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Full Length Article

Comparative Evaluation of Different Types of Soil Conditioners with Respect to Their Ability to Remediate Consecutive Tobacco Monoculture Soil

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Abstract

Consecutive monoculture cropping of tobacco has led to a decline in tobacco yield and quality. In this study, quicklime (900 kg/hm^2), calcium cyanamide (375 kg/hm^2) and microbial agents (210 kg/hm^2) were applied to soil where tobacco was continuously planted for 6 years. The potential of these soil conditioners to eliminate problems associated with consecutive tobacco monoculture was evaluated using agronomic traits, tobacco yield and quality, microbial diversity of tobacco rhizospheric soil and soil enzymatic activity as indicators. The quicklime, calcium cyanamide and microbial agent treatments significantly improved tobacco yield and quality compared with the control (CK). The quicklime treatment yielded the best results. Traditional culture methods, which were used to examine tobacco rhizospheric microecology, showed that the quicklime treatment resulted in the most abundant microbial population followed by the calcium cyanamide treatment. Further analysis using the BIOLOG method indicated that the greatest tobacco rhizospheric microbial functional diversity was measured in the quicklime treatment, i.e., the diversity index was higher compared with the other treatments. Soil enzyme measurements indicated that the highest enzymatic activity of tobacco rhizospheric soil occurred in response to the quicklime treatment. The application of quicklime was conducive to improving and restoring the rhizospheric microecological environment and this treatment promoted tobacco growth, improved tobacco yield and quality and alleviated the problems associated with consecutive tobacco monoculture.

Keywords: Soil conditioners; Quicklime; Calcium cyanamide; Consecutive monoculture problem; Functional diversity; BIOLOG

Introduction

Tobacco, which belongs to the Solanaceae family, is one of the most widely cultivated economic crops in the world (Zhao *et al*., 2014). Continuous tobacco cropping has resulted in a decline in yield and deterioration in quality (Yang *et al*., 2011). Therefore, alleviation of the problems associated with consecutive monoculture in the standardised cultivation of agricultural production has become critical.

Several studies have addressed the consecutive monoculture problem. Previous research has shown that certain methods, such as selecting breeds with high resistance (Perez *et al*., 2014), reasonable crop rotation (Xiao *et al*., 2012), intercropping and interplanting (Mundt, 2002) and soil sterilisation (Rosenbaum *et al*., 2014), are effective in mitigating or alleviating this problem. Some studies have focused on soil amendments, including vesicular arbuscular mycorrhizal fungi (Jie *et al*., 2013), calcium cyanamide and quicklime. These studies showed that calcium cyanamide can significantly reduce a variety of soil-borne diseases in vegetables such as eggplant (*Solanum melongena* L.) (Bletsos, 2006), melon (*Cucumis melo* L.) (Bletsos, 2005), cucumber (*Cucumis sativus* Linn.), cauliflower (*Brassica oleracea* L. var. botrytis L.) (Tremblay *et al*., 2005) and cabbage (*Brassica oleracea* L. var. capitata Linn.) (Donald *et al*., 2004). Moreover, it was reported that quicklime can be used as a restoration agent for heavy metals (Moon, 2005) and oil pollution (Schifano *et al*., 2007) in soil. In addition, quicklime can neutralise acidic soils (Mayfield *et al*., 2004) and increase soil enzymatic activity and soil nutrients (Li *et al*., 2008). However, there are few reports where quicklime was used to restore continuously cropped soil. Zheng *et al*. (2013) improved continuously cropped tobacco soil using quicklime water, which prevented and controlled tobacco bacterial wilt.

Yunnan is one of the major areas that produces tobacco that is characterised by an orange colour and fresh and elegant aroma. A single tobacco cultivation method is commonly used in the mountains of Yunnan. Moreover, this is a water-deficient area and a local saying states, "A more serious drought happens once every 5 years and the most serious drought happens once every 9 years". Particularly in recent years, frequent drought and pest and disease outbreaks

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have exacerbated the consecutive tobacco monoculture problem. It is thus necessary to identify effective methods to mitigate this problem.

Here, combined with the previous study, quicklime, calcium cyanamide and a number of microbial agents and other soil conditioners were applied to acidified and pest-ridden soil that was continuously (6 years) cropped with tobacco to investigate their potential to eliminate the problems associated with consecutive tobacco monoculture and to provide theoretical and technical support for these problems.

