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Full Length Article

Tobacco Stalk Biochar Application Improves Soil Fertility and Flue-Cured Tobacco Growth

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Abstract

Tobacco stalks the main agricultural waste after tobacco harvest, are generally discarded directly or returned to the field after burning. They are rarely processed into biochar, a product that could benefit soil properties. To explore the effects of applying tobacco stalk biochar on soil fertility and tobacco production, tobacco was grown at six biochar application levels (0, 3,000, 4,500, 6,000, 9,000 and 12,000 kg ha^{-1}) in three different sites (Jianchuan, Midu and Eryuan) in Dali County, Yunnan Province. Biochar decreased soil bulk density, increased large and small soil aggregate proportion, and increased soil organic carbon and nitrogen stocks. Biochar also improved the yield and quality of tobacco leaves at all sites. Biochar rates of 3,000, 4,500 and 6,000 kg ha^{-1} linearly improved soil fertility and agronomic traits while application rate of biochar exceeding 9,000 kg ha^{-1} reduced plant growth. Moreover, the optimum biochar application rates for better plant height, stem diameter, maximum leaf length and leaf width, yield, and average price differed by site. These rates were: 6,000 kg ha^{-1} (Midu), 3,000 kg ha^{-1}(Eryuan) and 4,500 kg ha^{-1} (Jianchuan), respectively. In conclusion, appropriate application of biochar could improve soil nutrients and contribute to tobacco growth in different soil nutrient conditions.

Keywords: Tobacco stalk; Biochar; Soil quality; Flue-cured tobacco; Growth

Introduction

Tobacco (*Nicotiana tobacum* L.) the most important economic crop in southwest regions of China, reached a cultivated area of 53, 0000 ha in 2019 (Zhang and Ma 2020). To achieve higher economic benefits and yield, tobacco growers usually apply large amounts of chemical fertilizer and practice continual tobacco monocropping. This causes constant loss of soil nutrients and deterioration of soil physical and chemical properties, leading to soil compaction, soil nutrient imbalance, soil microflora change, and frequent occurrence of soil-borne diseases (Cheng *et al.* 2013; Qin *et al.* 2015; Ma *et al.* 2017).

Tobacco stalks after leaf harvest are agricultural waste, with a biomass of 2250 ~ 3000 kg ha^{-1}. There are about 3 million metric tons of tobacco stalks in China's tobacco areas (Han and Wang 2016). In traditional management, most of these stalks are burned and returned to the field as ash because fresh stalks and roots left in field could cause disease for the next tobacco growing season (Zou *et al.* 2017). However, this management is resource wasteful and pollutes the environment. Balancing waste tobacco stalk production and use with continually decreased soil quality is a challenge and opportunity for sustainable tobacco production.

Biochar is a term for the solid material formed by pyrolysis or carbonization of biomass such as plant tissue, agricultural, and forestry residues, plant straw, *etc.* (El-Naggar *et al.* 2019). The typical carbon content of biochar is 65–90%, and it contains 3–20% of potassium, phosphorus, calcium, magnesium, silicon, manganese, zinc and other oxides and nitrogen compounds (Emma 2006; Yuan *et al.* 2016; Li *et al.* 2017). Biochar has good water absorption and adsorption capacity because of its large intermolecular distance (Wang and Sun 2016; Hussain *et al*. 2017; Farooq *et al*. 2020a). After application, biochar can loosen the soil increase the number of aggregates in soil, and increase the soil pH (Lin *et al.* 2018; Li *et al.* 2019; Farooq *et al*. 2020b). The nutrient elements in biochar could increase the content of organic matter in soil and reduce nitrogen leaching (Sarma *et al.* 2018; Zhang *et al.* 2018; Wei *et al.* 2019; Baruah *et al.* 2020; Liu *et al.* 2020). Biochar can reduce or inhibit soil borne diseases, promote carbon and nitrogen metabolism, and improve crop yield and quality even under less than optimum conditions (Wang *et al.* 2015; Farooq *et*

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al. 2020c).

