INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1458/2018/20–9–2110–2116
DOI: 10.17957/IJAB/15.0739
http://www.fspublishers.org

Full Length Article

Nutrients, Enzyme Activities, and Microbial Communities of Major Soils used for Tobacco Production in Yunnan, China

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Abstract

There are three major soil types used for tobacco production in Yunnan Province, which produces around 20% of all flue-cured tobacco in the world. To apply sound agronomic management, and to maintain a healthy soil environment for tobacco production, soil nutrients, enzyme activities, and microbial community structure were studied. The pH of the three major soil types varied from 5.7 to 7.3. The primary nutrient contents were sufficient to meet the minimum requirements for flue-cured tobacco production. The activities of all kinds of enzymes, including urease, phosphatase sucrase, catalase and dehydrogenase, in the three soil types were higher than in other regions, which may be related to the soil nutrient contents. The composition of the bacterial and fungal communities differed in different soil types, as determined by high-throughput sequencing analysis, but all comprised eight unknown bacteria and five unknown fungi. The difference in richness might be due to soil texture and nutrient levels. These results provide useful information about chemical and biological properties for soil management decisions in tobacco production.

Keywords: Flue-cured tobacco; High-throughput sequencing; Fungi; Bacteria

Introduction

Flue-cured tobacco is an important economic crop in Southwest China, which provides revenue through taxation amounting to 7−10% of national revenue (Li, 2015). As the main production area for tobacco, Yunnan Province annually plants an area of more than 250,000 ha, yielding up to 900,000 t. There were three major soil types found in tobacco production areas in Yunnan Province (red soil, purple soil, and paddy soil accounting for up to 57, 14 and 3% of the total growing region, respectively). The differences in soil nutrient levels and microbial properties among the soil types have complicated classification and fertilisation guidance (Hu *et al*., 2014); however, there are few studies systematically studying nutrients, enzyme activities, and microbial community structure among these soils with regard to flue-cured tobacco production.

There have been some reports evaluating different types of tobacco-growing soil in China (An and Ai, 2010; Wang *et al*., 2014; Wang *et al*., 2015); however, most of these studies remain at the level of nutrient analysis. Few studies are available on soil enzymes and microbes (Wang *et al*., 2008). Soil enzymes are considered the metabolic power of soil organisms, which play an important role in agricultural ecosystems (Xu *et al*., 2017), and they have been reported to have a close relationship to soil types, soil physical and chemical properties, fertiliser regime, and other agronomic practices. Bacteria and fungi are the most abundant component of soil microbial biomass. They are closely related to soil organic pool and enzyme activity, with various physiological, biochemical, and ecological functions (Acosta-Martínez *et al*., 2008; Shen *et al*., 2014; Wang and Bau, 2014; Cressey *et al*., 2018).

Research techniques used to evaluate bacteria have developed significantly (Manichanh *et al*., 2008). The plate isolation method, PLFA analysis, PCR-DGGE, and other conventional molecular biological techniques used to cultivate bacteria, cannot reflect the community composition soil microorganisms (Chen *et al*., 2015). High-throughput sequencing technology has a broad range of applicability, sensitivity, stability, can detect activity and dormancy in microorganisms and is much more likely to obtain the number of microbes compared to using traditional methods (Xia and Jia, 2014).

To cite this paper: Zou, C., Y. Li, Y. Jin, W. Huang, G. Zhao, X. Ji, R. Liu, X. Yang, Y. Chen, J. Li, X. Hu and Y. Duan, 2018. Nutrients, enzyme activities, and microbial communities of major soils used for tobacco production in Yunnan, China. *Int. J. Agric. Biol*., 20: 2110–2116

To improve soil management for flue-cured tobacco production, the objective of this present study was to analyse the differences in the physiological and biochemical characteristics, and the microbial community structure of three main soil types in Yunnan.

Materials and Methods

Study Site Description and Experimental Design

Three main tobacco-growing soils were chosen for this study in Yunnan Province, including red, purple, and paddy soil. The three tobacco growing soils are in three tobacco research stations that belong to the Yunnan Provincial Tobacco Monopoly Bureau. The red soil is from Shizong research station in Qujing County (coordinates: 24°49'53.36"N 103°59'16.23"E). The purple soil is from Donghua research station in Chuxiong County (coordinates: 24°56'45.36"N 101°29'29.21"E). The paddy soil is from Hongta research station in Yuxi County (coordinates: 24°21'26.24"N 102°32'38.26"E). Based on the USDA soil taxonomy, red, purple, and paddy soil were classified as Ultisols, Entisols, and Inceptisols, respectively (USDA, 2014). The three locations are all characterised by mild variation in mean monthly air temperatures, from 10°C in January to 25°C in June, but a relatively variable distribution of mean monthly precipitation, with an annual average rainfall of 850 mm: 80% of precipitation occurs from May to October in all three locations. This study belonged to the randomized block design with three replicates. The stations have similar altitudes and annual sunshine hours. Since 2010, all agronomic management practices at these three study sites, including tillage style, fertilizer and manure application, followed the same guidelines recommended by the Integrated Technology Promotion Centre at the Yunnan Academy of Tobacco Agricultural Sciences.