Materials and Methods

Experimental Materials and Sample Collection

The tobacco cultivar 'Hongda' was planted in 6-year continuously cropped tobacco fields in a tobacco experiment station in Pupeng Town, Xiangyun County (25°20′N, 100°54′E), Yunnan Province, China. The growing period extended from May 5, 2013 to November 30, 2013. The soil texture was sandy loam with the following basic physiochemical characteristics: soil pH of 5.1, 21.6 g/kg organic matter, 58.6 mg/kg active nitrogen, 19.6 mg/kg active phosphorus and 103.3 mg/kg active potassium.

Three soil conditioners 900 kg/hm^2 of quicklime (96% CaO, supplied by Makepolo, China), 375 kg/hm^2 of calcium cyanamide (containing 19.8% N, 50% CaO, provided by Ning Xia Yuan Da Xing Bo Chemical Co, NingXia, China.) and 210 kg/hm^2 of microbial agents (Heshenyuan multi-effect bacteria with an effective *Bacillus* content exceeding 2 billion/g, provided by Parti China (Beijing) Biotechnology Co., Ltd.) were applied to the replanted tobacco field. The control (CK) did not receive any soil conditioner. Each treatment was replicated three times (a total of 12 plots with 200 plants in each plot). The row spacing was 1.2 m X 0.5 m. The amount of pure nitrogen applied was 75 kg/hm, with an NPK ratio of 1:1:3. Prior to transplanting, 60% of pure nitrogen was applied using a special tobacco compound fertiliser and potassium sulfate; 40% of pure nitrogen was applied in the rosette stage according to local practice and the remainder of the fertiliser was applied by strict adherence to the local standardised practice. Rhizospheric soil was sampled during the mature stage of tobacco using the five-point sampling method and the samples were stored in a refrigerator at 4°C for the analysis of soil microbes and soil enzymatic activity. The agronomic traits of tobacco plants, including plant height, internode length, stalk perimeter, number of effective leaves and leaf area, were recorded during the mature period to determine the yield of flue-cured tobacco and analyse the tobacco quality.

Assessment of the Rhizosphere Microbial Community and Soil Enzymatic Activities

The tobacco rhizosphere soil microbial community, including bacteria, actinomycetes and fungi, was determined via the plate dilution method, in which bacteria were cultivated using beef extract peptone medium, the actinomycetes were cultivated using Gao 1 medium and the fungi were cultivated using Martin medium (Aneja, 2003). The soil enzyme activity was measured using Guan's method (Guan, 1986). The TTC (triphenyl tetrazoliumchloride) colourimetric method was used to determine dehydrogenase activity. The activity of peroxidase and polyphenol oxidase was measured using the purple gallic acid colourimetric method. The activity of urease, invertase, cellulose, catalase and sucrase was determined using the phenol sodium colourimetric method, nitro salicylic acid colourimetric method, anthraquinone colourimetric method, potassium permanganate titration, and 3.5-Dinitrosalicylic acid colorimetric method, respectively.

Functional Diversity Analysis of Microbial Communities

The BIOLOG ECO microplate method was used to determine the diversity of the microbial community (Choi and Dobbs, 1999). The BIOLOG ECO microplate was supplied by Biolog Inc., CA, USA. The procedures were carried out according to the method described by Yang *et al*. (2011). The plate was incubated at 28°C and the absorbance at 590 nm was recorded at 24 h intervals for 168 h using an ELISA reaction plate reader (Thermo Scientific Multiskan MK3, China). The microbial activity in each microplate was described as the average well colour development (AWCD) in each microplate. AWCD= $[\sum(C\text{-}R)]/n$, where C is the absorbance of the 31 carbon source wells; R is the absorbance of the corresponding control wells, and n refers to the number of carbon sources.

The functional diversity index for the soil microbial community was calculated using the data from the 96 h reaction (Ma *et al*., 1995). The following formulas were used: the Simpson index, $1/D=[\sum ni(ni\text{-}1)/N(N\text{-}1)]^{-1}$; Shannon-Wiener index, $H'=\sum(Pi*\log pi)$; Shannon evenness index, $E=H'/\ln S$; McIntosh index $U=(\sum n_i^2)^{1/2}$; Brillouin index, $H=(1/N)/\ln[N!(/n1!n2!n3!n4!.....nn!)]$; and McIntosh evenness index, $E=(N\text{-}U)/(N\text{-}N(S)^{-1/2})$. Here, pi is the ratio of the OD value of the well to the whole-plate relative OD value; S is the number of wells showing a colour change; n_i refers to the relative OD value (C-R) of the well; and N is the total OD value. The optical density after 96 h of incubation was used for principal component analysis (PCA) (Han *et al*., 2007) because this was the shortest incubation time that provided the best resolution among the treatments (Gomez *et al*., 2006).