Reports on biochar have mainly focused on reducing heavy metals in soil, remediation of soil pollution, soil nutrients, and the microbial community. There are few reports about using tobacco stalk biochar to influence soil aggregate structure and soil organic carbon and nitrogen stocks during tobacco growth. Three tobacco growing sites with different nutrient status were selected to explore the proportion of different particle size aggregates, the changes of organic carbon and soil nitrogen stocks, and tobacco yield and quality. Exploring the influence of tobacco stalk biochar on soil nutrients and plant growth would support sustainable and recyclable agricultural management in tobacco growing regions.

Materials and Methods

General situation of test sites

This study was conducted at three sites: Midu, Eryuan and Jianchuan, Dali City, Yunnan Province, China. The basic geographical conditions and soil physical and chemical properties in the three sites are in Tables 1 and 2. Sampling and measurement in this study occurred from April to September 2017.

Experiment design

Six different biochar levels were used at each site: 0, 3,000, 4,500, 6,000, 9,000, and 12,000 kg ha^{-1}. Biochar was produced from tobacco stalks by combustion in an open-top carbonization device under hypoxic condition at about 450°C for 30 min. The basic physico-chemical properties of tobacco stalk biochar are in Table 3.

The study sites were initially established in 2016, and sampling started in 2017. The experiment followed a randomized complete block design with factorial arrangement and was replicated three times with plot size of 14 m × 2 m. The biochar was scattered onto the soil surface by hand and then ploughed to achieve mixing to 40 cm depth soil. A flue-cured tobacco variety, ‘K326,’ was used as plant material. The planting density was 16,500 plants ha^{-1}, and plant spacing was 50 cm × 120 cm. A compound fertilizer (N: P_2O_5: K_2O = 1: 2: 2.5) amounting 75 kg ha^{-1} was strip-applied, with one third applied as a base fertilizer before transplanting and the remaining two thirds applied 30 days after transplanting.

Soil sampling and sample preparation

Soil samples were collected from April to September. Soil bulk density (BD) was measured with a cutting ring with a volume of 100 cm^3. The particle size distribution of aggregates in soil, soil organic carbon stocks (SOCs), and total soil nitrogen stocks (TSNs) were determined by separate random sampling to obtain 54 soil samples from depths of 0~20 cm and 20~40 cm in each block. After removing roots and pebbles, the soil was placed in self-sealing plastic bags, and stored in a refrigerator at 4°C. Within a week, the soil aggregates were screened by wet sieving method.

Sieving of wet aggregates

To avoid disruption during rewetting of dried soil field-moist soil was used to size grades within the aggregate samples (Zou *et al*. 2015).

The mean weight diameter (MWD) and the geometric mean diameter (GMD) of the aggregates, the SOC or TSN stock in each aggregate size fraction and whole soil were computed using Equation (1), (2), (3), and (4), respectively (Zou *et al.* 2015).

$$\mathrm{MWD} = \sum_{i=1}^{n} Xi \times wi \qquad (1)$$

$$\mathrm{GMD} = \exp\left[\frac{\sum_{i=1}^{n} \mathrm{Wi} \times \mathrm{LnXi}}{\sum_{i=1}^{n} \mathrm{Wi}}\right] \qquad (2)$$

$$\mathrm{SOCs(or\ TSNs)} = \mathrm{D} \times \mathrm{BD} \times \mathrm{SOC(or\ TSNi)} \times \mathrm{Wi} \qquad (3)$$

$$\mathrm{SOCsi(or\ TSNsi)} = \frac{\mathrm{D} \times \mathrm{BD} \times \mathrm{SOCi(or\ TSNi)} \times \mathrm{Wi}}{10} \qquad (4)$$

Agronomic traits were determined according to the Investigating and Measuring Methods of Agronomical Character of Tobacco in Tobacco Industry Standard of the People’s Republic of China YC/T142-2010. Fifteen flue-cured tobacco plants from each plot were evaluated in the rosette stage.

Yield refers to the total yield of tobacco leaves per unit area. The 5~8^{th} leaves (lower leaves), 10~13^{th} leaves (middle leaves), and 14~17^{th} leaves (upper leaves) were collected from the bottom to the top of tobacco stalk position when the tobacco leaves were mature. After flue-curing, the tobacco samples were graded and measured according to Chinese Standard GB 2635-1992, and indices, such as proportions (%) of top and middle-grade tobacco were obtained. The output was calculated according to the purchase price for that year (2017).

