Influence of *Panax ginseng* Continuous Cropping on Metabolic Function of Soil Microbial Communities

YING Yi-xin[1], DING Wan-long[1], ZHOU Ying-qun[2], LI Yong[1*]

1. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China
2. China National Corporation of Traditional and Herbal Medicine, Beijing 100195, China

Abstract: **Objective** To investigate the influence of *Panax ginseng* continuous cropping on the carbon substrate metabolic activity of microbes in soils sampled from Dafang, Huangni, and Wulidi in Jilin Province, China. **Methods** Soil metabolisms of soil communities were characterized by community level physiological profiles using BIOLOG^{TM} EcoPlate. **Results** Soils sampled from the three sites were analyzed and their metabolic activities were compared. Principal component analysis explored the significant variance in metabolic function of microbial communities in soils, though the Shannon index and the evenness index of them were similar. Futhermore, two principal components (PC1 and PC2), which contributed 67.83% and 10.78% of total variance, were extracted respectively. And also, substrates significantly correlated with PC1 and PC2 at the three sampling sites were identified. **Conclusion** Characteristic of soil is the primary factor influencing microbial communities, and *P. ginseng* continuous cropping has significant influence on microbial community. Though soil samples show similar microbial metabolic profiles, microbial communities in rhizosphere soil are changed obviously during the cultivation of *P. ginseng*, which would finally result in the unbalance of microbial community. Phytopathogens would gradually be the predominants in rhizosphere soil and make *P. ginseng* sick.

Key words: continuous cropping; metabolic function; microbial community; *Panax ginseng*; rhizosphere soil
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Introduction

Panax ginseng C. A. Mey., belonging to Araliaceae family, is one of the most valuable traditional Chinese herbal medicines. It is a perennial herb which matures after about five to six years. With significant re-plant problem, *P. ginseng* could be infected by various types of foliar and soil borne pathogens during the growing period resulting in syndromes of fallen fibre, taproot putrescence, and severe yield loss. In general, the survival rate of *P. ginseng* seedlings is no more than 25% after a growing season in replant model (Zhao, 2001). It has been reported that replant problem of *P. ginseng* was related to the deterioration of soil physico-chemical properties, nutrition unbalance, change of microbial community, accumulation of pathogens, and allelopathy (Han, Lei, and Yang, 1998).

Soil is a complex ecosystem in which microbial community sensitive to the soil chemical properties is an important measure of sustainable land use. Microbial parameters, e.g. microbial biomass and functional diversity, are considered to be potential indicators of the soil quality (Bending *et al*, 2004; Xue *et al*, 2008). Soil microorganisms play a crucial role in the cycling of plant nutrients, the energy flow of either natural or anthropogenically altered soils, and the maintenance of soil ecosystem (Smith and Paul, 1990; Bossio and Scow, 1995; Konopka, Oliver, and Turco, 1998). Also, soil microorganisms have significant influence on plants growing above-ground (Heckman *et al*, 2001; Lutzoni, Pagel, and Reeb, 2001). It will be helpful to study soil microbial community for the illustration of replant problem of *P. ginseng*.

* Corresponding author: Li Y Address: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, No.151 Malianwa North Road, Beijing 100193, China Tel/Fax: +86-10-5783 3360 E-mail: liyong@implad.ac.cn
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BIOLOG method is based on carbon substrates utilization by microbial communities, through analyzing data by multivariate statistics, such as principal component analysis (PCA) (Rogers and Tate, 2001) or by kinetic approaches, and dynamics of microbial community could be revealed by BIOLOG metabolic variance (Garland, Mills, and Young, 2001; Zabinski and Gannon, 1997). In our previous work (Ying, Ding, and Li, 2012), soil microbial communities were studied by randomly amplified polymorphic DNA (RAPD) and amplified ribosome DNA restrictive analysis (ARDRA) methods, and significant differences were detected between *P. ginseng* rhizosphere and non-phizosphere soils. The aim of the present work was to reveal dynamics of microbial community and their metabolic function by BIOLOG method.

Table 1 Soil samples used for BIOLOG analysis

No.	Age / year	Growing model
DF1	1	1
DF2	2	2
DF3	3	3
DF4	4	2 + 2
DF5	5	3 + 2
DF6	6	3 + 3
HN1	1	1
HN3	3	3
HN4	4	3 + 1
HN5	5	2 + 3
HN6	6	3 + 3
WLD1	1	1
WLD2	2	2
WLD4	4	4
WLD5	5	3 + 2
WLD6	6	3 + 3

In the column of growing model, a + b means *P. ginseng* growing at one place for "a" years, then transplanted to another place and growing for "b" years

Materials and methods

Soil samples

In total, 16 samples were from rhizosphere of one-, two-, three-, four-, five-, and six-year *P. ginseng* at Huangni (HN, 581.6 m, 42°31′54.2″N, 127°15′45.8″E), Dafang (DF, 570.8 m, 42°26′03.2″N, 127°20′00.1″E), and Wulidi (WLD, 649.9 m, 42°29′52.8″N, 127°20′42.1″E) at July, 2009 in Jilin Province, China (Table 1).