Statistical Analysis

A one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test ($P < 0.05$) was used for statistical analysis using DPS software version 7.05. The PCA was conducted using SSPS 11.5 software.

Results

The Effect of Soil Conditioners on the Main Agronomic and Economic Characters of Tobacco

The agronomic traits of tobacco measured during the mature period, including stalk perimeter, internode length, number of effective leaves and leaf area, were superior under quicklime, calcium cyanamide and microbial agent treatment compared with CK and the greatest leaf area was measured in the quicklime treatment (Table 1). The economic traits are presented in Table 2, which indicates that tobacco yield under quicklime, calcium cyanamide and microbial agent treatment was 19.99, 11.42 and 8.57% higher, respectively, than under CK and the output value in these treatments exceeded CK by 33.79, 23.36 and 18.04%, respectively. Notably, the average price and ratios of medium and high-grade tobacco under the three treatments were significantly higher than those under CK. In addition, quicklime, calcium cyanamide and the microbial agents improved soil, promoted tobacco growth and improved the yield and quality of tobacco leaves. The best results were observed in response to the quicklime treatment.

Rhizosphere Microbial Community Composition and Functional Diversity of the Microbial Community

The microbial population of the tobacco rhizospheric soil was assessed using traditional culture methods. The number of bacteria, actinomycetes and fungi in the rhizospheric soil under quicklime treatment was greater than that under the other treatments, followed by the calcium cyanamide treatment (Table 3). The CK treatment had the lowest microbial population. The quicklime treatment improved the microbial population of the tobacco rhizospheric soil, which in turn can improve soil microbial diversity.

The microbial functional diversity of the rhizospheric soil is shown in Fig. 1. The quicklime treatment was the most conducive to the growth of microbes with sugar, amino acids, amine, carboxylic acids and copolymers as substrates; the rhizospheric soil in the calcium cyanamide treatment was most conducive to the growth of microbes with copolymers, amino acids, amine and carboxylic acids as substrates and the rhizospheric soil in the CK treatment was the most conducive to the growth of microbes with phenolic acids as the substrate. In summary, the quicklime and calcium cyanamide treatments were conducive to the improvement of the tobacco rhizospheric microbial diversity and the quicklime yielded the best results.

The AWCD value of the soil microbial utilisation of sole carbon sources was assessed after 96 h of culture. The principal components analysis of the characteristics of microbial utilization of sole carbon source of tobacco rhizospheric soil with various soil conditioners was made, and the results showed that the first, second and third principal components, which were correlated with the functional diversity of the rhizospheric microbial carbon source utilisation, explained 37.67, 35.40 and 29.63% of the observed variable variances, respectively (Fig. 2). In addition, according to the results of the correlation analysis of the score coefficients of the principal components and the AWCD values of the sole carbon sources (Table 4), 14 types of carbon sources were significantly correlated with the three principal components, of which 5 types of carbon sources were significantly positively correlated with principal component 1. Of these, 2 types of carbon sources (D-galacturonic acid and α-D-lactose) were positively correlated with the principal component and soil microbes showed the most efficient utilisation of these carbon sources under calcium cyanamide treatment; the other 3 carbon sources (α-cyclodextrin, 2-hydroxy benzoic acid and L-asparagine) were negatively correlated with the principal component and soil microbes showed the most efficient utilisation of α-cyclodextrin and 2-hydroxy benzoic acid in the CK treatment (Table 5). Five types of carbon sources (D-cellobiose, N-acetyl-D-glucosamine, glucose-1-phosphate, L-arginine and L-threonine) were significantly positively correlated with principal component 2 and tobacco rhizospheric soil microbes showed the most efficient utilisation of these carbon sources under quicklime treatment. Four types of carbon sources were significantly correlated with principal component 3, of which 2 carbon sources (β-methyl-D-glucoside and D,L-α-glycerol phosphate) were significantly positively correlated with the principal component and tobacco rhizospheric soil microbes showed the most efficient utilisation of these sources under quicklime treatment; the other 2 carbon sources (γ-hydroxybutyric acid and D-xylose) (Table 5) were significantly negatively correlated with the principal component and tobacco rhizospheric soil microbes showed the most efficient utilisation of these sources under treatment with microbial agents. Under the four types of restoration techniques, 9 of the 14 carbon sources that played a major role were significantly positively correlated with the three principal components, including 7 types of carbon sources from the quicklime treatment and 2 types from the calcium cyanamide treatment; the remaining 5 carbon sources, which were mainly from CK, were significantly negatively correlated with the principal components. In summary, the quicklime treatment was more beneficial for the improvement of microbial functional diversity of rhizospheric soil compared with the other treatments.