Data analysis

Data were analyzed with the General Linear Model (GLM) procedure of the S.A.S. 9.3 computer package (S.A.S. Institute Inc., Cary, NC). This study used by factor design including study sites and biochar application rates. There were significant treatment effects if the probability (*P*) was < 0.05. Sigma Plot 14.0 (Systat Software Inc., Chicago, IL, USA) was used to produce all associated output plots.

Results

Effects of biochar on soil physical properties

When tobacco stalk biochar application rates were more than 4500 kg ha^{-1}, there were significant decreases in soil

Table 1: Basic geographical conditions of different test sites

	Longitude and latitude	Altitude (m)	Annual average temperature (℃)	Annual rainfall (mm)	Annual sunshine hours (h)
Jianchuan	N 24°47′, E 100°19′	1780	18.3	824.4	2750
Midu	N 25°23′, E 100°16′	1980	16.3	665.6	2740
Eryuan	N 28°56′, E 99°13′	1590	20.0	559.4	2720

Table 2: Physical and chemical properties of soil foundation

Sites	pH (2.5:1)	Soil organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Hydrolyzable nitrogen (g/kg)	Available phosphorus (g/kg)	Available potassium (g/kg)
Midu	6.5	56.2	2.8	1.1	17.6	210.8	91.3	285.5
Eryuan	6.7	27.4	1.3	0.9	10.4	154.3	36.7	62.4
Jianchuan	6.5	41.6	2.2	0.9	15.9	143.8	60.4	142.7

Table 3: The physical and chemical characteristics of tobacco stalk biochar in this study

Gravimetric Water content (%)	Carbon content (%)	Ash content (%)	Specific surface area ($m^2 \cdot g^{-1}$)	pH	Total nitrogen (%)	Mineralizable nitrogen (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (%)	Chloride content (%)
6.5	66.7	18.3	6.0	7.3	3.4	42.5	227	1.3	0.1

Table 4: The effect of biochar application on soil bulk density (BD), the mean weight diameter (MWD), and the geometric mean diameter (GMD) in 0-20 cm and 20-40 cm

Depth (cm)	Biochar levels (kg ha^{-1})	Bulk density (BD) (g/cm^3)			MWD (mm)			GMD (mm)		
		Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan
0-20	0	1.2 b	1.2 b	1.3 a	1.7 i	1.8 hi	1.8 g-i	0.5 j	0.5 j	0.5 j
	3000	1.2 b	1.2 b	1.2 b	1.8 f-i	2.0 d-h	2.0 d-g	0.6 h-j	0.7 g-i	0.6 h-j
	4500	1.2 b	1.3 a	1.2 b	1.9 e-h	2.1 c-e	2.1 b-e	0.7 f-h	0.8 d-g	0.7 e-h
	6000	1.2 b	1.2 b	1.2 b	2.0 c-f	2.1 c-e	2.3 ab	0.8 c-f	0.8 c-f	0.9 b-d
	9000	1.2 b	1.2 b	1.2 b	2.1 c-e	2.2 a-c	2.4 a	0.9 c-e	0.9 a-c	1.1 ab
	12000	1.2 b	1.2 b	1.2 b	2.1 b-d	2.3 a	2.4 a	0.9 b-d	1.1 a	1.1 a
20-40	0	1.2 b	1.3 a	1.2 b	2.0 h-j	1.6 k	1.9 ij	0.6 gh	0.4 i	0.4 hi
	3000	1.2 b	1.3 a	1.2 b	2.2 ef	1.8 ij	2.2 e-g	0.7 ef	0.5 g-i	0.6 gh
	4500	1.2 b	1.2 b	1.2 b	2.4 c-e	1.9 ij	2.3 c-f	0.9 cd	0.6 gh	0.7 ef
	6000	1.2 b	1.2 b	1.2 b	2.4 b-d	2.0 g-i	2.4 b-d	1.0 bc	0.6 fg	0.8 d-f
	9000	1.2 b	1.2 b	1.2 b	2.5 a-c	2.2 f-h	2.6 ab	1.1 ab	0.8 de	0.9 cd
	12000	1.2 b	1.2 b	1.2 b	2.6 ab	2.3 d-f	2.7 a	1.2 a	0.9 cd	1.2 a

