INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–0400/2017/19–5–992–998
DOI: 10.17957/IJAB/15.0374
http://www.fspublishers.org

Full Length Article

Effects of Tobacco Planting Systems on Rates of Soil N Transformation and Soil Microbial Community

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Abstract

Crop rotation is an efficient practice to control soil-borne pathogens and pests of continuous tobacco planting. For a wider and more detailed evaluation, the effects of four tobacco planting systems (T1, continuous cropping; T2, green manure-tobacco with crop rotation; T3, wheat inter-cropping; and T4 wheat-tobacco with crop-rotation) on soil N transformation and soil microbial community structure were evaluated using indoor culture and phospholipid fatty acids (PLFAs) technique in a four year field experiment. The results indicated that soil NH_4^+ and NO_3^- contents were both significantly affected by tobacco growth stages and planting systems, and were dependent upon form of N supplied. Rates of ammonification and nitrification and PLFA data from different soil microbial floras all varied with the tobacco planting systems and growth stages. Rates of ammonification increased at first, then decreased and increased in all treatments at the maturity stage. Rates of nitrification decreased at first and reached their lowest values at the fast-growing stage. The soil microbial community structure changed significantly in T4 throughout the whole tobacco growing season compared to that of T1. Correlation analysis indicated that the NH_4^+ content and ammonification rate were not or negatively and significantly correlated with the most parts of PLFAs except for ammonification rate with PLFAs of fungi and anaerobic bacteria, which was different from the NO_3^- content and nitrification rate. It was deduced that soil N transformation processes associated with soil microbial communities changed by differing tobacco planting systems, and the wheat-tobacco with crop rotation might be the optimized rotation system in the local area.

Keywords: Ammonification; Nitrification; Phospholipid fatty acids (PLFAs); Soil microbial community; Flue-cured tobacco

Introduction

Flue-cured tobacco (*Nicotiana tobacum* L.) is an important economic crop in the middle and southwest part of China. Tobacco planting is an effective way to improve the living standard of farmers in these mountainous areas (Hu *et al.*, 2007). But now, it is a growing challenge because of problems such as soil-borne disease, intensive fertilizer requirements, and multiple cropping pressures. To improve soil health and tobacco sustainability, several tobacco planting systems, such as crop rotation and intercropping, have emerged in these areas (Yang *et al.*, 2015). However, there is in need of wider and detailed evaluation, which includes determining the effects of tobacco planting systems and management practices on soil nutrient transformation and microbial community structure.

As one of the most important macronutrients for plant growth, nitrogen is always controlled by soil management practices. Anthropogenic disturbances (Yang *et al.*, 2008) and soil characteristics (Trap *et al.*, 2009) could result in changes in soil N transformation processes, such as ammonification and nitrification, which are essential functions that produce available nitrogen for plants and are driven by numerous soil microorganisms. And many soil microorganisms prefer NH_4^+ to NO_3^- as N sources (Omar and Ismail, 1999). But differently, NO_3^- is usually the preferred form for tobacco growth, since N supplied as NH_4^+ may reduce the yield and leaf quality (Cao *et al.*, 1992; Walch-Liu *et al.*, 2000).

Soil microorganisms play a key role in soil fertility and recycling of nutrients (Sotomayor-Ramirez *et al.*, 2009). To evaluate the soil quality improvement plans, the size and composition of soil microorganisms must be considered (Acosta-Martíneza *et al.*, 2010). Many studies have indicated that different crop rotation schemes have distinct effects on soil microbial biomass and community structure, when soils are augmented by the application of organic fertilizers (Jangid *et al.*, 2008; Yue *et al.*, 2016). Furthermore, one of the most important factors driving changes in soil microbiology is the crop or plant species present (Larkin, 2003; Baum *et al.*, 2013). And soil microbial community composition also changes with

To cite this paper: Zhang, J.G., Y.Z. Zhang, L.L. Zheng, Y. Shi, F.Y. Kong, X.H. Ma, L. Tian and Z.F. Zhang, 2017. Effects of tobacco planting systems on rates of soil N transformation and soil microbial community. *Int. J. Agric. Biol*., 19: 992-998

seasonal variations (Birgander *et al.*, 2014). Yet, little is known about the nature of changes in soil microbial ecosystems, including how they occur, or what their ultimate effects will be in planting systems (Olsson and Alström, 2000).

