

CHARACTERISTICS OF ORGANIC CARBON IN TOBACCO-GROWING SOILS WITH DIFFERENT pH

ZHENGGUI GOU[†], ZHEN ZHAI^{1†}, QINGZHU ZHANG, XINWEI FENG, YANHUI LI²,
YE ZHANG², YUTAO ZHANG³, XIANBO ZHAO^{2*} AND MENGJIAO DING^{1,4*}

Guizhou Tobacco Company, Qiannan Region Tobacco Company, Duyun 558000, China

Keywords: Characteristics of organic carbon, pH, Soil physico-chemical indicators

Abstract

This paper deals with the analysis of the relationship between acidity and alkalinity with organic carbon structure and humus components of tobacco-growing soils in Tengchong city. Under the same level of organic matter and nutrient content, soils with different pH values in Tengchong city were selected for the investigation. Results showed that the HA/FA values of the soil with low organic matter content gradually decreased with increasing pH, indicating a higher content of active carbon components and a lower content of stable carbon components in the soils. In soils with high organic matter content, pH value showed a highly significant negative correlation with HA/FA and PQ values. With increasing acidity, HA/FA became higher, and humus components with high stability increased. At the same level of organic matter content, the infrared A2920/A1630 ratio gradually increased with increasing pH. It indicates that at a certain pH range and the same level of organic matter content, the stable humus component increases, and the soil pH value decreases.

Introduction

Soil humus (the main form of soil organic matter) is one of the ecological factors affecting the quality of cured tobacco. It has significant effects on the nature of the tobacco-growing soil, the chemical composition of the tobacco leaf, and the formation of aroma and taste flavor (Zhang *et al.* 2019, Zhai *et al.* 2022, Ren *et al.* 2024). There is a significant positive correlation between the content of soil humus and its components, the degree of humification, and the content of alkaline nitrogen in soil (Liao *et al.* 2015). Soil physicochemical indicators (e.g., total nitrogen, total phosphorus, pH, and particles) have significant linear relationships with the content of organic carbon components (He *et al.* 2014). Previous studies demonstrated that the quality and aroma of cured tobacco are closely related to the humus compositional characteristics of tobacco-growing soil (Stevenson 1994, Nardi *et al.* 2021, Jia *et al.* 2024). The carbon content of humus components was significantly negatively correlated with nitrogen-alkali ratio, starch, and reducing sugar content of tobacco, and significantly positively correlated with nicotine content. In addition, the Humic Acid/Fulvic Acid (HA/FA) ratio was significantly positively correlated with nicotine content in the B2F tobacco (Liao *et al.* 2015). Therefore, this paper investigates the compositional characteristics of humus and its influencing factors in acidic tobacco-growing soils. The findings of the study are important for further characterizing and evaluating the quality of acidic tobacco-growing soils and help to elucidate the soil ecological factors that influence the quality of tobacco leaves (Liu *et al.* 2012).

*Author for correspondence: <zxb22935@fjtic.cn>; <952156929@qq.com>. ¹Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China. ²China Tobacco Fujian Industrial Co. Ltd, Xiamen, 361001, China. ³Xinzuobiao High School Liangshan, Jining, 272699, China. ⁴College of Tobacco Science of Guizhou University, Guiyang 550025, China.
[†]These authors have contributed equally to this work.

Different soil pH levels have a significant effect on soil humus components and organic carbon components, and soil humus levels tend to decrease with increasing pH (Liao *et al.* 2015). Different pH values can affect the degree of H dissociation in the carboxyl and phenolic hydroxyl groups of HA. In general, higher pH values result in greater dissociation (Li *et al.* 2019), while at low pH values, the protonation of amine and carboxyl groups can easily cause the coalescence of HA molecules. As a result, the specific surface area of HA colloids is reduced, thus decreasing the ability of HA to complex with metal ions (Li *et al.* 2018). Based on the above studies, studying the effect of pH on soil humus and carbon components has great significance in resolving the storage of soil organic carbon and the adsorption and complexation of metal ions.