Soil Sample Collection and Analysis

Soil samples for routine analysis were collected from the surface to 20 cm depth before plants were transplanted in 2014. The composite soil sample for each plot (comprised of 10-cores per plot) weighed 0.5 kg, and each soil included three replicated plots. Soil samples were air-dried for routine analysis. Soil samples for enzyme activity and microbial analysis were taken four times from the same range of depths, including right before harvest, one month after transplanting, two months after transplanting, and at tobacco harvest. Soil samples were immediately placed in refrigerators at 4°C for soil enzyme activity assay.

Routine Chemical Analysis

Twenty-gram air-dried soil samples were used to perform routine analysis for soil pH, soil organic matter, soil total nitrogen, phosphorus, and potassium, soil alkalised nitrogen, and soil available phosphorus and potassium using routine analysis methods. The pH of the soil was measured by the potentiometric method. The pH glass electrode and the temperature sensor were inserted into soil suspension with water to soil ratio of 1:1(w/v). Soil organic matter and total nitrogen were determined by dry combustion with a Vario Max CN analyzer (Elementar Co.) Soil alkalized nitrogen, and soil available phosphorus and potassium were determined using infrared spectroscopy. Soil total phosphorus and potassium were analysed using continuous flow analysis.

Soil Enzyme Activity Analysis

Soil enzyme activities were assayed within two weeks of sampling. During this time, samples were stored at 4°C. Urease activity was determined by indophenol blue colorimetric method and was indicated by the milligram number of NH_4^+-N in 1 g soil after 24 h. Phosphatase activity was determined by disodium phosphate benzene colorimetric method, and was indicated by the milligram number of phenol released in 1 g soil after 24 h. Sucrase activity was determined by 3,5-dinitrosalicylic acid colorimetry method, and was indicated by the milligram number of glucose produced in 1 g soil after 24 h. Catalase activity was determined by potassium permanganate titrimetric method, and was indicated by the number of millilitres of 1 g soil consuming 0.1 mol L^{-1} $KMnO_4$ per hour. Dehydrogenase activity was determined by TTC colorimetric method and was indicated by the milligram triphenyl formazan (TPF) in 1 g soil after 24 h.

Bacterial Sequencing Analysis

DNA was extracted from 5–10 g (dry mass equivalent) of each soil sample by using the Ultra-Clean Mega Soil DNA kit (MoBio Laboratories, Carlsbad, CA, USA). PCR was performed with 30 cycles of 30 s each at 94°C, 30 s at 55°C, and 2 min at 72°C, with 2 min of pre-heating at 95°C, and a final extension of 7 min at 72°C. DNA was further purified by using a Sepharose 4b column, as described in (Jackson *et al*., 1997) with DNA yields quantified by PicoGreen fluorometry (Molecular Probes). The HEX-labelled primer Bac8f (5'-AGAGTTTGATCCTGGCTCAG-3'), and unlabelled primer Univ1492r (5'-GGTTACCTTGTTACGACTT-3'), were used to amplify bacterial 16S rDNA (Reysenbach and Pace, 1994). Thereafter, the Roche Genome Sequencer FLX platform was used to sequence bacterial 16S rDNA (Herlemann *et al*., 2013) on a TBS-380 Mini-Fluorometer (Turner BioSystems, Sunnyvale, CA, USA). These primers yield DNA fragments of approximately 2.4 kb in length that span the nuclear ribosomal internal transcribed spacer regions and include approximately 1,000 bp of both the nuclear small subunit ribosomal DNA (nSSU rDNA) and nuclear large subunit ribosomal DNA (nLSU rDNA) genes.

Through cluster analysis of classification units (*i.e*., as operational taxonomic units, OTUs), the specificity of these primers was checked by searching for short, nearly exact, matches in BLAST and in comparisons with aligned reference sequences from GenBank. Accordingly, 97% of the 16S rDNA sequence similarity determinations were represented by bacterial species.