Therefore, a better understanding of the relationships among tobacco planting systems is necessary for a more sustainable production system. In this study, we have characterized and evaluated the effect of tobacco planting systems on rates and dynamics of soil N transformation and the microbial community.

Materials and Methods

Description of Experiments

The experiment was conducted from 2006 to 2009 in an upland area of Yishui County in Shandong Province of China (118 º40′ E, 35 º58′ N, approximately 220 m above sea level). The research area was representative of a typical temperate monsoon climate with an average annual rainfall of 792.1 mm, approximately 75% of which fell during the flood period (June to September). The mean annual temperature was approximately 12.3ºC, average annual hours of sunshine were 2468.2, and frost-free days were approximately 206 days each year. The soil developed from limestone and was classified as Alfisols (Soil Survey Staff, 1998). The cropland was continuous cultivated with flue-cured tobacco for more than 10 years. The soil chemical properties when the experiment began are shown in Table 1.

Treatments and Management

The experiment included four treatments (T1, T2, T3 and T4) arranged in a randomized complete block design with three replicates. T1 was continuous cropping of tobacco, T2 was green manure-tobacco with crop rotation, T3 was tobacco wheat inter-cropping, and T4 was wheat-tobacco with crop-rotation. Each experimental plot was 72 m^2 and separated by ridges to avoid cross contamination among plots. The tobacco was transplanted in mid-May and harvested in September every year. T2, T3 and T4 treatments were previously planted with crops other than tobacco. Furthermore, different rates of inorganic fertilizers, urea, superphosphate, and potassium sulfate were applied with these previous crops (Table 2). The mineral (potassium sulfate) and compound fertilizers for tobacco were applied at rates of 225 kg $ha^{-1}$and 800 kg ha^{-1}, respectively. All the potassium sulphate and 75% of the compound fertilizer were applied as basal fertilizer; the remaining 25% was applied as topdressing. The organic manure in the experiment included two sources: organic fertilizer (soya cake fertilizer) and green manure. The organic fertilizer was applied at a rate of 300 kg ha^{-1}as basal fertilizer for tobacco crop in each treatment. The green manure was annual ryegrass (*Lolium multifolorum)*, which was grown in T2 treatment during the winter fallow and then, it was ploughed back into soil (about 7500 kg ha^{-1} in dry weight) 30 days before the field preparation for tobacco transplantation.

Sampling and Analyses

Composite soil samples were collected from the surface layer (0–20 cm) in each plot (i.e., replicates) during the four stages of tobacco growth: ploughing (22 April), fast-growing (20 June), budding (28 July), and maturity (7 September) in 2009. Visible roots and fragments of organic debris were removed manually before the samples were ground until they could pass through a 2-mm sieve, after which they were divided into two parts. One part was kept fresh for ammonification and nitrification rate analysis, the other was stored at -20ºC for soil microbial community analysis.

Soil pH in distilled water (1:2.5 w/v) was measured using a pH meter equipped with a glass electrode. Soil organic carbon (SOC) , total N (TN) , alkali hydrolysable N (AH-N, NaOH), Olsen-P, and ammonia acetate extractable K (EK) were determined with air-dried soil using standard methods (Sparks *et al*., 1996). NH_4^+ and NO_3^- were determined with fresh soil using indophenol blue photometric method and UV-spectrophotometry, respectively (Lu, 2000).

Ammonification and nitrification rates were measured following Xu and Zheng (1986). Soil samples (three replicates) equivalent to 20 g of dry soil were placed in an Erlenmeyer flask, and 2 ml peptone solution of 2 mg g^{-1} and 1 ml culture medium of ammonifying bacteria was added. Distilled water was added to regulate soil moisture to 60% water-holding capacity (WHC). Samples were incubated for 14 days at 28ºC. Ammonium in soils was extracted with 1 mol L^{-1} KCl in a 1:4 soil: solution ratio (w/w). Ammonia was chlorinated to monochloramine, which reacts with phenol and nitroprusside to produce a green-colored complex, which was 630 nm (Zhu *et al.*, 2002). Soil samples (three replicates) equivalent to 20 g of dry soil were placed in an Erlenmeyer flask to which 5 mg N was added as a $(NH_4)_2SO_4$ solution. Distilled water was added in order to adjust soil moisture to 60% WHC. Soils were incubated for 14 days at 30ºC. Nitrates in soils were then extracted with 1.0 mol L^{-1} K_2SO_4 in a 1:4 soil: solution ratio (w/w). Nitrates were reduced to nitrites by cadmium powder. The nitrite reacts with sulfanilamide, N-(1-naphthyl) ethylnediamine dihydrochloride, and imidazole to form an azo-colored dye, which was 550 nm (Zhu *et al.*, 2002).

