlated with Firmicutes and Chloroflexi, while significantly negatively correlated with Proteobacteria (*Figure 4a*, $p < 0.05$). In addition, OM, AP and AK were positively correlated with carotenoid, lutein and chlorophyll a, and negatively correlated with total sugar and reducing sugar (*Figure 4b*, $p < 0.05$).

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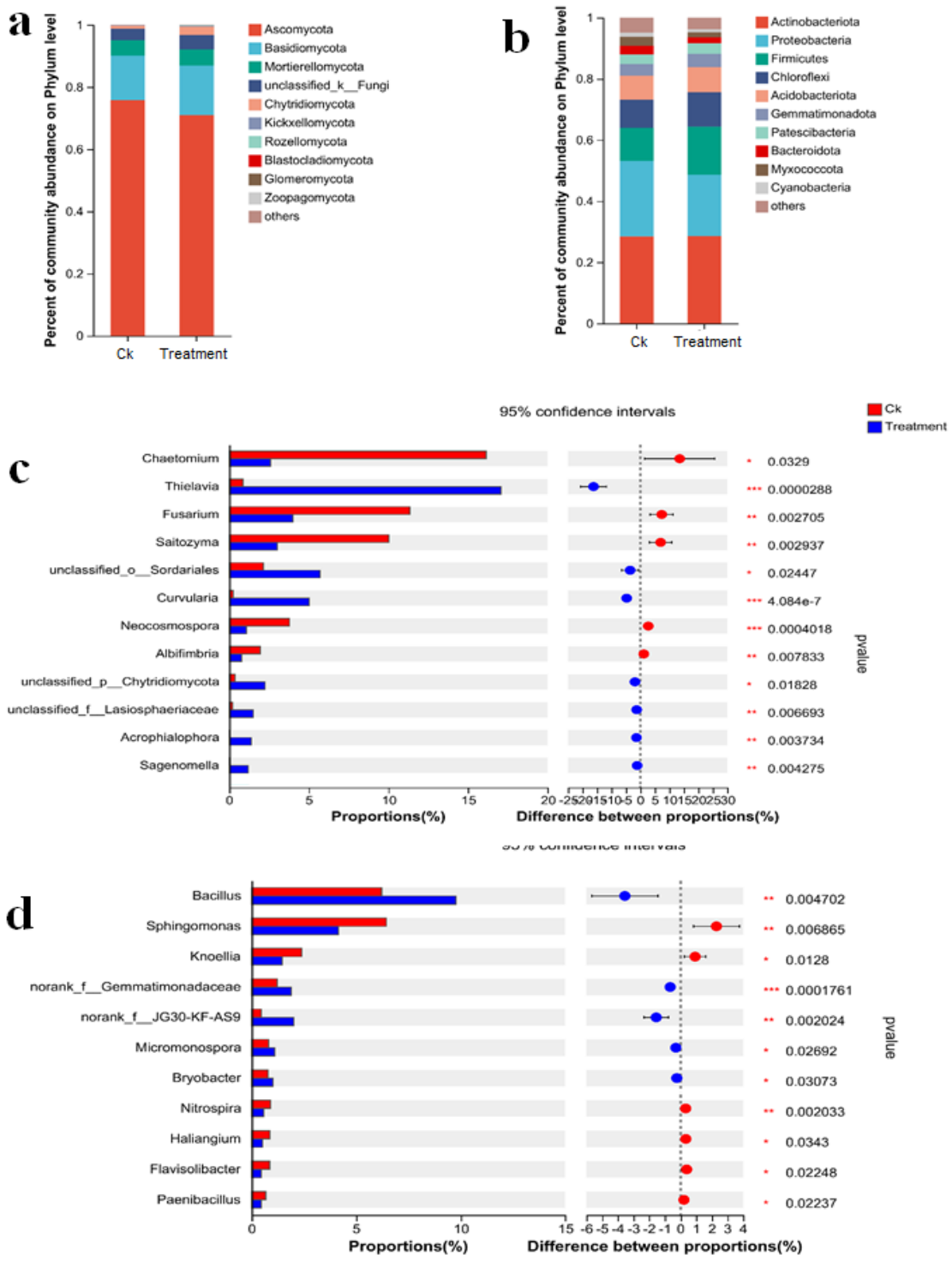