Means with different lowercase letters, within a column and rows for each trait, are statistically different from each other at $P < 0.05$ according to DNMR test

bulk density (BD) depending on site and depth (Table 4). At 0–20 cm depth, when biochar application rate reached 4500 kg ha^{-1}, the MWD and GMD in each site were significantly higher than the control. At 6000 kg ha^{-1}, the MWD became significantly different between sites (Table 4). At 20–40 cm depth, the effect of biochar on increasing MWD and GMD was more obvious than at 0–20 cm depth (Table 4).

Biochar to 4,500 or 6,000 kg ha^{-1} significantly increased the large macroaggregate proportion (LMP) and small macroaggregate proportion (SMP) in each site relative to the control and this trend at 20–40 cm depth was more obvious than that at 0–20 cm depth (Table 5). The SMP of each site increased significantly at 4,500 kg ha^{-1} biochar application with site specific differences at 20–40 cm depth. Biochar application reduced soil MIP and SCP in each site. At 0–20 cm depth, when the biochar rate was 4500 kg ha^{-1}, the soil MIP began to be significantly lower than the control. In the 20–40 cm depth, there were significant site differences in MIP. The SCP in soil was significantly reduced relative to the control when the biochar application rate was 3,000 kg ha^{-1}. At 20–40 cm soil depth, there were significant site differences in SCP.

Biochar could significantly increase SOCs and TSNs (Table 6). The SOCs in 0–20 cm depth at Midu increased most obviously (40–50%), followed by Eryuan and Jianchuan. Among three sites, the SOCs in Jianchuan were significantly less than in Midu and Eryuan. At 20–40 cm depth, the SOCs in Midu was significantly higher than in Eryuan and Jianchuan. Unlike SOCs, biochar addition most increased TSNs in Jianchuan at 0–20 cm depth (41–110%), followed by Midu and Eryuan. The TSNs in Midu were also significantly higher than in Jianchuan after biochar application.

Effects of biochar on agronomic traits of tobacco

Compared with the control, when the biochar application rate was 6,000 and 12,000 kg ha^{-1}, plant height of flue-cured tobacco significantly increased. When the biochar rate was 9,000 kg ha^{-1}, plant height was significantly lower than the control (Table 7). After applying biochar, plant height of flue-cured tobacco in Eryuan was

Table 5: The effect of biochar application on the large macroaggregate proportion, small macroaggregate proportion, microaggregate proportion and Silt-clay proportion in 0-20 and 20-40 cm depth