Fungal Sequencing Analysis

DNA was extracted from duplicate 0.5 g soil sub-samples using the method previously described by Griffiths *et al*. (2000). PCR amplification of fungal 18S rDNA was carried out using the primer pairs: 0817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and 1196R (5'-TCTGGACCTGGTGAGTTTCC-3') in the V5 to V7 region of the fungal 18S section. PCR amplification products were purified using the Qiaquick PCR purification kit (Qiagen) before cloning with the pGEMT Easy vector system (Promega) using a molar vector: insert ratio of 1:1. Purified DNA was then sequenced using a 454 high-throughput sequencing method. Cluster analysis was used to determine classification unit (as operational taxonomic units, OTUs). The specificity of these primers was checked by searching for short, nearly exact, matches in BLAST and in comparisons with aligned reference sequences from GenBank. Accordingly, 97% of the 18S rDNA sequence similarity determinations were represented by fungal species.

Statistical Analysis

Data were analysed with the General Linear Model (GLM) procedure of the SAS 9.3 computer package (SAS Institute Inc., Cary, NC, USA). Replicate measurements on composite soil and plant samples were averaged for statistical analysis of treatment effects. Treatment effects were declared significant when the probability (p) of a greater F-statistic were less than 0.05 and less than 0.001. For soil enzyme and microbial data, the average of four data points was used for statistical analysis. Means separation was performed by Tukey's honest significant difference (HSD) test at the 95% confidence level. All plots were produced with Sigma Plot 12.3 (Systat Software Inc., Chicago, IL, USA).

Results

Soil Nutrient Content

The three different soil types had pH values ranging from 5.7 to 7.3 (Table 1). Soil organic matter content, was extremely significant ($p< 0.01$), and in purple soil it reached to 35.9 g kg^{-1}; 28 and 59% higher, respectively, than paddy soil and red soil. The rank order for the amounts of soil alkalised nitrogen and soil available potassium was purple soil > paddy soil > red soil. Differences in the soil available phosphorus contents were significant varying from 24.2 to 29.9 mg kg^{-1}. The soil total nitrogen (0.10% to 0.21%) and potassium (0.54% to 1.82%) contents differed significantly. Both were lowest in red soil. The changes in soil total phosphorus contents were similar to those of available phosphorus, but with no significant difference.

Soil Enzyme Activities

Urease and dehydrogenase activity were highest (2.14 mg NH_4^+-N kg^{-1} h^{-1} and 0.048 g TPF kg^{-1} h^{-1}, respectively) in red soil, but the activities of phosphatase and sucrase were significantly lower than those in purple and paddy soils (Table 2). Phosphatase activity was highest in purple soil 227% higher than that in red soil. Sucrase and catalase activity were the highest in paddy soil; sucrase activity was 159% higher compared to that in red soil. There was no significant difference in catalase activity between red soil and purple soil.

Soil Bacteria Diversity

From 454 high-throughput sequencing analysis, 56,209 to 57,504 bacterial sequences were found. The bacteria 16S rNDA reflected 3729 taxa in red soil, 3169 taxa in purple soil, and 3770 taxa in paddy soil. The differences in amount therein in each type of soil were significant (Table 3). The richness index (Ace and Chao) of the bacterial community, in rank order, was: paddy soil > red soil > purple soil. The Shannon diversity index of the bacterial community was highest in the paddy soil (7.134), followed by the red soil (6.913) (with no significant difference between them). The purple soil had the lowest Shannon diversity index at 6.186. The Simpson index showed that paddy soil (0.0996) > purple soil (0.0991) and red soil (0.0991).

The composition of the bacterial community in each soil type was different, but all comprised eight well-defined bacterial groups. The richness of eight main phyla bacteria was 94−96%, over the 90% level of the total soil bacteria (Fig. 1). The top four bacteria richness ranks in three types of soil were consistent, followed by Proteobacteria, Actinobacteria, Chloroflexi, and Acidobacteria, and in other types of soil these were also higher (Acosta-Martínez *et al*., 2008; Souza *et al*., 2013).

Soil Fungal Diversity

The ITS amplicon sequencing in fungi showed significant differences between soil with 978 taxa in red soil, 842 taxa in purple soil, and 749 taxa in paddy soil (Table 4). The richness index (Ace and Chao) of the fungal community ranked: red soil > purple soil > paddy soil. Similarly, the Shannon diversity index of the fungal community was also highest in the red soil (4.190) and purple soil (3.891) (with no significant difference between them) and lowest in paddy soil (3.443).