The physicochemical characteristics of the soils sampled from the three geographic sites were listed in Table 2. For each sample, five *P. ginseng* plants distributed symmetrically in the field were pulled up, after shaken off redundant soil, the soil adhered tightly on root surface was collected. All soils sampled were stored in icebox and analyzed immediately back to the laboratory.

Table 2 Characteristics of main nutrient components in soils tested

Soil samples	TN / %	TP / %	TPS / %	QN / (mg·kg^{-1})	QP / (mg·kg^{-1})	QPS / (mg·kg^{-1})	pH
DF	0.841	0.157	1.974	358.49	37.15	428.50	4.85
HN	0.528	0.100	1.938	300.06	23.80	250.50	4.72
WLD	0.231	0.068	2.025	246.50	38.65	325.00	4.09

TN: total nitrogen TP: total phosphorus TPS: total potassium QN: quick acting nitrogen QP: quick acting phosphorus QPS: quick acting potassium

BIOLOG analysis

Soil metabolisms of soil microbial communities were characterized by community level physiological profiles (CLPP) using BIOLOG^{TM} EcoPlate (Schutter and Dick, 2001). In summary, fresh soil (10 g) was suspended in sterile 0.85% saline solution (90 mL), shaken at 120 r/min for 30 min, and then the suspensions were diluted by 1000-fold. Each well of a BIOLOG^{TM} EcoPlate was inoculated with 150 μL of the diluted soil suspensions, and incubated at a constant temperature of 25 ℃ in dark without agitation. The plates were scanned at wavelength of 590 nm by a BIOLOG reader on OmniLog Plus (BIOLOG Inc., US) at a 24 h interval for 168 h. Each soil sample using one plate has 31 carbon substrates arranged in triplicates.

Data analysis

The average well color development (AWCD) was calculated according to Garland and Mills (1991) and evaluated the total ability of microbial community on carbon substrates utilization. AWCD of each well was calculated using the following formula.

$$\mathrm{AWCD}=[\sum(A_i-A_{A1})]/31$$

Where A_i was the absorbance of i well and A_{A1} was the absorbance of A1 well following the incubation measured in terms of optical density at wavelength of 590 nm

The metabolic profile of microbial communities includes the Shannon index (H') and the evenness index (E) (Li, Wu, and Chen, 2007; Zak *et al*, 1994). The diversity of microbial community was evaluated by H' (Shannon and Weaver, 1949) and calculated by the following formula.

$$H' = -\sum p_i \cdot \ln p_i$$

Where p_i was the principal color development of i well relative to the total color development, i.e. $p_i = (C-R) / \sum(C-R)$

The E value was calculated as $E = H' / \ln S$, where diversity S was the total number of carbon substrates utilized by microbial community in a given soil sample, and only the positive data ($A \geq 0.2$) was used to calculate E value (Ratcliff, Busse, and Shestak, 2006). The AWCD value at 96 h was used to calculate the H', and SPSS 17.0 and SIMCA-P 11.5 Demo softwares were used for PCA analysis (Schutter and Dick, 2001). Three replicates were set for all tests.

Results

Carbon substrates metabolic profiles of soil microbial communities

The carbon substrate metabolic profiles of soil microbial communities were evaluated by AWCD, and results showed that AWCD increased with the culturing time without exception, and carbon substrates utilized by microbes were very limited at the beginning (Fig. 1).

Carbon substrates metabolic profiles of soil microbial communities

The H' suggested the carbon substrates metabolic profile of soil microbial communities. The H' and E had no significant variance among microbial communities in soils sampled from DF, which was the same as soils sampled from WLD. Except for the lower H' of HN5 and HN6, the E values of them had no significant variance in HN (Table 3).

As a result, the accumulative contribution of principal components (PC) was 78.61% including 67.83% PC1 and 10.78% PC2, respectively. The analyzing results were shown in Fig. 2.

Through correlation analysis, substrates significantly correlated with PC1 and PC2 were identified (Table 4).