Lipid extraction and phospholipid fatty acids (PLFAs) were analyzed following the method of Frostegård *et al*. (1993). Briefly, aliquots of 3.0 g of fresh soil on a dry weight basis was extracted with a chloroform-methanol-citrate butter mixture (1:2:0.8) to separate the phospholipids from other lipids on a silicic acid column. The phospholipids were further subjected to mild-Alkali methanolysis and the resulting fatty acid methyl esters were

Table 1: Pre-experiment physical and chemical properties of soil in the research plot

pH	SOC (g kg^{-1})	TN (g kg^{-1})	AH-N (mg kg^{-1})	Olsen P (mg kg^{-1})	EK (mg kg^{-1})
6.18	7.17	0.43	35.62	10.04	65.24

SOC: soil organic carbon; TN: total nitrogen; AH-N: alkali hydrolyzable nitrogen; Olsen P: Olsen phosphorus; EK: ammonia acetate extractable potassium

Table 2: Amounts of fertilization and previous crops of different planting systems

Treatment	Previous crop	Transplant time	Amount of fertilizer for previous crops (kg ha^{-1})			Amount of fertilizer for tobacco (kg ha^{-1})			
						Base fertilizer			Top- dressing
			Urea	Super phosphate	K_2SO_4	Soya	K_2SO_4	Comp. fertilizer	Comp. fertilizer
T1	tobacco	05-20	-	-	-	300	225	600	200
T2	ryegrass	05-20	97.8	74.2	83.3	300	225	600	200
T3	wheat	05-20	326.1	192.4	216	300	225	600	200
T4	wheat	06-04	326.1	192.4	216	300	225	600	200

Time = mm/yy; Urea contained N 46.0%; superphosphate contained P_2O_5 16.0%; potassium sulfate contained K_2O 51%; Soya = soya cake fertilizer, which contained N 7.06 %, P_2O_5 1.30%, K_2O 2.13%; compound fertilizer contained N 15.0%, P_2O_5 15.0%, K_2O 15.0%

separated via a Hewlett Packard 6890 gas chromatography equipped with a flame ionization detector. The microbial community was divided according to different PLFAs. The bacterial biomass was the total of the following PLFAs: *i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, cy17:0, 10Me17:0, 10Me18:0, 18:1ω7* and *cy19:0* (Frostegård and Bååth, 1996). PLFA 18:2ω6 was as an indicator of fungal biomass and the sum of PLFAs of *10Me16:0, 10Me17:0* and *10Me18:0* defined the actinomycin biomass (Federle, 1986). The ratio of PLFA 18:2ω6: bactPLFAs represented the ratio of fungal: bacterial biomass (Bardgett *et al.*, 1996). Fatty acids with *iso* or *anteiso* branching were used as markers for Gram-positive (G^+) bacteria, and cy17:0 was used for Gram-negative (G^-) bacteria (Frostegård *et al.*, 1993).

Date Analyses

Analysis of variance (ANOVA) was used to determine which means differed significantly between treatments with Fisher's LSD. The relationships between soil microbial communities and soil parameters were evaluated with correlation analysis. All these statistical analyses were conducted using SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Mineral N Dynamics

Both NH_4^+ and NO_3^- content in soil were significantly different among growth stages and planting systems, and were dependent upon form of N supplied (Fig. 1). Mean NH_4^+ content in all treatments increased and reached a peak at the budding stage, and then declined as growth progressed. The NH_4^+ content in T2 was greater than other treatments during all growth stages, except for the ploughing stage, which occurred because of the return of ryegrass in T2, coupled with soil management throughout all tobacco growth stages. The highest NH_4^+ content (121.1 mg kg^{-1}) appeared at the budding stage in T2, and the lowest value (18.6 mg kg^{-1}) occurred at the ploughing stage in T1.

The NO_3^- content in different treatments was significantly different among growth states (Fig. 1). There was a similar trend for NO_3^- content in all treatments, which decreased as growth stages progressed. And the highest value occurred at the ploughing stage. The lowest value occurred at the maturity stage, which might be caused by the root uptake and leaching loss.