Table 1: Nutrient contents in three different types of tobacco-planting soil

Soil type	pH	Organic matter (g/kg)	Available nutrients (mg/kg)			Total nutrients (%)		
			Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
Red soil	7.3±0.20 aA	22.6±1.40 cC	96.6±14.46 cB	29.2±3.54 aA	152.3±36.15 bA	0.10±0.01 cC	0.11±0.06 aA	0.54±0.04cC
Purple soil	5.7±0.15 cC	35.9±1.32 aA	191.5±11.93 aA	24.2±2.10 aA	258.7±11.70 aA	0.21±0.00 aA	0.07±0.02 aA	1.34±0.04bB
Paddy soil	6.7±0.09 bB	28.2±1.14 bB	139.2±20.36 bA	29.9±1.71 aA	246.7±47.64 aA	0.19±0.01 bB	0.08±0.01 aA	1.82±0.06aA

Table values are the measured mean ± S.E.M., with two degrees of freedom due to soil type. Different lower-case letters indicate a statistically significant difference at $p< 0.05$ across soil type; and different upper-case letters a statistically significant difference at $p< 0.001$ across soil type

Table 2: Enzyme activities in three different types of tobacco-planting soil

Soil type	Urease(mg NH_4^+-N$kg^{-1}h^{-1}$)	Phosphatase(mg *p*N$Pkg^{-1}h^{-1}$)	Sucrase(g glucose$kg^{-1}h^{-1}$)	Catalase(molL^{-1} $K_2MnO_4h^{-1}g^{-1}$)	Dehydrogenase (g TP$Fkg^{-1}h^{-1}$)
Red soil	2.14±0.18 aA	3.49±0.73 cC	92.97±6.74 cC	1.19±0.08 bB	0.048±0.017 aA
Purple soil	1.57±0.11 cC	11.42±0.53 aA	191.25±11.18 bB	1.25±0.31 bB	0.012±0.002 bB
Paddy soil	1.72±0.14 bB	9.84±0.58 bB	240.49±12.26 aA	1.56±0.26 aA	0.036±0.005 aA

Table values are the measured mean ± S.E.M., with two degrees of freedom due to soil type. Different lower-case letters indicate a statistically significant difference at $p< 0.05$ across soil types; and different upper-case letters indicate a statistically significant difference at $p< 0.001$ across soil type

Table 3: Bacterial 16S rDNA sequences, taxonomic units and their community characteristics

Soil type	Reads	OTUs	Richness index		Diversity index	
			Ace	Chao	Shannon	Simpson
Paddy soil	57504a	3770a	4624a	4410a	7.134a	0.0996a
Red soil	56209a	3729a	4490a	4311a	6.913a	0.0991a
Purple soil	56225a	3169b	3850b	3694b	6.186b	0.0991a

Table values are the measured mean. Different lower-case letters indicate a statistically significant difference at $p< 0.05$ across soil types

Table 4: Fungi ITS rDNA sequence number, taxonomic unit, and its community eigenvalue

Soil type	Reads	OTUs	Richness index		Diversity index	
			Ace	Chao	Shannon	Simpson
Red soil	45823a	978a	1279a	1237a	4.190a	0.0943a
Purple soil	44339a	842b	1154b	1112b	3.891a	0.0946a
Paddy soil	52068a	749c	1061b	1020b	3.443b	0.0895a

Table values are the measured mean. Different lower-case letters indicate a statistically significant difference at $p< 0.05$ across soil types

The Simpson index was ranked: purple soil (0.0946) > red soil (0.0943) >paddy soil (0.0895) but was not significantly different between soils. The population structure of the fungi showed opposite pattern to that of the bacterial community in these three types of soil.

Similar to the bacteria, by 454 high-throughput sequencing analysis, 44339 to 52068 fungal sequences, and 749 to 978 species (genera) fungi were obtained. By far the fungal OTUs belonging to Ascomycota dominated (Fig. 2).

Discussion

The basic soil chemical properties play an importance role on soil nutrients supply capacity. The pH values of three soil types range greatly but are within the range of pH values required for normal growth and development of flue-cured tobacco (Ren *et al*., 1995). The lowest pH was found in purple soil associated with highest available N, which indicates the acidification process through nitrification. Soil organic matter contains nutrients required for plant growth and nutrient release. Appropriate amounts of organic matter are beneficial to the maintenance of soil quality and increased crop yield. A high organic matter content, especially in the latter part of crop maturation, could lead to late tobacco maturation, yellowing difficulties, and excessive nicotine levels (Shi *et al*., 2004). In this study, soil organic matter contents ranged from 2.3 to 3.6% indicating the potential to reduce the amount of nitrogen fertiliser and phosphate fertiliser therein. Chen *et al*. (2014), in contrast to this study, found that the organic matter content in paddy soil was the highest, which might be due to its intensive crop rotation scheme and high residue biomass input. Total phosphorus and available phosphorus in the three soils are suitable for high-quality tobacco production, but the contents of nitrogen and potassium in purple soil and paddy soil were high to the recommendation level, so reducing the amount of nitrogen and phosphorus is possible (Gao *et al*., 1995).

The description of soil extracellular enzyme activity on a global scale provides a frame of reference for comparing ecosystems. Soil enzymes, mainly released from soil microbes, can promote organic matter decomposition, humus synthesis, nutrient transformation, biological nitrogen fixation, and contaminant removal processes (Kennedy and Smith, 1995). Bacteria, as the numerically dominant and most diverse species found in soil microbe communities, were significantly associated with soil enzyme activities (Meng and Wu, 2004).