Depth (cm)	Biochar levels (kg ha^{-1})	Large macroaggregate proportion (LMP) (%)			Small macroaggregate proportion (SMP) (%)			Microaggregates proportion (MIP) (%)			Silt-clay proportion (SCP) (%)		
		Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan
0-20	0	24.9 i	26.7 hi	28.5 f-i	40.1 d-f	37.2 fg	33.0 h	9.1 b-d	11.1 a	10.5 ab	26.0 ab	25.1 ab	28.1 a
	3000	26.5 hi	30.0 e-h	31.2 e-g	43.0 b-d	39.6 d-g	35.8 gh	8.1 c-f	9.9 a-c	9.5 a-d	22.4 b-d	20.5 c-e	23.6 bc
	4500	27.8 g-i	31.7 e-g	32.6 c-f	45.2 ab	41.6 b-e	38.4 e-g	7.9 d-f	8.8 b-d	8.5 c-e	19.2 d-f	17.9 e-g	20.4 c-e
	6000	29.3 f-h	31.9 d-g	36.0 a-d	47.4 a	43.2 b-d	40.8 c-f	6.9 e-g	8.2 c-f	6.9 e-g	16.4 f-h	16.7 e-h	16.3 f-h
	9000	30.2 e-h	33.7 b-e	37.5 ab	48.0 a	44.9 a-c	41.9 b-e	5.7 g	6.8 e-g	6.5 fg	16.1 f-h	14.7 gh	14.1 gh
	12000	31.0 e-g	36.2 a-c	38.0 a	48.5 a	45.3 ab	42.5 b-d	5.4 g	5.9 g	6.5 fg	15.1 gh	12.6 h	13.0 h
20-40	0	31.8 gh	25.1 i	33.4 fg	31.8 e-g	27.2 hi	20.7 j	10.0 cd	16.8 a	14.4 b	26.5 bc	31.0 a	31.5 a
	3000	36.5 ef	27.9 hi	37.5 d-f	32.9 d-f	31.9 e-g	23.3 ij	8.4 d-f	13.7 b	10.9 c	22.2 d-f	26.6 bc	28.3 ab
	4500	38.9 c-e	29.1 hi	40.0 b-e	35.0 b-e	34.8 c-e	28.2 gh	7.9 e-g	11.0 c	7.6 fg	18.2 gh	25.1 b-d	24.0 cd
	6000	40.2 b-e	30.9 gh	41.9 bc	37.3 a-c	36.5 a-d	28.5 gh	6.3 g-i	9.8 c-e	6.5 gh	16.2 g-i	22.8 c-e	23.5 c-e
	9000	41.4 b-d	33.9 fg	44.0 ab	39.1 ab	39.6 a	29.8 f-h	5.1 hi	7.9 e-g	6.5 g-i	14.3 h-j	18.5 fg	19.8 e-g
	12000	43.0 a-c	36.4 ef	46.3 a	40.2 a	40.5 a	33.9 c-e	4.6 i	6.3 g-i	6.3 f-h	12.2 j	16.9 g-i	13.2 ij

Means with different lowercase letters, within a column and rows for each trait, are statistically different from each other at $P < 0.05$ according to DNMR test

Table 6: Effects of biochar application rates on soil organic carbon stock (SOC) and total soil nitrogen stock (STNs) in 0-20 and 20-40cm depth

Depth (cm)	Biochar levels (kg ha^{-1})	SOCs (g C/m^2)			TSNs (g N/m^2)		
		Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan
0-20	0	3628.9 i	4194.4 h	3803.7 i	324.8 g	382.2 e	212.3 i
	3000	5070.1 d-f	4963.1 d-f	4250.6 h	451.0 cd	411.0 d	300.4 h
	4500	5091.4 c-e	5078.6 de	4349.6 h	478.8 b	422.6 d	340.9 f
	6000	5266.1 b-d	5283.6 b-d	4399.1 gh	510.9 b	479.2 b	378.1 ef
	9000	5290.3 b-d	5540.8 ab	4769.0 ef	551.7 b	529.3 b	433.8 cd
	12000	5440.7 a-c	5715.6 a	4712.5 fg	532.8 b	572.8 a	457.7 c
20-40	0	3466.7 f-h	2892.3 i	2891.5 i	304.3 de	212.0 f	101.6 h
	3000	4249.4 cd	3316.1 h	3317.1 h	400.8 c	257.6 e	145.7 h
	4500	4461.2 bc	3388.5 h	3425.1 gh	457.6 bc	269.0 e	191.8 g
	6000	4522.6 a-c	3411.2 h	3207.1 hi	441.2 bc	269.5 e	160.9 gh
	9000	4793.9 ab	3770.5 e-g	3517.4 f-h	480.4 ab	341.2 d	261.4 e
	12000	4836.0 a	3816.7 ef	3940.1 de	482.0 a	349.9 d	333.8 d

Means with different lowercase letters, within a column and rows for each trait, are statistically different from each other at $P < 0.05$ according to DNMR test

Table 7: Effects of different biochar application rates on plant height, stem circumference, maximum leaf length, and maximum leaf width at rosette stage of flue-cured tobacco grown at different sites