The carbon source utilisation of a soil microbial community can be expressed using a variety of diversity indices (Jussila *et al*., 2006). The results showed calcium cyanamide and quicklime treatment resulted in a significant increase in these indices (e.g., SIMPSON (J), SHANNON (H), EVENNESS, BRILLOUI) compared with the control, although there were some exceptions (Table 6). The SIMPSON (J), EVENNESS and McIntosh indices were

Table 1: Analysis of flue-cured tobacco's main agronomic characters under soil conditioners

Treatments	Plant height (cm)	Stem thickness (cm)	Internodal length (cm)	Productive leaves (leaf)	Leaves area (cm^2)
CK	102.8±3.1b	7.8±0.6c	4.8±0.1b	20.2±0.9b	1104.4±57.7b
Quicklime	117.0±3.7a	8.6±0.4b	5.3±0.3a	22.0±1.0a	1364.3±88.6a
Calcium cyanamide	120.4±4.7a	9.4±0.5a	5.4±0.3a	21.8±1.3a	1301.2±104.2a
Microbial agents	118.4±2.8a	9.0±0.6ab	5.6±0.4a	22.2±0.8a	1351.1±126.2a

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at $P < 0.05$

Table 2: Comparison of economic character under soil conditioners

Treatments	Yield ($kg.hm^{-2}$)	Output value ($yuan.hm^{-2}$)	Average price (yuan)	Mid-high grade leaves (%)
CK	2625.4±40.6c	54090.2±1514.2c	20.6±0.3b	80.5±0.9c
Quicklime	3150.2±89.9a	72156.1±2881.5a	22.9±0.4a	90.3±1.0a
Calcium cyanamide	2925.2±28.3b	66701.8±1696.3b	22.8±0.4a	88.1±0.9b
Microbial agents	2850.3±58.5b	63856.2±2001.4b	22.4±0.2a	87.7±1.2b

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at $P < 0.05$

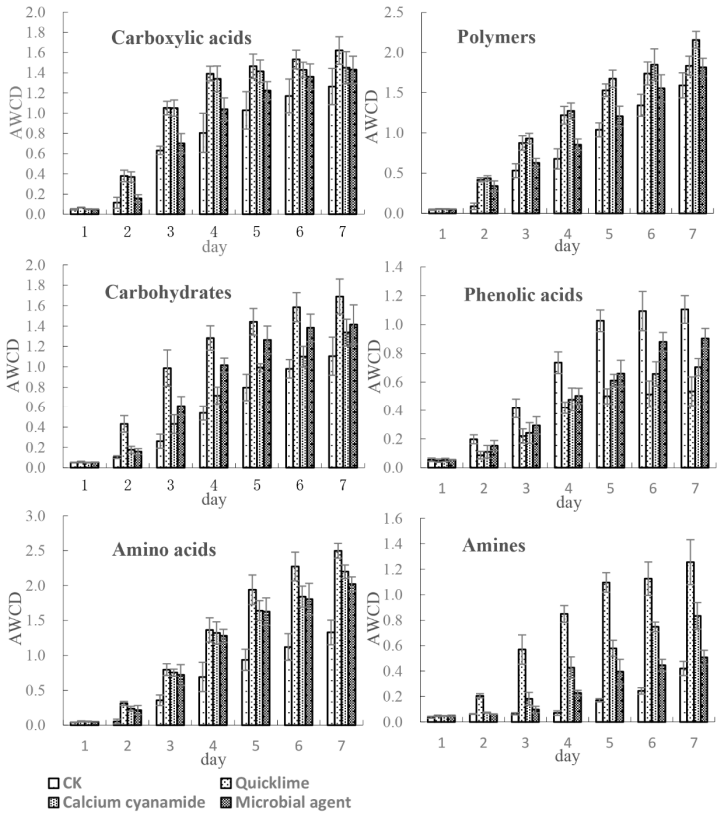

Fig. 1: Changes in the average well colour development (AWCD) for different types of carbon sources in rhizospheric soil treated with various soil conditioners

higher under the quicklime treatment than under the other treatments and the SHANNON (H) and BRILLOUIN indices were highest under the calcium cyanamide treatment, followed by the quicklime treatment. The highest microbial functional diversity of tobacco rhizospheric soil occurred under the quicklime treatment.