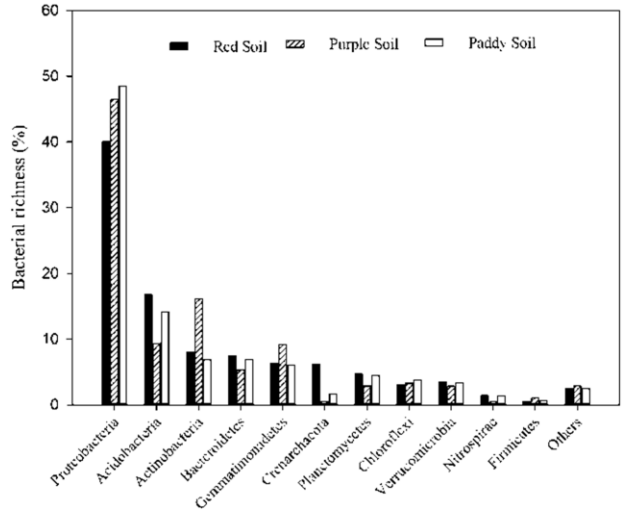

Fig. 1: Classification and distribution of bacterial communities across three soil types

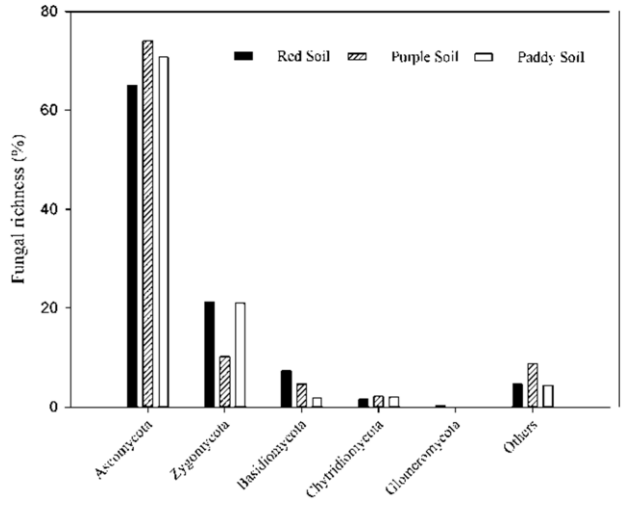

Fig. 2: Classification and distribution of fungal communities across three soil types

The soil enzyme activities in the three soil types were generally all higher, compared with other tobacco-planting regions, which might be due to the mild climate in Yunnan (Ye *et al*., 2013; Chen *et al*., 2015).

In this study, there were significant differences between soil type in terms of enzyme activity. Urease catalyses the hydrolysis of urea, which is a critical step in the nitrogen cycle. The relative ranking of soils in terms of urease activity is inverse to the relative ranking of soils with respect to available N and total N. Dehydrogenase, as a general indicator of redox reactions in cells, can only occur in living cells and is closely related to cell metabolic activity (Garcia *et al*., 1997; Guo *et al*., 2000). The relative ranking of soils with respect to dehydrogenase activity mirrors the relative ranking of soils with respect to pH (high to low), which reflects that neutral to slightly alkaline pH is normally associated with higher respiratory activity. Sucrase, and catalase are involved in soil organic matter decomposition (Meng and Wu, 2004; Yang *et al*., 2011). The relative ranking of soils with respect to sucrase and catalase activity is similar. The catalase measure is looking at labile C, which is what sucrose evaluates, hence the similarity. It is higher in paddy soil possible because in a wet soil the extent of complete C mineralization is retarded. Phosphatase plays a key role in P cycling in soils by releasing soluble P from organic matter. The relative ranking of the three soils with respect to phosphatase mirrored the relative ranking of the soils with respect to % SOM. Available P suppresses phosphatase activity, and although the differences between soils was usually not significant the relative ranking of soils with respect to phosphatase was inverse to the relative ranking of the soils with respect to total and available P. Most likely there is more phosphatase activity in the purple soil because it has a higher SOM content and, because it is acid, soluble P is diminished relative to what the other soils have so the microbial population thinks it is limited. There was few significant differences between activity and soil nutrient levels in different soil types, which was similar to the results of Qiu *et al*. (2004).

The Ace and Chao index represents the richness of microbial communities: the higher its value, the richer the community in species count terms (Anne, 1984). Number of organisms is not necessarily indicative of species richness. The diversity index indicates the number of species in the biocoenosis, and the larger the index, the richer the species variety in the community, and the higher the dominance index, the greater the dominant population in the alien community (Paul and Michael, 1988). Among these three soil types, the purple soil, the most acid soil, has the fewest OTU and significantly lower richness and diversity, even if no one group dominates. This phenomenon could be explained that pH has an effect on soil variable in purple soil.