Figure 3. *Comparative analysis of the top species phyla and genera. (a) Relative abundance of the fungal phyla. (b) Relative abundance of the bacterial phyla. (c) Comparison of fungal genera with significant differences. (c) Comparison of bacterial genera with significant differences*

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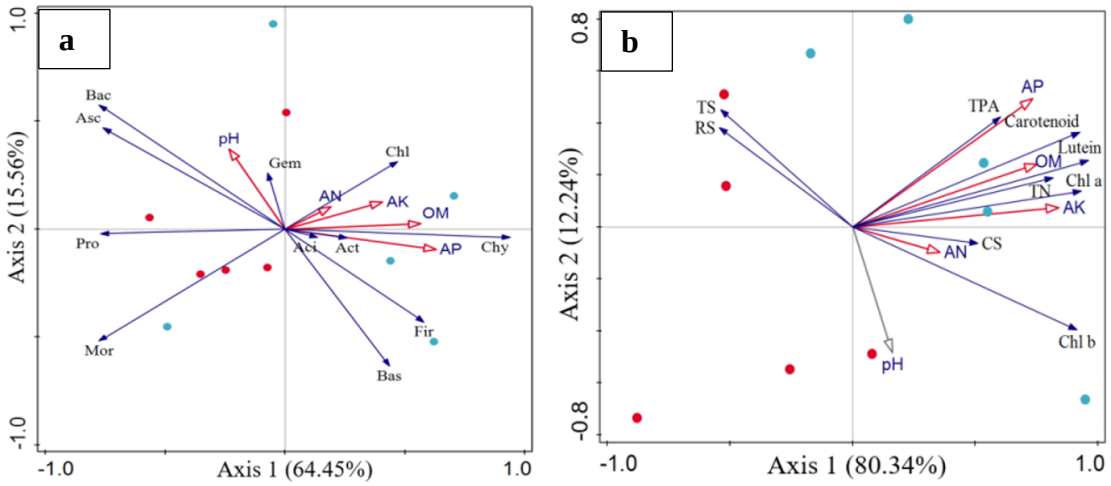

Figure 4. *The redundancy analysis of soil physicochemical properties, microorganisms and cigar tobacco growth. (a) Correlation between soil physicochemical properties and microorganisms. (b) Correlation between soil physicochemical properties and cigar tobacco growth. Aci: Acidobacteriota; Act: Actinobacteriota; Asc: Ascomycota; Bac: Bacteroidota; Bas: Basidiomycota; Chl: Chloroflexi; Chy: Chytridiomycota; Fir: Firmicutes; Gem: Gemmatimonadota; Mor: Mortierellomycota; Pro: Proteobacteria; TS: Total sugar; RS: Reducing sugar; TN: Total nitrogen; TPA: Total plant alkaloids; CS: Chlorine starch; Chl a: chlorophyll a; Chl b: chlorophyll b*

Discussion

Soil is one of the important ecological conditions affecting the tobacco growth, and soil physicochemical properties directly affect the yield and quality of tobacco (Wan et al., 2018). Mulching increased crop yield, water productivity, and nitrogen use efficiency, by increasing soil temperature and moisture, and improving the availability of soil nutrients (Zhang et al., 2019; Qin et al., 2022). In this study, compared with the control, the content of soil OM, AP and AK was significantly increased by mulching, which was consistent with Yin's study (Yin et al., 2022). In our study, mulching promoted the chlorophyll of tobacco leaves increased the ratio of nitrogen to base (*Figure 1f*). Hooper et al. (2015) indicated that the mulching treatment increased the nitrogen content in soil, promoted the nitrogen absorption of tobacco plants, and caused the increase of nicotine and total nitrogen content. It is therefore expected that the shift observed in the composition of the microbial community, due to an increase in beneficial microorganisms like Bacillus and a decline in pathogenic species such as Fusarium, indicate that mulching could indeed favor the health of the soil by promoting a disease-suppressive microbial community (Zhang et al., 2019; Qin et al., 2022). Overall, an improvement in microbial balance, reduced fungal richness, and bacterial diversity would thus suggest that plastic mulching acts selectively on beneficial organisms in the soil for plant health and resilience. Accordingly, through the collation of such findings, plastic film mulching could demonstrate potentials not only in increasing the soil nutrient profile but also in building a microbial community suitable for tobacco production in less favored climates (Briški et al., 2012; Beutel et al., 2021; Zhu et al., 2024). At the same time, the ratio of sugar to base were also decreased, which may be because the increase of soil nitrogen content was conducive to the conversion of nitrogen-containing compounds, but was not conducive to the conversion of carbohydrate to beneficial components (Lisuma et al.,

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2021; Qadeer et al., 2023; Younus et al., 2024). Our results indicated that film mulching could harmonize tobacco chemical composition by improving soil physicochemical properties.

