



### Full Length Article

## Effect of Intercropping of *Angelica sinensis* with Garlic on its Growth and Rhizosphere Microflora

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### Abstract

**Aims and Methods** A pot experiment was carried out to determine the effect of *Angelica sinensis* intercropped with garlic on *A. sinensis* growth and soil microbial communities in rhizosphere soil of *A. sinensis* under the scheme of *A. sinensis* intercropped with garlic. **Results** Soil microbial community was changed and bacteria functional group diversity was increased, and *A. sinensis* growth was improved when *A. sinensis* intercropped with garlic under the continuous cropping condition. Also, it shows that intercropping significantly increased root dry weight, and much less substantially shoot dry weight and plant height. Relative to the control, the activity of superoxide dismutase, peroxidase and catalase was increased by 80.00, 243.41 and 37.13%, respectively, but the content of malondialdehyde was decreased by 11.67%, along with a 70.13% yield improvement of *A. sinensis*, and 50.00% and 10.20% essential oil and alcohol-soluble extract increasing, respectively. The result also showed that the population of bacteria, aerobic cellulose-decomposing bacteria, organic phosphorus-solubilizing bacteria, inorganic phosphorus-solubilizing bacteria and potassium-solubilizing bacteria in rhizosphere soil were promoted in intercropping in vigorous growth stage. The abundance of aerobic cellulose-decomposing bacteria, organic phosphorus-solubilizing bacteria and potassium-solubilizing bacteria in intercropping soils changed at the rootstock thickening. Although the total population of functional groups in intercropping *A. sinensis* rhizosphere soils was lower than in monocropping system, community diversity and evenness increased and dominance concentration decreased. **Conclusions** Intercropping *A. sinensis* with garlic can alleviate the soil sickness of *A. sinensis*. © 2015 Friends Science Publishers

**Keywords:** *Angelica sinensis*; Soil sickness; Soil microbial community; Intercropping

**Abbreviations:** AB – Ammonifying bacteria; ACDB – Aerobic cellulose-decomposing bacteria; OPSB – Organic phosphorus-solubilizing bacteria; IPSB – Inorganic phosphorus-solubilizing bacteria; KSB – Potassium-solubilizing bacteria; SOD – Superoxide dismutase; POD – Peroxidase; CAT – Catalase; MDA – Malondialdehyde; FM – Fresh mass; NBT – Nitroblue tetrazolium; CFU – Colony forming units; LSD – Least significant difference; ROS – Reactive oxygen species

### Introduction

*Angelica sinensis* (Oliv.) Diels, a perennial herb belonging to family Apiaceae, is commonly used in Traditional Chinese Medicine since ancient times (Zhang and Cheng, 1989). *A. sinensis* is widely used as an ingredient in cosmetic and health beverage at present (Ma *et al.*, 2002; Chen, 2004). In order to meet its demand, continuously cropped area with *A. sinensis* has increased sharply in the last decade. Continuous cropping of *A. sinensis* in the same fields has resulted in plant growth inhibition, plant mortality and *Ditylenchus destructor* infestation, which result in the reduced yield and quality (Zhang *et al.*, 2009). At present, the soil sickness has become one of the major constraints in

*A. sinensis* cultivation.

It is generally known that continuous cropping can affect crop growth and development, decrease yield and quality, and increase disease occurrence in most conditions (Wu *et al.*, 2007; Dai *et al.*, 2009; Zhang *et al.*, 2011). Soil sickness is usually found in agricultural crops, especially in medicinal plant (Zhang *et al.*, 2005; He *et al.*, 2009; Yin *et al.*, 2009). Soil sickness may be attributed to autotoxicity, soil microflora imbalance and changes in soil physiochemical properties (Young, 1984; Yu and Matsui, 1994). Previous studies report that build-up of plant pathogens is one of the major causes in soil sickness in *Cistus ladanifer* (Hassan *et al.*, 1989), peach (Benizri *et al.*, 2005), cucumber (Yao *et al.*, 2006), *Rehmannia glutinosa*