According to the evaluation results of nutrient content and fertility adaptability of tobacco-growing soils in Baoshan city, it can be obtained that tobacco-growing soils in Tengchong city have high organic matter content (Calderón *et al.* 2011) and serious acidification (Wang *et al.* 2014). Tobacco-growing soils exhibit strong acidity, slight acidity, neutrality, and alkalinity at different pH values under the same organic matter level. In this study, tobacco-growing soils with different pH values under the same organic matter and soil nutrient content levels in Tengchong city were selected as the research object. By analyzing the differences in organic carbon structure, humus components, and the contents of active and inert carbon components of acid-alkaline soils under the same organic matter level, the characteristics of different active carbon components and molecular structures of acid-alkaline soils in Tengchong city, as well as the relationship between soil acidity and alkalinity, organic carbon structure, and humus components are elucidated. The findings of this study can provide statistical support for the practice of tobacco production in high organic matter acidic tobacco-growing soils.

Materials and Methods

The Baoshan tobacco-growing soil was categorized into 16 and 8 groups based on pH and organic matter content. The 16 groups were divided by four pH levels (<5.5, 5.5-6.5, 6.5-7.5, >7.5) and four organic matter levels (low <15 g/kg, medium 15-25 g/kg, high 25-35 g/kg, rich >35 g/kg). The 8 groups were defined by combining the low and medium (≤ 25 g/kg) and high and rich (> 25 g/kg) organic matter levels. Sampling was conducted in July 2017 at Jietou Town, Tengchong City, selecting soils with varying pH but consistent nutrient levels for further analysis.

10 g of air-dried, 0.25 mm sieved soil was mixed with 100 mL of 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide solution, shaken for 30 min, and left to stand for 12 hrs before centrifugation to obtain a clear filtrate. 3-10 mL of filtrate was adjusted to pH 7 with H_2SO_4 and evaporated. It was then oxidized using $\text{K}_2\text{Cr}_2\text{O}_7$ and concentrated H_2SO_4 , and titrated with FeSO_4 to determine total HA-FA carbon content. 25 mL of the filtrate was acidified with H_2SO_4 , heated to induce HA precipitation, and left overnight. The precipitate was washed, dissolved in NaOH, and measured for HA carbon content using the same oxidation and titration procedure.

The humic acid (HA) precipitate was dissolved in a hot 0.05 M sodium bicarbonate solution (instead of sodium hydroxide) in a 25-100 mL volumetric flask. The concentration of the solution was adjusted to $0.136 \text{ mg}\cdot\text{mL}^{-1}$ through dilution or concentration. A GBC Cintra 1010 UV-Vis spectrophotometer was calibrated with 0.05 M sodium bicarbonate solution as a reference. Optical density (absorbance) was measured at wavelengths of 726, 665, 655, 619, 600, 574, 533, 496, 465, and 400 nm using a 1 cm cuvette. Absorption spectral curves were plotted using absorbance values at 726, 655, 619, 574, 533, 496, and 465 nm.

The $\Delta\log K$ calculation method is as follows:

$$\Delta\log K = \log A_{400} - \log A_{600}$$

The E4/E6 value calculation method is as follows:

$$E4/E6 = A_{465}/A_{665}$$

where A400, A460, A600, and A665 are the absorbance values at wavelengths 400, 465, 600, and 665 nm, respectively.

The SOC was determined with Fourier transform infrared transmission spectrometry method following Ndzelu *et al.* (2021) and Sharaf *et al.* (2021).

The mid-infrared spectrum is typically divided into two main regions: the 4000–1300 cm^{-1} region, known as the group frequency region, functional group region, or characteristic region, and the 1300–600 cm^{-1} region, referred to as the fingerprint region (Song *et al.* 2022). The group frequency region is primarily used to identify functional groups present in the sample, making it an essential area for studying the characteristics of soil organic carbon (SOC) and humus components. In contrast, the fingerprint region is more complex, with subtle variations in absorption patterns that allow for the differentiation of compounds with similar structures. This region is particularly useful for distinguishing between different substances based on their molecular structures.