In a healthy, stable, soil environment, the microbial diversity index should be high but the dominance index should be low. This result was found in the red soil, which further shows that the red soil offers a benign ecological environment, with high diversity and a rich structure to its bacterial community. The largest number of bacteria in the three types of soils belong to the Proteobacteria, which are ubiquitous in the natural environment and contain members that can participate in subsequent nitrification reactions, oxidation-reduction reactions, plant nitrogen fixation, stimulation of plant growth, *etc*. (Rudnick *et al*., 1997; Chen *et al*., 2012). The representation of actinomycetes in the three soil types of soil was also different, being highest in purple soil and lowest in paddy soil. This result was consistent with highest SOM content in purple soil. Actinomycetes play an important role in soil and are involved in the transformation of soil organic matter and the formation of soil aggregates, as well as the secretion of plant

growth hormone and antibiotics. Chloroflexi belonging to aerobic bacteria, are found in activated sludge, and help to degrade toxic substances in the soil (Roller *et al*., 1994). Acidobacteria contribute greatly to the stability of ecosystems and have high metabolic and genetic diversity (Susan *et al*., 1999). In addition, most of the bacteria belonging to Planctomycetes and Nitrospira, which might be due to the manure application history in three study sites, have beneficial effects on the removal of ammonia nitrogen and nitrite nitrogen in water; bacteria belonging to Gemmatimonadetes and nitrospirae may play an important role in resisting banana wilt disease. Therefore, the composition of bacteria in different tobacco soils is the same, but its richness will be different due to soil texture and nutrient content variations.

In a healthy and stable soil environment, various fungi compete with each other, which can prevent the over-propagation of some pathogenic fungi and reduce the incidence of crop diseases and benefit soil organic carbon (Li *et al*., 2017). By far the fungal OTUs belonging to Ascomycota dominated (Fig. 2). The fungal taxa, diversity, and richness in paddy soil are significantly less than the red and purple soil, and this phenomenon could be explained by that selective environmental pressure of flooding on fungi (Jiang *et al*., 2016). Penicillium can break down the cellulose, pectin, and starch in the soil (Jiang *et al*., 2010); Zygomycetes can break down sugars and simple polysaccharides, such as Mucor, Rhizopus, and Mortierella (Puget *et al*., 1999). Xiao *et al*. (2012) found that, the addition of *Ceratobasidium stevensii* B6 mycelia to the soil can improve the soil micro-environment and increase watermelon yield. *Synchytricum endobioticum* can cause potato wart disease (Günaçti and Erkiliç, 2010). *Glomus intraradices* can infect cucumber roots and form arbuscular mycorrhiza, enlarge the absorption range of the root system, and promote root absorption of nutrients (Li *et al*., 2014). The number of beneficial fungi in the three types of soil was higher, while those of harmful fungi was lower. Generally speaking, the number, and type, of fungi in red soil are better than those in purple soil and paddy soil, so, we can conclude that the red soil offers the better ecological environment.

Conclusion

There were the differences in soil nutrient levels, enzyme activities, and microbial community structure between three major soils evaluated for tobacco production potential in Yunnan Province. The activities of soil extracellular enzymes could reflect the characteristics of each soil. The population structure, and richness, and diversity of the fungi and the bacteria showed that the red soil is the healthiest among three major soil types. Also, the content of organic matter, available nitrogen, and available potassium in purple soil and paddy soil were higher than those in red soil. However, flue-cured tobacco production wouldn't require too much nutrients which would delay leaf maturity. Therefore, the amount of fertiliser should be controlled during tobacco-production on purple soil and paddy soil, but not on red soil.

Acknowledgements

This study was financially supported by several projects from the National Natural Science Foundation of China (No. 41601330), Yunnan Applied Basic Research Projects (No. 2017FB074) and Tobacco Monopoly Bureau China (110201302016, 2014YN13, 2016YN28, 2016YN31, 2017YN06 and 2017YN09).