Biochar levels (kg ha^{-1})	Plant height (cm)			Stalk girth (cm)			Maximum leaf length (cm)			Maximum leaf width (cm)		
	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan
0	7.0 e	7.0 e	7.1 e	4.3 d	4.1 e	3.1 g	26.0 g	30.8 b	21.5 k	12.4 h	14.5 ef	10.7 j
3000	7.0 e	7.5 d	7.0 e	4.3 c	4.3 d	3.0 h	26.2 g	31.7 a	21.9 jk	12.8 g	16.3 b	10.1 k
4500	7.0 e	7.5 d	7.0 e	2.7 i	4.4 bc	3.0 h	26.0 g	30.7 b	23.9 h	11.3 i	15.2 d	12.1 h
6000	10.0 b	11.5 a	10.0 b	4.8 a	3.2 g	3.0 h	30.0 c	28.0 ef	22.2 ij	15.9 c	17.0 a	11.5 i
9000	5.5 g	6.5 f	5.6 g	4.0 e	4.4 c	2.7 i	29.0 d	27.5 f	20.9 l	14.6 e	14.2 f	11.2 i
12000	8.5 c	8.5 c	8.5 c	3.8 f	4.5 b	3.2 g	28.1 e	30.8 b	22.6 i	14.7 e	15.5 d	11.3 i

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significantly higher than in Midu and Jianchuan. The difference of stem girth among the three sites was significant, with the order of Midu > Eryuan > Jianchuan. The stem girth (SG) decreased significantly compared to the control in each site when the biochar rate was 4,500 (Midu), 6,000 (Eruyuan), and 9, 000 (Jianchuan) kg ha^{-1} respectively. The maximum leaf length and maximum leaf width of flue-cured tobacco in Midu decreased significantly at the 4,500 kg ha^{-1} biochar rate, while in the Eryuan and Jianchuan sites they began to decrease at rates of 9,000 kg ha^{-1}. The maximum leaf length and maximum leaf width of the three sites were also significant in the order of Eryuan > Midu > Jianchuan.

Effects of biochar on flue-cured tobacco yield

When the biochar rate was 6,000 and 12,000 kg ha^{-1}, the yield of flue-cured tobacco in Midu significantly increased, by 280–300 kg ha^{-1} compared to the control (Table 8). Under the same biochar application rate, the yield in Jianchuan was significantly less than in Midu and Eryuan. Similar to the yield, there was a maximum value of the proportion of superior leaves at 6,000 and 3,000 kg ha^{-1} biochar application rates in Midu and Eryuan, respectively. In Jianchuan, the maximum value of the superior leaves appeared when the biochar application rate was 4,500 kg ha^{-1}. Except for the application rate of 6,000 and 9,000 kg ha^{-1}, the proportion of

Table 8: Effects of different biochar rates yield, proportion of superior leaves and average leaves price of tobacco grown at different locations

Biochar levels (kg ha^{-1})	Yield (kg ha^{-1})			Proportions of superior leaves (%)			Average price (dollar kg^{-1})		
	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan	Midu	Eryuan	Jianchuan
0	2892 b-d	2853 c-e	2533 gh	58.6 d	53.7 g	49.8 k	4.3 d	3.7 i	3.6 j
3000	2890 b-d	2923 bc	2781 de	59.0 cd	58.8 d	49.3 k	4.5 b	4.3 d	4.0 g
4500	2988 b	2859 b-e	2745 ef	60.0 bc	55.1 f	52.1 h	4.5 bc	4.0 g	3.9 h
6000	3174 a	2910 b-d	2533 gh	61.8 a	58.5d	50.0 jk	4.5 c	4.3 e	3.6 j
9000	2750 ef	2922 bc	2488 h	57.2 e	56.4 e	51.2 hi	4.2 f	4.2 e	3.5 k
12000	3193 a	2780 de	2635 fg	61.0 ab	54.7 fg	50.9 ij	4.6 a	3.9 h	3.7 i

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superior leaves in each site was significant with the order: Midu > Eryuan > Jianchuan. The trend in the average price was the same as that of the proportions of superior leaves.