The determination of soil physicochemical indicators (Moisture content, pH, Organic matter, Total nitrogen, Hydrolyzable nitrogen, Available phosphorus, Available potassium, Exchangeable calcium, Exchangeable magnesium, Available sulfur, Total exchangeable bases, Cation exchange capacity, Base saturation) involves several established methodologies (XU *et al.* 2008, Atallah *et al.* 2017, Zhang *et al.* 2019, Propa *et al.* 2021, Alam *et al.* 2024). The scanned spectral lines were analyzed and plotted using Fourier transform infrared spectroscopy software OMNIC and Origin 8.5. SPSS 18.0 was applied for data analysis. Infrared spectroscopy principal component analysis was performed using MATLAB.

Results and Discussion

With the application of organic fertilizers, the change in nutrient content of fast-acting nutrients was positively correlated with organic carbon (Hernández-Montoya *et al.* 2012, Li *et al.* 2012). According to the principle of control variables, the nutrient content of each soil sample must be consistent when identifying the differences in soil carbon components and structure at different pH values under the same organic matter level. In other words, the effective nutrient content of soils with different pH values under the same organic matter level should be consistent. In order to select soils with the same organic matter content and different pH values, test results of soils in Jietou Town, Tengchong city, were divided into four groups according to pH values. Basic soil physicochemical indexes determined by soil testing and formula fertilization of tobacco-growing soils in Baoshan city during 2011-2017 were considered for the division. The percentage of each group was as follows: 19.35% for $\text{pH} < 5.5$ (strongly acidic soil), 78.8% for $\text{pH} = 5.5-6.5$ (slightly acidic soil), 0.92% for $\text{pH} = 6.5-7.5$ (neutral soil), and 0.92% for $\text{pH} > 7.5$ (alkaline soil). Based on the nutrient data of neutral and alkaline soils, the range of basic physical and chemical indicators for sampled soils was determined. Moreover, the on-site sampling was performed based on the coordinates of the sampling sites in previous years and the description of the relevant sampling leaders. The actual number of samples taken was 134. Based on the measured soil pH and organic matter content, soils were categorized into the following eight treatment groups.

According to Table 2, the optimum pH range for the growth of tobacco is 5.5-6.5, with soils of high, rich organic matter content (Group IV) accounting for the highest proportion in this range.

The second optimum range is pH <5.5, with soils of high, rich organic matter content (Group II) accounting for 86.57%. Soils with high organic matter content account for 79.85% of the selected samples. Under the same level of organic matter content, soil pH includes different gradients of strongly acidic, slightly acidic, neutral, and alkaline. Differences in soil acidity and alkalinity may lead to different absolute and relative contents of soil humus components. The content of active components, molecular structures, and functional groups in organic carbon may also vary. Soils at the same pH also cover samples with low, medium, high, and rich organic matter levels. The soil humus components, the visible spectral properties of HA, and the functional group structure of carbon were further analyzed. The analysis results further explained the differences in soil carbon components for different organic matter contents under the same pH (especially for acidic soils with pH < 6.5) and for different pH values under the same organic matter content (especially for high carbon soils with organic matter content > 25 g/kg).

Table 1. The range of basic physical and chemical indicators of soil for experiment.

Organic matter	Alkali hydrolyzed nitrogen	Available Phosphorus	Availability potassium	Effective boron	Exchangeable magnesium	Effective zinc	Water-soluble chloride ion
g/kg				mg/kg			
<15	48.62-65.14	11.95-15.53	44.75-130.90	0.14-0.46	78.62-106.23	0.96-2.17	17.77-23.38
15-25	89.95-91.65	4.80-8.83	46.30-137.00	0.13-0.30	52.50-80.80	0.73-0.88	11.56-18.94
25-35	93.73-102.06	8.38-24.72	97.99-192.90	0.32-0.47	90.98-145.40	0.78-2.18	19.96-23.84
>35	174.22-220.17	14.47-42.01	136.00-291.01	0.44-1.40	75.98-121.00	0.83-1.65	9.97-15.41
Total	48.62-220.17	4.80-42.01	44.75-291.01	0.13-1.40	52.50-145.40	0.73-2.18	9.97-23.84

Table 2. Grouping of actual sampled soil from Jietou town, Tengchong.