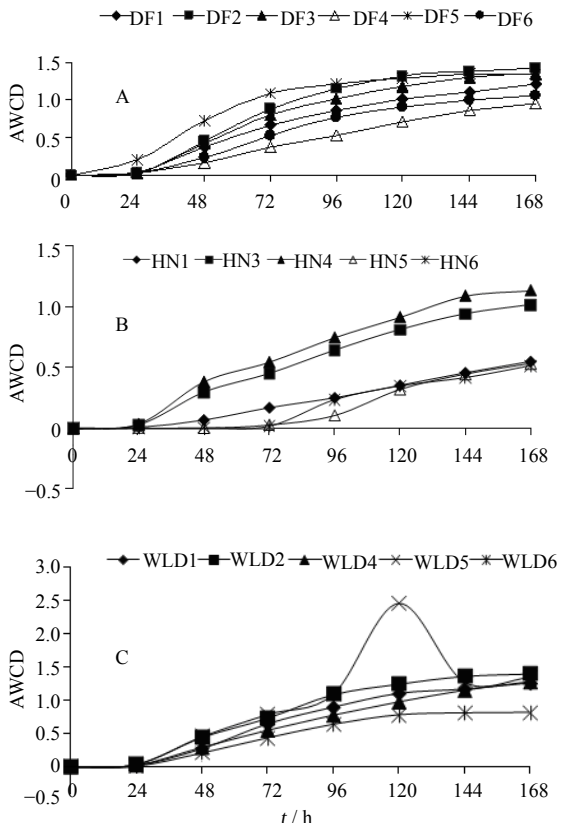

Fig. 1 AWCD in BIOLOG EcoPlates for rhizospheric soils sampled from DF (A), HN (B), and WLD (C)

Table 3 H' and E of soils sampled from DF, HN, and WLD

Soil samples	H'	E
DF1	2.79 ± 0.020^a	0.85 ± 0.010^a
DF2	2.79 ± 0.100^a	0.84 ± 0.030^a
DF3	2.77 ± 0.090^a	0.85 ± 0.040^a
DF4	2.72 ± 0.160^a	0.85 ± 0.040^a
DF5	2.78 ± 0.120^a	0.82 ± 0.040^a
DF6	2.66 ± 0.030^a	0.82 ± 0.010^a
HN1	2.02 ± 0.032^a	0.77 ± 0.054^a
HN3	2.71 ± 0.056^a	0.85 ± 0.009^a
HN4	2.73 ± 0.048^a	0.82 ± 0.029^a
HN5	2.41 ± 0.097^b	0.80 ± 0.036^a
HN6	2.43 ± 0.128^b	0.82 ± 0.049^a
WLD1	2.75 ± 0.096^a	0.82 ± 0.030^a
WLD2	2.73 ± 0.098^a	0.80 ± 0.029^a
WLD4	2.72 ± 0.058^a	0.81 ± 0.031^a
WLD5	2.79 ± 0.112^a	0.82 ± 0.030^a
WLD6	2.67 ± 0.020^a	0.84 ± 0.001^a

Different letters in each column indicate $P < 0.05$

Discussion

Sole carbon metabolic profile is a useful tool to study the dynamic of microbial community by community-level physiological profiles analysis, and the BIOLOG EcoPlate was used to simulate the complicated soil ecosystem (Buyer *et al*, 2011;

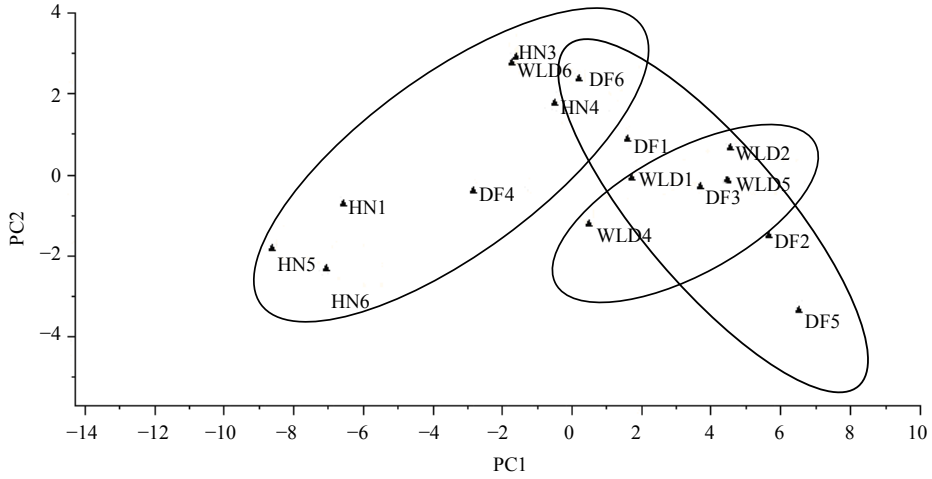

Fig. 2 PCA of BIOLOG EcoPlates data

Table 4 Substrates highly correlated with PCs for soils sampled from DF, HN, and WLD