Discussion

Soil structure plays an important role in plant growth and soil water movement, but also has a vital role in improving soil physical and chemical properties, and biological processes (Wei *et al.* 2006; An *et al.* 2008). In this experiment, biochar application decreased the soil bulk density (Table 4), was due to the porous structure of biochar, which effectively increase soil porosity and improve soil aeration. This result is similar to the results of Meng *et al.* (2020). The mean weight diameter and geometric mean diameter of soil aggregates increased with the biochar application rate in each site, indicating that biochar application could improve soil aggregate stability; these results are consistent with Zhu *et al.* (2018). The MWD and GMD at Eryuan were significantly lower than at Midu and Jianchuan for the 20–40 cm depth (Table 4). We believe the main reason for this difference among the three sites was the variation of soil nutrients (Shinjo *et al.* 2000). Compared with Midu and Jianchuan, the soil organic matter and total nitrogen content in Eryuan were significantly less than in the other two sites. This limited the availability of nutrients to microorganisms in soil, and because the secretion of microbial products and the production of hyphae are the core of soil aggregation, this limited the increase of aggregate mean weight diameter and geometric mean diameter (Wang *et al*. 2020).

Many studies show that the quantity and distribution of water stable aggregates determine the stability of soil structure and its resistance to erosion, especially the number of aggregates with particle size > 0.25 mm, which is one of the important indexes to determine the quality of soil (Li *et al.* 2014; Amundson *et al.* 2015). Applying biochar significantly increased the proportion of large and small aggregates and decreased the proportion of microaggregates and silty clay -sized aggregates. This result was similar with previous studies (Chen *et al.* 2008). The main reason for the change in aggregate proportion was that the biochar having a nutrient holding capacity effect, offering sufficient nutrient for the growth and reproduction of microorganisms. The hyphae of fungi and actinomycetes can mechanically

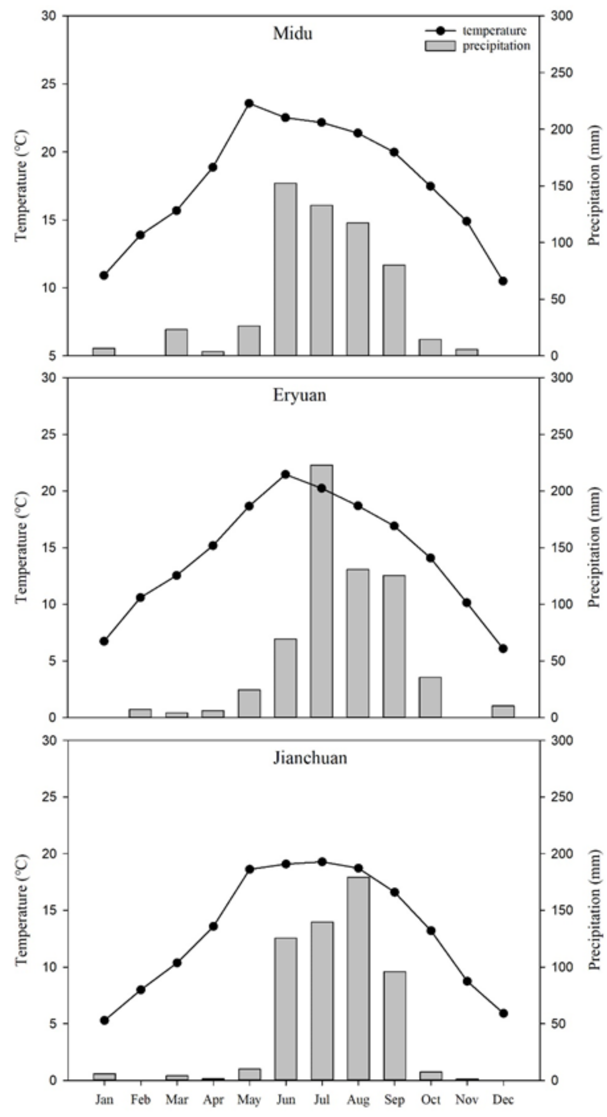

Fig. 1: The average monthly temperature and monthly accumulated precipitation at Midu, Eryuan and Jianchuan

entangle particles in the soil to form aggregates, thus increasing the proportion of large and small aggregates. However, the proportion of large aggregates in Jianchuan was significantly higher than the other two sites (Table 5).