Number	pH	Organic matter content	Sample quantity (pieces)
I	<5.5	Low, Medium	18
II	<5.5	High, Rich	28
III	5.5-6.5	Low, Medium	2
IV	5.5-6.5	High, Rich	68
V	6.5-7.5	Low, Medium	5
VI	6.5-7.5	High, Rich	6
VII	>7.5	Low, Medium	2
VIII	>7.5	High, Rich	5

The high and low standards for organic matter content: low and medium organic matter ≤ 25 g/kg, high and rich organic matter > 25 g/kg.

Based on the soil physicochemical indexes in Group III (pH=5.5-6.5, low and medium organic matter content) and Group VII (pH >7.5, low and medium organic matter content), the range of soil nutrient contents was determined (Table 3). Samples from other treatments within this nutrient content range (a total of 29 samples) were selected for humus component, HA visible spectroscopy, and soil FTIR spectroscopy analyses (Table 4).

Table 3. Physical and chemical indicators of soil for carbon composition analysis.

Alkali hydrolyzed nitrogen	Available Phosphorus	Availability potassium	Effective boron	Exchangeable magnesium	Effective zinc	Water-soluble chloride ion	Cation exchange capacity
(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(cmol/kg)
50.82-98.13	5.58-14.82	45.83-136.96	0.14-0.68	50.45-90.60	0.79-1.53	10.23-21.25	22.21-28.30

Table 4. Sample information for soil carbon composition analysis.

Group	Organic matter content	pH	Sample quantity (pieces)
1		<5.5	5
2	Low, Medium	5.5-6.5	2
3		6.5-7.5	2
4		>7.5	2
5		<5.5	6
6	High, Rich	5.5-6.5	7
7		6.5-7.5	2
8		>7.5	3

Soil samples with different organic matter contents were compared at the same soil nutrient level, and differences between absolute and relative contents of humus components were studied at the same pH level. The comparison results showed the effect of different organic matter contents on the levels and composition of soil humus components. As can be seen from Tables 5, the HA, FA, and HM, HA/FA, and PQ (the proportion of HA in the extractable humus) increase with the total organic carbon content at the same pH level, but their relative contents are different. Under the same pH environment, the HA/FA and PQ of soil samples with an organic matter content of more than 25 g/kg are higher than those with an organic matter content of less than 25 g/kg. Soils with high organic matter content present lower FA content and higher HA content. FA is also a more active substance than HA because of its smaller molecular weight, lower degree of aromatization, and stronger acidity. The above results indicate that the stable humus content is higher in soils enriched with organic matter than in soils less enriched with organic matter at the same pH value. In contrast, low organic matter content can lead to high levels of active humus components. When amending acidic tobacco-growing soils, a higher organic matter content can lead to higher levels of stable humus components. However, increasing the active carbon component is more favorable for improving acidic soils. Therefore, it is more difficult to improve acidic tobacco-growing soils with high organic matter content than acidic soils with low organic matter content. Acidified tobacco-growing soils with different organic matter contents and different organic carbon activity components require the addition of plant materials with different carbon compounds and molecular activities to achieve the desired results rather than being improved solely by straw return.

As can be seen from Table 5, the HA/FA and PQ of the soil with low organic matter content and pH >5.5 gradually decrease with increasing pH, indicating a gradual increase in the relative content of highly active humus components (FA) and a gradual decrease in the relative content of highly stable humus components (HA). The analysis of the absolute HA content shows no significant differences between HA carbon contents at different pH levels. The HA content of the pH<5.5 group is lower than that of the pH=5.5-6.5 group, suggesting a less effect of pH on HA in the group with low organic matter contents. In such groups, acidic soils have significantly higher FA content than neutral and alkaline soils.

Table 5. Summary table of variance analysis of humus components in eight grouped soil samples.