Ammonification and Nitrification Rate Dynamics

Ammonification rate in all treatments exhibited a similar trend, which increased at first, decreased, and then increased again, reaching its peak at the maturity stage (Fig. 2). The T3 had the lowest value at both the ploughing and budding stage, and there were no significant differences among treatments at other growth stages. Nitrification rate in all treatments decreased and reached its lowest point at the fast-growing stage, after which it increased as growth stages progressed. The nitrification rates were not significantly different in all treatments among growth stages, except for T2 and T3 at the fast-growing stage. The higher soil nitrification rates were found at the ploughing and fast-growing stages in T1, it suggested that T1 treatment might lead to high N losses through nitrate leaching.

Soil Microbial Community Dynamics

The total soil PLFAs and individual PLFAs in different treatments responded to the planting systems across growth stages (Fig. 3). The total PLFAs did not show a particular trend among treatments, except for T4 at the ploughing stage and T2 at the fast-growing stage. And the value in T2 (127.92 nmol g^{-1}) was significantly higher than that of other treatments. The trends of bacterial PLFAs, fungal PLFAs, G^+ bacterial PLFAs, G^- bacterial PLFAs, and total PLFAs were similar to one another within growth stages. They exhibited the highest values at the ploughing stage, declined dramatically at the fast-growing stage, and then remained

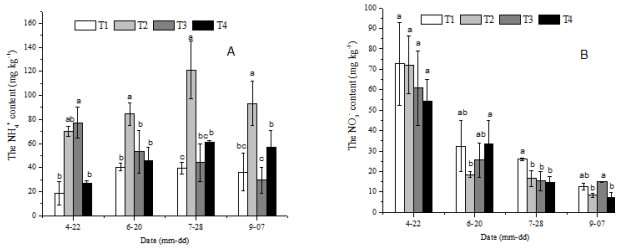

Fig. 1: Dynamics of soil NH_4^+ (A) and NO_3^- (B) content in different tobacco planting systems. Only statistically significant differences are represented by different letters (a, b, c)

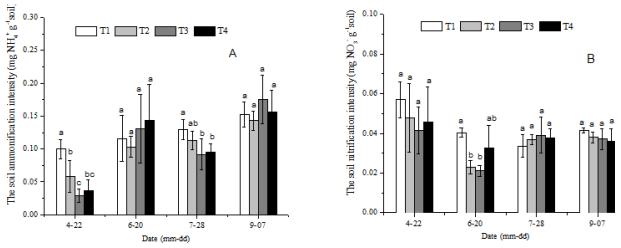

Fig. 2: Dynamics of soil ammonification (A) and nitrification (B) rate in different tobacco planting systems. Error bars within treatments with different letters were significantly different ($p < 0.05$)

constant. However, the actinomycete PLFAs decreased as growth stages progressed. The trend for aerobic bacterial PLFAs was similar to that of total PLFAs. However, the anaerobic bacterial PLFAs exhibited nearly the opposite trend with the lowest value (0.30 nmol g^{-1}) occurring at the fast-growing stage. The difference between aerobic and anaerobic bacterial PLFAs was related to aerobic conditions that originated from tillage at the ploughing stage.

Soil Microbial Community Structure Change

Changes in the ratio of fungi to bacteria (F/B biomass ratio) and gram positive to gram negative bacteria (G^+/G^- bacteria) across growth stages were showed in Fig. 4. Generally, the ratios of fungi to bacteria PLFAs showed little fluctuation among treatments during growth stages, and the values of them were all less than 0.3, which meant that the soil microbial community in this experiment was strongly dominated by bacteria. The ratio in T4 was lower than that of other treatments at the budding and maturity stages, as well as lower than the former growth stage within the same treatment. Furthermore, the ratios at the maturity stage implied that the soil microbial community structure was altered by tobacco planting systems across growth stages.