(Chen *et al.*, 2007), Liriope (Zhao *et al.*, 2010), while autotoxicity has been reported to be one of the major causes in soil sickness in cucurbit crops (Yu *et al.*, 2000), alfalfa (Segiun *et al.*, 2002), *Cistus ladanifer* (Alías *et al.*, 2006), cucumber (Yao *et al.*, 2006) and wheat (Wu *et al.*, 2007). In many cases, soil microorganism played either direct or indirect role in yield reduction (Manici *et al.*, 2003; Hu *et al.*, 2006). In conclusion, the cause of soil sickness is complex. Previous studies have identified some methods to manage soil sickness, such as root stock grafting (Lv *et al.*, 2000), breeding for resistant varieties (Duan *et al.*, 2002), and biocontrol (Haggag, 2002). However, these methods have their limitations, and there was no a method could be solve soil sickness completely.

Intercropping is a system, where two or more crop species are grown in the same field in the same season (Ofori and Stern, 1987). It is an ancient and traditional agronomic practice, which if utilized correctly, can contribute significantly to reduce pest problems and consequently increase crop production. In recent years, intercropping is becoming popular as it offers the possibility of yield increase compared to monocropping (Egbe and Adeyemo, 2006; Saddam, 2009), such as peanut yields were increased by 39% and 35%, respectively when intercropped with *Atractylodes lancea* and *Euphorbia pekinensis*, respectively, in a field experiment (Dai *et al.*, 2009). In addition, intercropping also helps maintain the soil fertility (Patra and Chatterjee, 1986), efficient use of nutrients (Nazir *et al.*, 1997; Zhu *et al.*, 2006), improve soil microbial communities (Song *et al.*, 2006; Dai *et al.*, 2009) and reduce weed problems (Baumann *et al.*, 2002). At present, intercropping is one option for alleviating soil sickness. Su *et al.* (2008) suggested that rain fed rice could be intercropped with watermelon to alleviate soil sickness compared to watermelon monoculture by restraining pathogen (*Fusarium oxysporum* f.sp. *cucumerinum*) development and regulating the microflora in rhizosphere of watermelon. Similar phenomena have been observed for peanut intercropped with *Atractylodes lancea* and *Euphorbia pekinensis* (Dai *et al.*, 2009). However, few studies have focused on the *A. sinensis* soil sickness (Zhang *et al.*, 2010).

The aims of the study were: (1) to evaluate the feasibility of alleviate soil sickness in *A. sinensis* production through intercropping with garlic, (2) to estimate the effect of *A. sinensis* intercropped with garlic on the rhizosphere soil microbial communities, and the diversity of bacteria functional group, and (3) to examine the effect of *A. sinensis* intercropped with garlic on *A. sinensis* growth, antioxidant enzyme activity.

## Materials and Methods

### Soil

Top soil (about 0-20 cm) cropped with *A. sinensis* for two

years, with known soil sickness problem was collected in March 2008 from the Qingshui village, Min county (103°34' E, 34°27' N, 2300 m above Mean Sea Level), Gansu province, China. Thirty plots of 30 by 30 cm were randomly selected to collect experimental soil from 14 cm depth. Analysis of the soil indicated that it contain 6.6 g·Kg<sup>-1</sup> organic matter, 0.89 g·Kg<sup>-1</sup> total N, 1.15 g·Kg<sup>-1</sup> total P, 20.74 g·Kg<sup>-1</sup> total K, 29.26 mg·Kg<sup>-1</sup> alkali-hydrolyzable N and 15.84 mg·Kg<sup>-1</sup> rapidly available P with pH=7.7. The soil was screened through a 1 mm sieve for removing the plant residues and stones.

### Pot Experiment

The experiment was carried out at the experimental site of the Institute of Radix Angelicae Sinensis in Min County, Gansu province in China, during the growing season (April to October) of 2008. The average temperatures for day and night were 24 and 13°C, and the light and dark periods were 14 and 10 h each day during the whole growing season, respectively. No fertilization was applied during the experiment. *A. sinensis* grown alone served as the control.