Analysis of Enzymatic Activity of Tobacco Rhizospheric Soil under Different Soil Conditioners

The enzymatic activity of urease, acid phosphatase, catalase and dehydrogenase in the rhizospheric soil was higher under quicklime than under the other treatments (Table 7).

Table 3: Changes of rhizosphere cultivable microbes under soil conditioners

Treatments	Microbes		
	Bacteria (*10^8 cells·g^{-1} soil)	Fungi (*10^5 cells·g^{-1} soil)	Actinomycetes (*10^7 cells·g^{-1} soil)
CK	1.3±0.1c	4.1±0.1d	1.7±0.1c
Quicklime	2.4±0.3a	9.9±0.3a	3.9±0.5a
Calcium cyanamide	1.8±0.1b	8.3±0.5b	2.3±0.1b
Microbial agents	1.6±0.1bc	4.9±0.2c	2.2±0.1b

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at $P < 0.05$

Table 4: Main components in media significantly correlated with *PC1, PC2* and *PC3* in each soil

Principal components	Different carbon resources	Correlation coefficient
Principal component 1 (*PC1*)	D-Galacturonic Acid	1.00**
	α-Cyclodextrin	-0.99**
	α-D-Lactose	0.91*
	2-Hydroxy Benzoic Acid	-0.93*
	L-Asparagine	-0.91*
	D-Cellobiose	0.88*
Principal component 2 (*PC2*)	N-Acetyl-D-Glucosamine	0.92*
	Glucose-1-Phosphate	0.95*
	L-Arginine	0.96*
	L-Threonine	0.90*
	γ-Hydroxybutyric Acid	-0.88*
Principal component 3 (*PC3*)	β-Methyl-D-Glucoside	0.94*
	D-Xylose	-0.96**
	D,L-α-Glycerol Phosphate	1.00**

**Significant at P<0.01, *Significant at P<0.05

Table 5: Effects of soil conditioners on various principal components in flue-cured tobacco soil microorganisms with AWCD

Carbon sources		Treatments			
		CK	Quicklime	Calcium cyanamide	Microbial agent
Carbohydrates	D-Cellobiose	0.8470±0.0056b	1.2140±0.0072a	0.8930±0.0728b	1.1977±0.0295a
	α-D-Lactose	1.1560±0.0282b	1.1577±0.0051b	1.4440±0.0233a	1.1450±0.0101b
	β-Methyl-D-Glucoside	0.7290±0.0214b	1.0493±0.0747a	0.6543±0.0210b	0.0820±0.0040c
	D-Xylose	1.0637±0.0104c	1.0093±0.1015c	1.2570±0.1246b	1.7547±0.0114a
	N-Acetyl-D-Glucosamine	1.6330±0.1057c	2.6520±0.0318a	1.5947±0.0670c	2.3870±0.0680b
	Glucose-1-Phosphate	0.0997±0.0061d	1.6127±0.1201a	0.4170±0.0590c	1.3150±0.0606b
	D,L-α-Glycerol Phosphate	2.1177±0.1005b	2.3287±0.0254a	2.1443±0.1016b	1.2857±0.0075c
Amino acids	L-Arginine	0.655±0.01410d	3.0783±0.0980a	1.7580±0.0986c	2.5363±0.0397b
	L-Asparagine	2.7607±0.0633b	2.8447±0.0370a	2.4143±0.0070d	2.6620±0.0477c
	L-Threonine	0.4953±0.0586d	3.1513±0.1010a	2.1790±0.0493c	2.7793±0.1020b
Polymer	α-Cyclodextrin	1.6790±0.0298a	1.6273±0.0059b	1.4017±0.0183d	1.5447±0.0023c
Phenolic acids	2-Hydroxy Benzoic Acid	0.2263±0.0110a	0.1903±0.0169b	0.0493±0.0021d	0.1017±0.0093c
Carboxylic acids	D-Galacturonic Acid	0.4770±0.0072d	0.5450±0.0036c	0.7083±0.0327a	0.5807±0.0064b
	γ-Hydroxybutyric Acid	1.5443±0.0313b	1.0827±0.0234c	0.785±0.0101d	2.2777±0.0951a

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at P < 0.05

The highest enzymatic activity of invertase and dehydrogenase was measured in the microbial agent treatment followed by the quicklime.