Compounds	*r*		
	DF	HN	WLD
PC1			
L-arginine	0.848	–	–
L-diphenylala	0.918	–	–
L-threonine	0.838	0.861	–
I-erythritol	0.895	–	0.964
glycogen	0.835	–	0.953
D-cellobiose	0.891	–	–
γ-hydroxybutyric acid	0.930	0.822	0.930
D-glucosaminic acid	0.859	–	–
itaconic acid	0.959	–	–
α-ketobutyric acid	0.907	–	–
α-cyclodextrin	0.886	–	0.957
β-methyl-*D*-glucoside	–	0.978	–
D-xylose	–	0.966	–
D-mannitol	–	0.979	–
N-acetyl-*D*-glucosamine	–	0.980	0.823
L-asparagine	–	0.969	–
L-serine	–	0.972	–
glycyl-*L*-glutamic acid	–	0.990	0.937
D-malic acid	–	0.878	–
D, *L*-α-glycerol	–	0.958	–
putrescine	–	0.928	–
α-*D*-lactose	–	–	0.979
4-hydroxybenzoic acid	–	–	0.908
phenylethylamine	–	–	0.857
PC2			
N-acetyl-*D*-glucosamine	0.804	–	–
L-phenylalanine	–	0.844	–
α-ketobutyric acid	–	0.924	–
D-galacturonic acid	–	–	−0.904
D-mannitol	–	–	0.921
D-cellobiose	–	–	0.852
D-xylose	–	–	0.878
glucose-1-phosphate	–	–	0.816
putrescine	–	–	−0.918

Preston-Mafham, Boddy, and Randerson, 2002). AWCD of substrates arranged on the BIOLOG EcoPlate depicted the metabolic activity of microbes in soil, and AWCD data reflected the metabolic activity of microbial community as a whole (Chen *et al*, 2007; Wang *et al*, 2011).

Due to the large amount of data, it was difficult to interpret the sole carbon metabolic profile properly, and usually a number of univariate and multivariate methods were required to resolve the intractable hurdle. As the fluctuant characteristic of AWCD on BIOLOG EcoPlate, it was not appropriate to carry out microbial community level analysis directly. The optimal characteristic of BIOLOG required approximately equivalent inoculum density (Garland and Mills, 1991), but it was practically impossible, for counts of microbes delayed the process, and microbial community would change during the inoculation (Preston-Mafham, Boddy, and Randerson, 2002). A solution of compensating for differences in inoculum density and reflecting the real variance is to use a fixed level of AWCD to determine the data for further analysis (Garland, 1996).

The present study revealed the variance of microbial communities in *P. ginseng* rhizosphere soils, and the utilization of six types of substrates also showed the same conclusion. In addition, soil characteristics also had significant influence on soil microbial community (Girvan *et al*, 2003; Li, Wu, and Chen, 2007). In the present study, though the *H′* and *E* of microbial communities in soils tested showed indistinct differences and significant variance on

microbial communities, and microbial metabolic activity were detected. Chen *et al* (2007) also reported that microbial community and carbon metabolic activity might probably present variance even with similar *H′*.

Along with the development of molecular biology, a number of molecular methods, such as RAPD, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), ARDRA, and terminal restriction fragment length polymorphism (T-RFLP), have been successfully applied to microbial diversity research (Buchan *et al*, 2002; Crecchio *et al*, 2007; Nakatani *et al*, 2011; Wei *et al*, 2007; Yao, Jiao, and Wu, 2006). These techniques may characterize the microbial community more accurately than traditional culture-dependent methods. However, whether the genetic diversity could reflect the microbial community-level changes and what was the relationship between the genetic diversity and the carbon substrates metabolic diversity were still unknown. However, as a culture-dependent method, BIOLOG data could not represent the microbial community adequately (Nannipieri *et al*, 2003), which provided us much valuable information for further research.

In conclusion, microbial community and their metabolic profiles exhibited obvious variance during the cultivation of *P. ginseng*, and the influence was correlated with the age of *P. ginseng* and quantity of root exudates released by *P. ginseng*, significantly. We deduced that the change of microbial community in rhizosphere soil of *P. ginseng* weakened the original inhibition on phytopathogens, and made them dominant gradually, then resulted in severe diseases in *P. ginseng*. In another way, the change of microbial community may have influence on the cycle of nutrition, substance, energy, and so on. Furthermore, we studied the dynamics of bacterial communities by RAPD and ARDRA methods (data not shown), and the dynamics of microbial communities in *P. ginseng* rhizospheric soil were partly revealed.

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