In our study, film mulching significantly inhibited bacterial wilt (*Figure 1e*), and influenced the community structure of rhizosphere soil microorganisms (*Figure 2*). The diversity and composition of soil microorganisms play a key role in soil health regulation (Bui et al., 2023; Sindesi et al., 2023). Here, the relative abundance of Proteobacteria was significantly decreased, while Chytridiomycota, Firmicutes and Chloroflexi were increased (*Figure 3a,b*). Firmicutes promote the development of soil fertility, plant growth, and environmental protection through nitrogen fixation, organic degradation, stress resistance, biological control, and environmental restoration (Gupta et al., 2018). Moreover, the abundance of pathogen (Fusarium) increased, and the abundance of beneficial bacteria (especially Bacillus) significantly decreased in the samples with severe bacterial wilt (*Figure 3c,d*). Bacillus is an important agricultural microbial resource, which can dissolve insoluble phosphorus, potassium and other compounds, effectively resist the growth of pathogen Fusarium, and antagonistic effect on tobacco bacterial wilt (Klein et al., 2013; Wu et al., 2016; Fan et al., 2022). Previous studies reported that the balance of microbial community composition is important for the ability of host plants to resist disease and remain healthy. Therefore, these data indicated that film mulching could enhance the antagonistic activity against soil-borne pathogens by enriching antagonistic bacteria, thereby alleviating the occurrence of bacterial wilt (Muhammad et al., 2023; Ali, 2024).

Soil microbial diversity and community composition were affected by soil nutrient, temperature, moisture and mulching conditions. The results showed that film mulching could improve the richness and diversity of microbial community in tobacco rhizosphere soil, in which the diversity of fungal community was significantly increased, while the diversity of bacteria was decreased (*Table 2*), which was consistent with the studies of other crops such as maize and potato (Sun et al., 2013; Yin et al., 2022). Mulching increased soil nutrient content and air permeability, which was conducive to the propagation and growth of fungi, and led to the increase of soil fungal abundance under mulching conditions (Lela et al., 2024). In addition, RDA demonstrated that soil AP and AK were the main factors affecting the fungal and bacterial community structure at phylum level. Specifically, AP and AK were significantly and positively correlated with Firmicutes and Chloroflexi, while significantly negatively correlated with Proteobacteria. Chloroflexi can produce energy through photosynthesis, and the relative abundance of the bacteria in the soil with good physicochemical properties is significantly increased (Ren et al., 2018).

These results suggested that plastic film mulching was efficient in modifying both physicochemical properties and microbial community composition in soil, likely toward a better growth environment for cigar tobacco plants under high temperature and high humidity. Improvement in the content of soil organic matters, available phosphorus, and available potassium under mulching could support the idea that increased nutrient availability for plants would promote increased chlorophyll and nicotine content in tobacco leaves. These results further indicated that film mulching improved rhizosphere soil microorganisms and the stability of soil ecological community by increasing soil nutrients.

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Conclusions

Mulching with plastic film is regarded as one of the most effective methods to improve the cultivation of cigar tobacco, especially under conditions with a huge temperature difference and fluctuating rainfall. Under film mulching, the improvement of soil physicochemical properties-such as SOM content and the availability of phosphorus and potassium-was directly contributed to by tobacco. This positive effect extends into the makeup of the tobacco leaf, too, by way of increased chlorophyll and nicotine yet decreased sugars and starches. Besides, film mulching has promoted favorable changes in the rhizosphere microbial community, such as increasing the abundance of advantageous microorganisms like Bacillus and suppressing injurious pathogens like Fusarium, hence the control of bacterial wilt. It therefore provides a critical role of soil microbial diversity in relation to soil health and crop production. Although no information was obtained about soil moisture variation, this result signals film mulching among the adaptative and sustainable practices that could be helpful for the improvement of the quality and enhancement of tobacco yield in a high-temperature and humid environment. Further research is therefore suggested taking into consideration the dynamics of soil moisture and the long-lasting effects of mulching in agriculture for sustainability around the world.

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Conflict of interest. The authors declare no conflict of interest.

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