The ratio of G^+/G^- bacterial PLFAs also differed among the planting systems and growth stages. No significant differences were found in the ratio among all treatments at

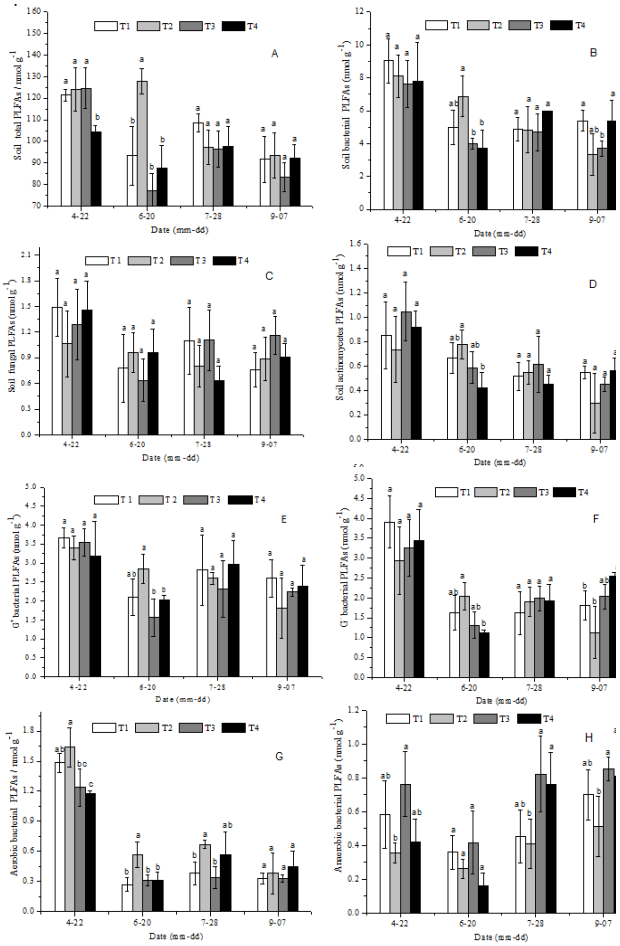

Fig. 3: Dynamics of different PLFAs data of soil microbial communities (A→H) in different tobacco planting systems. Error bars within treatments with different letters were significantly different ($p < 0.05$)

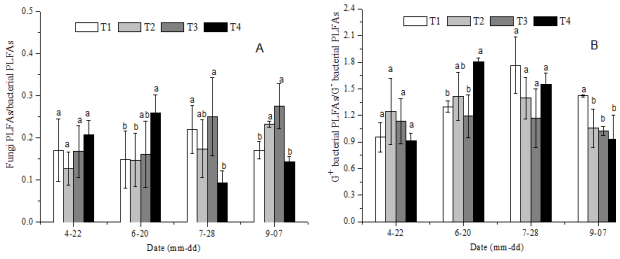

Fig. 4: Dynamics of soil microbial community (A) and bacteria community (B) composition in different tobacco planting systems. Error bars within treatments with different letters were significantly different ($p < 0.05$)

the ploughing stage. The ratio in T4 was increased at the fast-growing stage, and then decreased with progressive growth stages, which implied that the community structure of soil bacteria in T4 treatment changed substantially.

Table 3: Correlations between soil microbial properties and soil nitrogen characteristics

N transformation parameters	Total PLFAs	Bacterial PLFAs	Fungal PLFAs	Actinomycete PLFAs	G+ bacterial PLFAs	G- bacterial PLFAs	Aerobic bacterial PLFAs	Anaerobic bacterial PLFAs
NH_4^+ content	0.17	-0.14	-0.40	-0.13	-0.08	-0.25	0.01	-0.20
NO_3^- content	0.53*	0.72**	0.57*	0.72**	0.59*	0.67**	0.83**	-0.28
Ammonification rate	-0.67**	-0.75**	-0.47	-0.80**	-0.70**	-0.66**	-0.75**	0.10
Nitrification rate	0.37	0.59*	0.60*	0.36	0.64**	0.70**	0.67**	0.26

*and** means significant at $p < 0.05$ and $p < 0.01$ individually using two-way analysis of variance

The Correlations

The NH_4^+ content was not significantly correlated with all the PLFAs (Table 3). However, the NO_3^- content had significant positive correlations with all the PLFAs, except for the anaerobic bacterial PLFAs. Significant negative correlations were found between ammonification rate and all the PLFAs, except for the fungal and anaerobic bacterial PLFAs, whereas inverse correlations were found between nitrification rate and all the PLFAs, except for the total, actinomycete and anaerobic bacterial PLFAs. And the results also indicated that soil N transformation rates were highly related to soil microbial community among different planting systems.