References

Acosta-Martínez, V., S. Dowd, Y. Sun and V. Allen, 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. *Soil Biol. Biochem.*, 40: 2762–2770

An, D.Y. and F.Q. Ai, 2010. Kinds and fertility of soils for tobacco cultivation in Tongren Region. *Guizhou Agric. Sci*., 38: 126–129

Anne, C., 1984. Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.*, 11: 265–270

Chen, D.M., X.M. Chen, Y.H. Liang, X.J. Huo, C.H. Zhang, Y.Q. Duan, Y.H. Yang and L. Yuan, 2015. Influence of crop rotation on soil nutrients, microbial activities and bacterial community structures. *Acta Pratac Sin*., 24: 56–65

Chen, R.X., J.K. Su, J.B. Wang, J.B. Cai, H.Q. Yang, Z.Y. Ren, D.Q. Wang, H.T. Luo and Y. Ni, 2014. Effect of soil enzyme and edaphon on flue-cured tobacco growth and quality. *Acta Tab. Sin.*, 20: 73–78

Chen, X.B., Y.R. Su, X.Y. He, W.G. Qin, Y.W. Wei, Y.M. Liang and J.S. Wu, 2012. Effect of human disturbance on composition of the dominant bacterial group—Proteobacteria in Karst soil ecosystem. *Acta Pedol. Sin*., 49: 354–363

Cressey, E.L., J.A.J. Dungait, D.L. Jones, A.P. Nicholas and T.A. Quine, 2018. Soil microbial populations in deep floodplain soils are adapted to infrequent but regular carbon substrate addition. *Soil Biol. Biochem.*, 122: 60–70

Garcia, C., T. Hernandez and F. Costa, 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. *Commun. Soil Sci. Plant Anal*., 28: 123–134

Gao, Z.M., G.S. Liu, G.X. Zhang and D.P. Xie, 1995. The effects of phosphate levels on development and structure of flue-cured tobacco leaves. *Chin. Tob. Sci*., 4: 19–23

Günaçti, H. and A. Erkiliç, 2010. Determination of variety reaction to potato wart disease (*Synchytrium endobioticum*) in potato planting areas of Nevsehir Province, Turkey. *J. Turk Phytopathol*., 39: 39–44

Guo, M., H.J. Chen and C.L. Wang, 2000. Effect on soil dehydrogenase activity of four pesticides. *Environ. Chem*., 19: 523–527

Griffiths, R.I., A.S. Whiteley, A.G. O'Donnell and M.J. Bailey, 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl. Environ. Microbiol*., 66: 5488–5491

Herlemann, D.P., D. Lundin, M. Labrenz, K. Jürgens, Z. Zheng, H. Aspeborg and A.F. Andersson, 2013. Metagenomic*de novo*assembly of an aquatic representative of the verrucomicrobial class *Spartobacteria*. *MBio*., 4: e00569-12

Hu, L., L.J. Zhou, J. Wang, X.L. Zhang, T. Wang, Y.H. Pan and X.Y. Shan, 2014. Comprehensive evaluation of soil fertility in tobacco-growing areas in Yunnan Province. *J. Henan Agric. Sci*., 43: 52–59

Jackson, C.R., J.P. Harper, D. Willoughby, E.E. Roden and P.F. Churchill, 1997. A simple, efficient method for the separation of humic substances and DNA from environmental samples. *Appl. Environ. Microbiol.*, 63: 4993–4995

Jiang, H.Y., W. Yan, X.T. Li and Y.J. Fan, 2010. Diversity and community structure of soil fungi in Larixgmelinii Forest. *J. Northwest For. Univ*., 25: 100–103

Jiang, Y., Y. Liang, C. Li, F. Wang, Y. Sui, N. Suvannang, J.Z. Zhou and B. Sun, 2016. Crop rotations alter bacterial and fungal diversity in paddy soils across East Asia. *Soil Biol. Biochem.*, 95: 250–261

Kennedy, A.C. and K.L. Smith, 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil*, 170: 75–86

Li, C., C.X. He, Y. Yan, Y.S. Li, X.C. Yu and J.M.V. Martina, 2014. Effects of arbuscularmycorrhizal fungi inoculation at different stage on yield and nutrient quality of cucumber (*Cucumiss ativus L.*) in plastic tunnel. *Chin. Veget*., 1: 21–24

Li, Y.B., 2015. *Effect of Fertilizer Application for the Yield and Quality of Flue-Cured Tobacco and Soil Microbial*. Southwest University, Kenner, Louisiana, USA

Li, Y., Y. Li, S.X. Chang, X. Liang, H. Qin, J.F. Chen and Q.F. Xu, 2017. Linking soil fungal community structure and function to soil organic carbon chemical composition in intensively managed subtropical bamboo forests. *Soil Biol. Biochem.*, 107: 19–31

Manichanh, C., C.E. Chapple, L. Frangeul, K. Gloux, R. Guigo and J. Dore, 2008. A comparison of random sequence reads versus 16S rDNA sequences for estimating the biodiversity of a metagenomic library. *Nucl. Acids Res*., 36: 5180–5188

Meng, L.J. and F.Z. Wu, 2004. Advances on soil enzymes. *J. Northeast Agric. Univ*., 35: 622–626

Paul, R.H. and A.G. Michael, 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol*., 26: 2465–2466

Puget, P., D.A. Angers and U.C. Chen, 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biol. Biochem*., 31: 55–63