The highest enzymatic activity of cellulase and polyphenol oxidase was measured in the calcium cyanamide treatment and the polyphenol oxidase activity was higher under the quicklime treatment than under the calcium cyanamide or CK treatment. Higher enzyme activity was measured with quicklime, calcium cyanamide and microbial agent treatment compared with the control. This indicates higher rhizospheric soil biological activity and intensity of biochemical reactions, especially in response to quicklime.

Discussion

Changes in soil microbial population and structure directly affect the overall function of rhizospheric microbes that play an important role in the transformation of matter and energy in the micro-ecological environment of the rhizosphere. However, consecutive monoculture significantly changes the diversity of microbial communities (Wu *et al*., 2011). Previous studies showed that the problems associated with consecutive monoculture could be alleviated or eliminated by improving soil microbial community structure and increasing microbial diversity and activity (Cardinale *et al*., 2006; Enwall *et al*., 2007).

Table 6: Microbial diversity of utilised substrates in rhizospheric soil treated with various soil conditioners

Treatments	SIMPSON(J)	SHANNON(H)	EVENNESS	BRILLOUIN	McIntosh
CK	0.9401±0.0017c	2.3263±0.0419b	0.9115±0.0319c	1.3161±0.0461c	0.9507±0.0017d
Quicklime	1.1565±0.0405a	2.418±0.0417ab	0.9761±0.0066a	1.6720±0.0585a	1.2501±0.0107a
Calcium cyanamide	0.9816±0.003b	2.5233±0.0883a	0.9697±0.0047ab	1.7168±0.0601a	0.9800±0.0024b
Microbial agents	0.9793±0.001b	2.5067±0.0877a	0.9417±0.0076bc	1.4625±0.0512b	0.9654±0.0024c

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at $P < 0.05$

Table 7: Enzyme activity in rhizospheric soil treated with various soil conditioners

Treatments	Urease mg/(g·24h)	Cellulase mg/(g·24h)	Sucrase mg/(g·24h)	acid phosphatasemg/(g·24h)	Polyphenol oxidase mg/(g·24h)	Catalase mL/(g·1/3h)	Peroxidase mg/(g·24h)	Dehydrogenase mg/(g·24h)
CK	27.79±2.66b	0.11±0.04ab	5.64±0.21c	16.21±1.57b	0.39±0.02d	0.92±0.11b	0.22±0.02b	0.09±0.01c
Quicklime	38.46±2.89a	0.09±0.01b	6.89±0.27b	23.82±3.34a	0.61±0.04b	1.32±0.09a	0.29±0.03a	0.13±0.02a
Calcium cyanamide	33.19±5.69a	0.14±0.02a	6.04±0.12c	21.02±0.58a	0.84±0.01a	1.31±0.14a	0.27±0.03a	0.12±0.01b
Microbial agents	37.23±1.58a	0.10±0.03b	7.53±0.36a	22.92±0.94a	0.55±0.01c	0.93±0.04b	0.30±0.02a	0.11±0.01b

Mean values ± standard deviation (SD). Different lowercase letters represent significant differences at $P < 0.05$. The mg/(g·24 h) indicates the content of a corresponding compound that was generated per gram of dry soil cultured for 24 hours, e.g., the amino nitrogen, glucose, glucose, p-nitrophenol, pyrogallol, pyrogallol, and triphenyl methyl board contents represent the activity of urease, cellulase, sucrase, acid phosphatase, polyphenol oxidase, peroxidase, and dehydrogenase, respectively. The ml/ (g·1/3 h) indicates the number of milliliters of 0.1 N potassium permanganate consumed per gram of dry soil in 20 minutes