Group	pH	HA (g/kg)	FA (g/kg)	HM (g/kg)	HA/FA	PQ
1	5.12±0.08d	1.74±0.65bcd	3.18±0.21bcd	7.29±0.71cd	0.57±0.22bc	13.30±3.24bc
2	5.74±0.02c	2.33±0.11bcd	2.92±0.33cd	10.47±0.56bcd	0.80±0.05bc	14.84±0.78abc
3	7.10±0.20b	0.64±0.01d	1.31±0.15d	4.84±1.32d	0.50±0.06bc	9.94±2.31c
4	7.70±0.05a	0.19±0.01d	1.71±0.02d	3.93±0.25d	0.11±0.03c	3.18±0.89c
5	5.26±0.06d	9.63±1.25a	5.01±0.68abc	19.36±1.50a	1.94±0.38a	26.68±3.80a
6	5.85±0.11c	7.32±0.75ab	5.37±0.61ab	14.89±1.55ab	1.41±0.14ab	26.63±1.21a
7	6.91±0.21b	6.53±0.01abc	6.22±0.08a	16.54±0.96ab	1.05±0.01abc	22.33±0.77ab
8	7.94±0.10a	1.51±0.58cd	4.19±0.57abc	12.79±1.22abc	0.34±0.08c	7.69±1.19c

Data are expressed as mean ± standard error (n ≥ 3), and different letters in the same column indicate significant differences between treatments (p < 0.05)

The effect of pH on soil HA content is not significant in groups with low organic matter content. Differences in humus components of soil with high organic matter content were primarily analyzed. As can be seen in Tables 5, treatments with high organic matter content exhibit a significantly higher FA than those with low organic matter content, indicating a strong relationship between FA content and organic carbon content. In the group with high organic matter content, the HA gradually decreases with increasing soil pH. The HA carbon content of soil samples with pH > 7.5 is significantly lower than that of the pH < 5.5 group by 84.32%. In addition, HA/FA and PQ values gradually increase with increasing acidity in soils with high organic matter content. A greater pH difference represents more significant differences between HA/FA and PQ, indicating an increase in the highly stable humus component.

Correlation analysis results in Tables 6, 7, and 8 reveal a positive correlation between the organic matter content and the content of humus components. At low organic matter levels, pH is negatively correlated with the content of humus components but is not significantly correlated with HA carbon, HA/FA, and PQ values. Moreover, a highly significant negative correlation is found between pH and HA carbon in soils with high organic matter content. A higher pH leads to smaller HA/FA and PQ values, which is consistent with the above results. For soils with organic matter ≤ 25 g/kg, pH shows a highly significant negative correlation with total humus carbon content; for soils with organic matter > 25 g/kg, pH exhibits a significant negative correlation with organic matter content. This result suggests that treatment schemes with different organic matter contents have differences in improving the acidification of acidic soils. Therefore, different amending substances should be determined based on differences in soil organic matter content and other indicators.

Table 6. Correlation analysis of 29 samples.

	HS	HA+FA	HA	FA	HM	HA/FA	PQ
pH	-0.342	-0.380*	-0.410*	-0.226	-0.293	-0.470*	-0.485*
HS		0.978**	0.943**	0.853**	0.980**	0.761**	0.750**
HA+FA			0.976**	0.845**	0.916**	0.798**	0.827**
HA				0.708**	0.873**	0.890**	0.870**
FA					0.825**	0.404*	0.546**
HM						0.695**	0.647**
HA/FA							0.924**

Table 7. Correlation analysis of soil under different pH conditions (organic matter content \leq 25 g/kg).

	HS	HA+FA	HA	FA	HM	HA/FA	PQ
pH	-0.773**	-0.781**	-0.517	-0.884**	-0.678*	-0.378	-0.428
HS		0.903**	0.792**	0.748**	0.953**	0.677*	0.627*
HA+FA			0.902**	0.796**	0.730*	0.746**	0.797**
HA				0.456	0.624*	0.947**	0.957**
FA					0.629*	0.209	0.301
HM						0.550	0.436
HA/FA							0.965**

** and * indicate significant correlation at 0.01 and 0.05 level, respectively.

Table 8. Correlation analysis of soil under different pH conditions (organic matter content $>$ 25 g/kg).