Qiu, L.P., J. Liu, Y.Q. Wang, H.M. Sun and W.X. He, 2004. Research on relationship between soil enzyme activities and soil fertility. *Plant Nutr. Fertil. Sci*., 10: 277–280

Ren, Y.H., J.J. Chen and J.F. Han, 1995. Influence of pH value on growth of flue-cured tobacco. *Chin. Tob. Sci*., 3: 1–5

Reysenbach, A.L. and N.R. Pace, 1994. Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by the polymerase chain reaction. *In: Archaea – A Laboratory Manual: Thermophiles*, pp: 101–105. Robb, F.T., K.R. Sowers, A.R. Place, H.J. Schreier (eds.) Cold Spring Harbor Laboratory Press, New York, USA

Roller, C., M. Wagner, R. Amann, W. Ludwig and K.H. Schleifer, 1994. *In situ* probing of Gram-positive bacteria with high DNA G+C content using 23S rRNA-targeted oligonucleotides. *Microbiology*, 140: 2849–2858

Rudnick, P., D. Meletzus, A. Green, L. He and C. Kennedy, 1997. Regulation of nitrogen fixation by ammonium in diazotrophic species of proteobacteria. *Soil Biol. Biochem*., 29: 831–841

Shen, Z.Z., D.S. Wang, Y.Z. Ruan, C. Xue, J. Zhang, R. Li and Q.R. Shen, 2014. Deep 16S rRNA pyrosequencing reveals a bacterial community associated with banana Fusarium wilt disease suppression induced by bio-organic fertilizer application. *PloS One*, 9: 1–8

Shi, J.X., S.Q. Zheng, Z.Q. Daio, M.X. Zhang and W.C. Zheng, 2004. Effects of manure application on quality and usability of leaf tobacco under different levels of nitrogen. *Chin. Tob. Sci*., 25: 42–45

Souza, R.A., L.C. Babujia, A.P. Silva, M. de Fátima Guimarães, C.A. Arias and M. Hungria, 2013. Impact of the ahas transgene and of herbicides associated with the soybean crop on soil microbial communities. *Transg. Res*., 22: 877–892

Susan, M.B., L.T. Shannon and R.K. Cheryl, 1999. Wide distribution and diversity of members of the bacterial kingdom *Acidobacterium* in the environment. *App. Environ. Microbiol*., 65: 1731–1737

USDA, 2014. *Keys to Soil Taxonomy*, 12th edition. USDA, Washington DC, USA

Wang, D.Q., L. Cheng, Z.C. Xu, Q.W. Bi, J. Wang and B.X. Wang, 2008. Analysis of the fertility status of different soil types in Shiyantobacco-growing areas of Hubei province. *J. Anhui Agric. Sci*., 36: 7322–7325

Wang, F. and T. Bau, 2014. Research advances in the diversity of soil fungi. *J. Fungal Res*., 12: 178–186

Wang, X.Z., Z.D. Fan, Z.G. Li, D.X. Wang, Y.X. Hu and F.H. Xu, 2015. Analysis of nutrient status in different types of tobacco-growing soil in Dali. *Acta Tab. Sin*., 21: 54–58

Wang, Y., J.J. Chen, F.J. Li and J. Luo, 2014. Analysis on nutrient status in the main types of tobacco-planting soil in Nanxiong of Guangdong province. *Guangdong Agric. Sci*., 41: 37–41

Xia, W.W. and Z.J. Jia, 2014. Comparative analysis of soil microbial communities by pyrosequencing and DGGE. *Acta Microbiol. Sin*., 54: 1489–1499

Xiao, Y., X.X. Wang, H.W. Wang, F.Y. Liu and C.C. Dai, 2012. The effects of the addition of *Ceratobasidum stevensii* B6 and its growth on the soil microflora at a continuously cropped watermelon (*Citrullus lanatus*) site in China. *Acta Ecol. Sin*., 32: 1185–1192

Xu, Z.W., G.R. Yu, X.Y. Zhang, N.P. He, Q.F. Wang, S.Z. Wang, R.L. Wang, N. Zhao, Y.L. Jia and C.Y. Wang, 2017. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South transect in eastern China (NSTEC). *Soil Biol. Biochem*., 104: 152–163

Yang, L.F., Q. Zeng and H.B. Li, 2011. Measurement of catalase activity in soil by ultraviolet spectrophotometry. *Chin. J. Soil Sci*., 42: 207–210

Ye, X.F., C. Yang, Z. Li and H.X. Jing, 2013. Effects of green manure incorporation on soil enzyme activities and fertility in tobacco-planting soils. *Plant Nutr. Fertil. Sci*., 19: 445–454

(Received 22 December 2017; Accepted 16 April 2018)