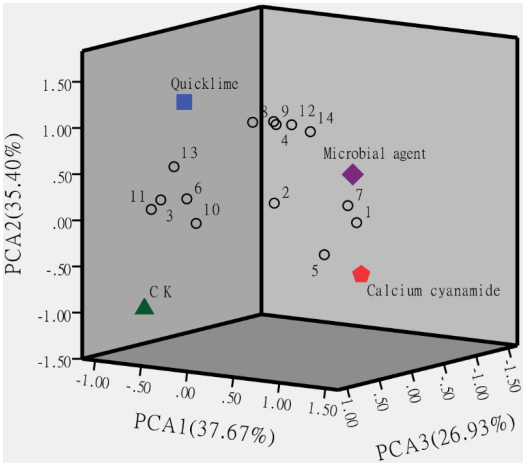

Fig. 2: Loadings for the principal component analysis (PCA) of microorganisms from tobacco rhizospheric soil treated with various soil conditioners

Numbers represent different carbon sources: 1 D-galacturonic acid; 2 γ-hydroxybutyric acid; 3 α-cyclodextrin; 4 D-cellobiose; 5 α-D-lactose; 6 β-methyl-D-glucoside; 7 D-xylose; 8 N-acetyl-D-glucosamine; 9 glucose-1-phosphate; 10 D,L-α-glycerol phosphate; 11 2-hydroxy benzoic acid; 12 L-arginine; 13 L-asparagine; 14 L-threonine. The distance between the carbon sources and soil conditioners can be used to distinguish the degree of utilisation of the carbon sources by the soil microbes. Smaller distances indicate treatments that were more conducive to the growth of microbes

In this study, the quicklime and calcium cyanamide treatments increased the microbial population and activity in tobacco rhizospheric soil. These microbes included bacteria, actinomycetes and fungi. The highest microbial functional diversity was measured in the quicklime and calcium cyanamide treatments.

Wu *et al.* (2011) reported autointoxication of root exudates as a result of consecutive monoculture. Phenolic acids, one of the major allelopathic toxic substances that commonly leads to the accumulation of phenolic acids in soil, are one of major factors that contribute to the problems associated with continuous tobacco cropping (Wang *et al.*, 2009). The rhizospheric soil in the CK treatment was the most conducive to the growth of microbes with phenolic acids as a substrate. However, the application of quicklime, calcium cyanamide and microbial agents were detrimental to the growth of these microbes, indicating that these treatments can reduce the content of phenolic compounds in the soil, which could alleviate the problems associated with consecutive tobacco monoculture.

The principal component analysis (PCA) distinguished the microorganisms in the rhizospheric soil treated with soil conditioners by 14 single carbon sources. In addition, the first, second and third principal components accounted for 100% of the total variation. Nine of the 14 carbon sources that played a major role in component separation were significantly positively correlated with the three principal components and 7 were from the quicklime treatment. This indicates that the quicklime treatment was conducive to the improvement of the microbial functional diversity in the rhizospheric soil.

The diversity index of the soil microbial community utilisation of carbon sources (including the SIMPSON (J), SHANNON (H), EVENNESS, BRILLOUIN and McIntosh indices) was higher under the quicklime treatment. In conclusion, the quicklime and, to a lesser extent, the calcium cyanamide treatment improved the tobacco rhizospheric microbial population and diversity. The microbial diversity of rhizospheric soil was relatively low under the microbial agent treatment, possibly as a result of an extended drought period following application.

Soil enzymes play an important role in the soil nutrient cycle metabolism and efficient release of nutrients

(Von Mersi and Schinner, 1991). The enzymatic activity in soil can, to some extent, reflect biological activity and the intensity of biochemical reactions. In this study, soil enzymatic activity and soil nutrients were increased by treatment with quicklime. Similar results were presented by Li *et al*. (2008).

Previous studies have shown that soil pH decreases with the duration of continuous cropping (Wu *et al*., 2013), and quicklime can aid in neutralising acidic soil (Mayfield *et al*., 2004). The current study showed that quicklime can improve and stabilise rhizospheric microecology and mitigate the consecutive monoculture problem.

Therefore, we suggest that quicklime can be used as a favorable biochemical treatment for tobacco and related crops, especially those that are subjected to consecutive monoculture.

Conclusion

The application of quicklime (900 kg/hm^2) and calcium cyanamide (375 kg/hm^2) to 6-year continuously cropped tobacco soil can improve the microbial population and microbial functional diversity. The quicklime treatment yielded the best results and improved soil enzymatic activity. This treatment can be widely applied because it can improve the rhizospheric microecology of continuously cropped soil, mitigate the consecutive monoculture problem, increase tobacco growth and improve tobacco yield and quality.

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