	HS	HA+FA	HA	FA	HM	HA/FA	PQ
pH	-0.535*	-0.585*	-0.649**	-0.165	-0.439	-0.690**	-0.722**
HS		0.963**	0.924**	0.684**	0.958**	0.635**	0.604**
HA+FA			0.968**	0.688**	0.845**	0.698**	0.754**
HA				0.484*	0.803**	0.841**	0.833**
FA					0.625**	-0.001	0.219
HM						0.517*	0.397
HA/FA							0.897**

** and * indicate significant correlation at 0.01 and 0.05 level, respectively.

Similar to other polymer compounds, the absorption spectra of HA in the visible wavelength band are non-characteristic. There is no maximum or minimum on the spectral curve, and the optical density often decreases with increasing wavelength. However, the slope of the absorption spectrum curve can reflect the condensation degree of the aromatic ring in the HA molecule, the aromatization degree, and the molecular weight. A larger slope (E4/E6) indicates a smaller condensation degree of the aromatic ring in the molecule, a lower aromatization degree, and reduced molecular weight. In addition, the slope of the HA absorption spectrum curve varies with soil type. E4/E6 values of soil humus reflect the condensation degree, aromaticity, and molecular weight of the soil humus. A smaller E4/E6 value indicates a higher molecular complexity, more aromatic nuclei groups, and a higher condensation degree. ANOVA was performed for groups with low and high organic matter contents. As shown in Table 9 and Fig. 1, the E4/E6 values gradually

increase with the pH value at the same organic matter content. It indicates that the content of highly active organic carbon components in the soil increases with increasing pH, and the stability of humus decreases. ANOVA results for the eight groups reveal that organic matter-rich soils have lower E4/E6 values and higher stable humus components compared to soils with low organic matter content at the same pH. According to the above analysis, the highly active humus components in soil increase with increasing pH at the same organic matter content. With the same pH value, the highly active humus components in soil increase with decreasing organic matter content.

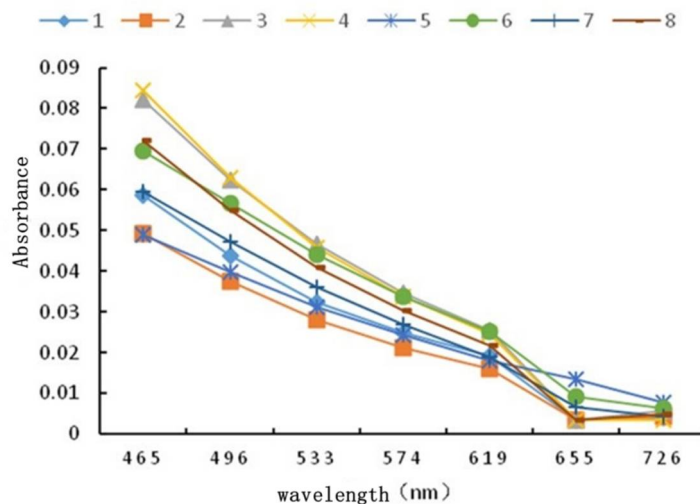


Fig. 1. Spectral optical density curves of different groups of soils (average of each group)

Table 9. Analysis of variance of optical density of humic acid and A2920/A1630 in each group.

Organic matter	pH	A2920/A1630	E4/E6
C≤25 g/kg	<5.5	0.19	17.94±1.35b
	5.5-6.5	0.33	15.05±0.07b
	6.5-7.5	0.35	25.11±1.53a
	>7.5	0.40	25.88±0.97a
C>25 g/kg	<5.5	0.23	3.74±0.14c
	5.5-6.5	0.22	8.79±1.23bc
	6.5-7.5	0.24	13.86±9.64ab
	>7.5	0.27	22.11±4.74a

The unstable functional groups include alcohol-phenol free-OH, C-O of polysaccharides, and aliphatic-CH, with absorption peak positions at 1007, 1015, and 1031 (1029, 1009) cm^{-1} , respectively, representing the C-O stretching vibration of polysaccharides. According to the results in Table 10, the relative content of functional group structures representing active carbon gradually increases with increasing pH at the same organic matter level. It indicates a significant effect of pH on the functional group structure of active carbon. Stable functional group structures include the aromatic C=O and C=C. By analyzing the relative integral area of the absorption peak position

at 1630.52 cm^{-1} , it can be found that the relative content of rA1630 gradually increases with decreasing pH at the same organic matter level. This result indicates a gradual increase in the relative content of functional group structures representing inert carbon. With smaller absorbance A2920/A1630, the relative content of inert carbon functional groups is higher. According to the obtained absorbance A2920/A1630, the treatment ratios of each group are 4, 3, 2, 8, 7, 5, 6, and 1 in descending order, which also illustrates that the content of inert carbon fraction increases with decreasing pH.