This may be related to the difference of precipitation and temperature in three sites (Fig. 1). Previous studies showed that soil aggregate stability was negatively correlated with temperature and soil moisture. High temperature and high humidity will reduce soil aggregate stability (Ye *et al*. 2013; Xu *et al*. 2019). In this study, the temperature and precipitation in Jianchuan during soil sampling period (Apr. to Sep.) were lower than in Midu and Eryuan (Fig. 1), which was conducive to maintaining the activity of soil microorganisms and the stability of soil structure. It was beneficial to the formation of large aggregates.

Many studies have shown that biochar can significantly enlarge the SOCs and TSNs in topsoil (Shinjo *et al.* 2000; Mao *et al.* 2008). As an exogenous organic matter, biochar can directly increase the soil organic matter content and maintain the relative stability of soil organic carbon stocks (Wang and Sun 2016). The present study showed that biochar application could significantly enlarge soil SOCs (Midu) and TSNs (Jianchuan). It is consistent with the results of previous studies (Wang and Sun 2016). Ji *et al.* (2018) hold the opinion that adding high C/N ratio biochar into low organic matter soil or adding low activity biochar in high organic matter soil could inhibit the mineralization of soil organic matter. In this study, the soil organic matter content in Midu was highest. Adding biochar could inhibit organic matter mineralization in soil, thus contributing to the stability of SOCs. However, the content of soil organic matter, animal, plant, and microbial residues in 20–40 cm depth are less than topsoil, which makes its storage capacity smaller.

Plants growth depends on good soil environment. The plant height increased significantly when the biochar rate was 6000 kg ha^{-1}. This result is consistent with previous studies (Chen and Du 2015; Minhas *et al*. 2020). However, when the biochar rate was 9000 kg ha^{-1}, the plant height decreased significantly compared with no biochar application. That is because biochar has a threshold effect (Zhang *et al*. 2015). When the biochar application rate was low, it provided tobacco with nutrients for the growth; when the rate was high, the soil structure was destroyed, and the crop growth affected negatively (Wei *et al*. 2019). Excessive carbon accumulated in the soil will affect the absorption of water and nutrients by plant roots, resulting in the decrease of yield and its components (Liang *et al.* 2006; Qiu *et al.* 2020). The stalk girth at a certain biochar concentration decreased compared with the control (Table 7), which may be related to nitrogen leaching caused by biochar. With benefits from the improvement of soil physical and chemical properties, the yield of tobacco leaves and the proportion of upper leaves also increased, which contributed to the increase of average price.

Previous research showed that after applying biochar, the availability of soil nutrients and the efficiency of plant nutrient absorption were improved (Jeffery *et al.* 2011). In this study, the effects of biochar on yield and quality of flue-cured tobacco in different locations were in the order of Midu > Eryuan > Jianchuan. This is mainly because at the rosette stage (late May to early June), the Midu site had the highest SOM, total nitrogen content and total soil nitrogen stock among three sites, which provided sufficient nutrition for the tobacco roots growth. Moreover, the precipitation in Midu area is less than that in Eryuan site, which reduces the nitrogen leaching in topsoil (Table 6).

Conclusion

Tobacco stalk biochar improved soil physical structure. Biochar decreased soil bulk density, increased the proportion and diameter of large and small soil aggregates, and increased soil organic carbon and nitrogen stocks. Biochar addition also improved the yield and quality of tobacco leaves. However, if the biochar application rate exceeded 9,000 kg ha^{-1}, it deteriorated conditions conducive to plant growth. The optimum biochar application rates for better plant height, stem diameter, maximum leaf length and leaf width, yield, and average price differed by site. These rates were: 6,000 kg ha^{-1} (Midu), 3,000 kg ha^{-1}(Eryuan) and 4,500 kg ha^{-1} (Jianchuan), respectively.

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Author Contributions

Y Li and CM Zou designed the study and collected the data; X He and MY Hu conceived the idea; JE Su, TY Xu and R Liu carried out the field experiment; TX Liu and KY Gu analyzed the data; all authors contributed to the writing and revisions.

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