Discussion

Ammonification and nitrification as important N transformation processes are of great importance in maintaining soil fertility and plant development (Kara *et al*., 2004). In this study, the ammonification and nitrification rates both varied with the tobacco planting systems and growth stages. And other studies were also found that soil cultivation and different cropping practices could significant affect soil N transformation processes (Mendelssohn *et al*., 1999; Raiesi, 2006). Because the cropping practices are known to modify factors that affect most biological processes in soil. And the difference in N transformation among the planting systems might be affected by soil properties and substrate quality (Mikha *et al*., 2006).

Phospholipid fatty acids (PLFAs) were widely used as the biological mark to analyze changes in soil microbial biomass and community structure (Bu *et al*., 2010). In this study, the total PLFAs in T2 was significantly higher (127.92 nmol g^{-1}) than that of other treatments, which was likely caused by the decomposition of the perennial ryegrass. Because green manure could increase soil organic matter, C and N availability, and then increase soil microbial biomass and activity (Shah *et al.*, 2010). And researchers also found that higher soil microbial biomass was related to C sequestration and soil organic material levels under agriculture systems focusing on minimizing fallow periods (Sotomayor-Ramirez *et al*., 2009; Acosta-Martíneza *et al*., 2010). Furthermore, the greater total PLFAs was found in T2 during the early growth stages, which were accompanied by an increase in bacterial and aerobic bacterial PLFAs, and a decrease in fungal and anaerobic bacterial PLFAs (Fig. 3). This was in accordance with other observations on the effects of straw incorporation (Bossio and Scow, 1998) and crop rotation (Bunemanna *et al.*, 2004).

The ratio of fungi to bacteria is a measure of what proportion of the microbial community is dominated by bacteria or fungi, which can be used as an index of shifts in microbial community structure (Bardgett *et al*., 1996). And the soil bacterial PLFAs was more than fungal PLFAs in our study (Fig. 2), and the fungi biomass only consists of less than 30% of the soil microbial biomass in these soils. Which meant the soil micro-flora was dominated by bacteria, and it was accord with the results in Netherlands (Bloem *et al*., 1994; Velvis, 1997). Zou *et al*. (2005) reported that continuous cropping soybean could result in the transition from bacteria dominated to fungi dominated soil. And the F/B biomass ratio of T4 was lower than that of other treatments at budding and maturity stages, which showed that the biomass of bacteria in T4 increased more than the fungi biomass, yielding the shifts in microbial community structure, which might be relate to the soil management, straw incorporation and soil type (Beare *et al*., 1997; T de Vriesa *et al*., 2006). And the most ratios of G^+/G^- bacterial PLFAs were greater than 1 in this study, which did not agree with the findings of Peacock *et al*. (2001), who reported that the ratios were generally lower than 1 for cultivated soils with large C content or soils being treated with organic substances. But the SOC content in the field was very low (Table 1). Furthermore, there was a notable increase of G^- bacterial PLFAs than G^+ bacterial PLFAs in T4 at the maturity stage, yielding a net decrease of the ratio of G^+/G^- bacterial PLFAs (Fig. 4), which showed that the bacterial community structure changed substantially in T4. In addition, G^- bacteria are also considered to be fast-growing microorganisms that utilize various C sources and adapt quickly to a changing environment (Hinojosa *et al*., 2005). Thus, the T4 with decreased ratio of G^+/G^- bacterial PLFAs could have elevated bacterial community tolerance to environmental stresses.

Conclusion

Soil NH_4^+ and NO_3^- content, rates of soil ammonification and nitrification, and PLFAs data from different soil microbial floras were all varied with the tobacco growth stages and planting systems. And the soil microbial community structure changed significantly in T4 throughout

the whole tobacco growing season compared to that of T1 through the ratios of F/B biomass and G^+/G^- bacterial PLFAs. Furthermore, soil NO_3^- content and nitrification were positively significantly correlated with most parts of PLFAs different from the NH_4^+ content and ammonification rate. So, the wheat-tobacco with crop-rotation might be the optimized rotation system, which could lead to the sustainable improvement of local tobacco farms for its decreased ratios of F/B biomass and G^+/G^- bacteria. The results also indicated that the PLFA technique provided a better insight into the effects of tobacco planting systems on the soil microbial community structure.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (41201291), the Agricultural Science & Technology Innovation Program (ASTIP-TRIC06) and the Basic Research Fund of Chinese Academy of Agricultural Sciences (TRIC2016). We thank the anonymous reviewers and the editor for their valuable comments and suggestions, which distinctly contributed to the improvement of this paper.

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(Received 02 August 2016; Accepted 20 April 2017)