Table 10. Infrared spectroscopy analysis of each soil samples.

Number of absorption peaks cm^{-1}	Ascription	Relative area
1107、1029、1010	C-O stretching vibration of polysaccharides	3>4>1>8>2>7>6>5
1630	C=C, Stretching vibration of phenolic aromatic ring (Aromatic C=C)	5>6>7>2>1>8>3>4

Differences in the content of each active carbon fraction in different acidic and alkaline soils were identified by determining the molecular structure of humus components, active functional groups, and compounds with large molecular weights (e.g., lignin) in different soil sample groups under the same climatic conditions. Under the same pH condition, the effect of organic matter content on soil humus components and their structure was obtained by comparing soil samples with the same soil nutrient content and different organic matter contents. The comparison results indicate that at a certain pH range and the same level of organic matter content, the stable humus component increases, and the soil pH decreases. By comparing the differences in carbon components and carbon structure of soil samples with the same organic matter content at different pH values, the effect of pH on soil humus components could be revealed.

Tobacco-growing soils under the same climatic conditions, same soil formation material, and the same level of organic matter content in Tengchong city have different gradients of pH, including strongly acidic, slightly acidic, neutral, and alkaline. The humus components of soils with different acidity and alkalinity at the same organic matter level were analyzed, and the functional group spectra of soil carbon were analyzed using the Fourier infrared method. The results show that HA/FA gradually decreases with increasing pH in soils with low organic matter content, indicating a gradual increase in the highly active carbon components and a gradual decrease in the highly stable carbon components. Soils with high organic matter content exhibit a gradual increase in HA/FA with increasing acidity and an increase in stable humus components. The comparison results indicate that at a certain pH range and the same level of organic matter content, the stable humus component increases, and the soil pH decreases. Increasing the content of FA (active carbon component) in soil can facilitate the increase of soil pH and the improvement of acidified soils.

Soil acidity and alkalinity affect the redox potential of soil. Theoretically, 1 unit increase in pH results in 59 mv decrease in Eh. The redox capacity of humus depends on the range of redox potential distribution and redox functional groups, and the molecular structure of humus is influenced by soil pH (Bernard *et al.* 2022). Soil acidification inhibits the growth and activity of microorganisms and reduces their number in soils, thus affecting the decomposition of soil organic matter and the cycling of carbon, nitrogen, phosphorus, and sulfur (Haynes 1984). In addition, microorganisms involved in organic carbon cycling and related enzyme activities are inhibited, decreasing the utilization of carbon sources by microorganisms and promoting the burial of soil organic carbon (Wu *et al.* 2016). After long-term acid rain drenching and continuous soil

acidification, a series of physical, chemical, and biological processes in the soil are affected, which also changes the soil carbon cycle process and ultimately affects soil carbon storage (Lu *et al.* 2015).

Acknowledgements

We would like to thank the anonymous reviewers for their valuable comments on this study. The work was supported by the National Natural Science Foundation of China (Grant No. 42467007), Guizhou Science and Technology Partnership Initiative, Basic Research Grant [2024] Youth 177, the Science and Technology Project of Guizhou Tobacco Company (Grant No. 2024XM17, 2021XM21), the Science and Technology Project of Bijie Tobacco Company (Grant No. 2023520500240161, 2024XM20) and the Science and Technology Project of Zunyi Tobacco Company (Grant No. 2022XM06), the Science and Technology Project of Yunnan Wenshan Tobacco Company (Grant No. 20245326002), the Science and Technology Project of China tobacco Fujian industrial Co., Ltd (Grant No. FJZYKJJH2022ZD029), China National Tobacco Corporation Key Research and Development Project (110202102037), China National Tobacco Corporation Guizhou Provincial Branch Science and Technology Project (2024520000240027).

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(Manuscript received on 03 August, 2024; revised on 19 September, 2024)