Plastic pots (30×30×28 cm) were used, and each pot contained 10 Kg of soil, and the pots were embedded into the soil leaving the top of the pot at the same height with the ground for eliminating the effect by the external conditions, using a completely randomized design. Four *A. sinensis* seedlings were planted into each pot under control, four *A. sinensis* seedlings with six seeds of garlic were planted in each pot under treatment, garlic was planted along the edge of pots. The pots were watered by drip irrigation when necessary, during the experiment.

### Sampling

The stem height was measured using the tap measure at 18 days (8 May), 65 days (23 June) and 107 days (4 August) after seedling emergence, respectively. At seedling stage (23 June), healthy leaves of *A. sinensis* were collected, and stored at 4°C until analyzed for antioxidant enzyme activity. At harvest stage (25 October), the plants were harvested and divided into shoot and root, and dried in an oven at 65°C to constant weight.

At seedling stage (23 June), rootstock thickening (15 August) and harvest stage (25 October), the soil adhering to the root, designated as 'rhizosphere soil' (Fujii *et al.*, 2005), was collected, and the soil samples were mixed, sieved through a 1 mm mesh sieve. The soil samples were stored in refrigerator at 4°C until analyzed.

### Antioxidant Enzyme Activity and Lipid Peroxidation Determination in Leaves of *A. Sinensis*

Extraction and activity determination of antioxidant enzyme were carried out following the method of Nakano and Asada

(1981). Commonly, weighed 0.5 g of leaf material, and homogenized with 10 mL extraction buffer containing 50 mM phosphate buffer (pH 7.4), 1 mM ethylene diamine tetraacetic acid, 1 g polyvinyl pyrrolidone and 0.5% (v/v) triton  $\times$  100. The homogenate was centrifuged for 20 min at 12 000 g and the supernatant obtained was used for analysis of enzyme activity.

Superoxide dismutase activity was measured by its ability to inhibit the photochemical reaction of nitroblue tetrazolium at 560 nm (Becana *et al.*, 1986). One unit of SOD activity was defined as the enzyme amount causing 50% inhibition of *NBT* reduction. SOD activity is expressed as units per mg fresh mass of leaves.

Peroxidase activity was measured by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation at 25°C (Becana *et al.*, 1986). One unit of POD activity was defined as the increase in absorbance at 470 nm for 1 min due to guaiacol oxidation. POD activity expressed as units per min per g FM.

Catalase activity was assayed by monitoring the disappearance of  $H_2O_2$  at 240 nm at 25°C (Becana *et al.* 1986). One unit of CAT activity was defined as the decrease at 240 nm for 1 min due to  $H_2O_2$  consumption. CAT activity expressed as units per min per g FM.

The content of malondialdehyde (MDA) was determined by TBA test (Zhou *et al.*, 2004).

### Yield and Quality of *A. sinensis*

At harvest stage (25 October), all plants in pot were taken out of the soil and separated into shoots (leaves and stems were combined) and roots. Roots were first cleaned using distilled water. Then the sample was oven-dried at 60°C for 48 h and weighed. Content of essential oils and alcohol-soluble extract were measured according to Zhang *et al.* (2010).

### Enumeration of Culturable Microbial Populations in Rhizosphere Soil of *A. sinensis*

Enumeration of culturable microbial populations was determined with traditional plate-dilution frequency technique on agar media in Petri plates (Harris and Sommers, 1968). Well mixed 0.05 mL samples of dilutions from  $10^{-3}$  to  $10^{-7}$  (in sterile deionized water) were spread in triplicate onto the following media for culturable microbe enumerations, bacteria were determined in the culture medium of Beef-cream and Peptone, actinomycete were determined in the culture medium of improved Gao 1, fungi on Martin's agar, azobacter was determined on Ashby-Sucrose agar medium, ammonifying bacteria on protein agar medium, aerobic cellulose-decomposing bacteria on agar according to Waksman, organic phosphorus-solubilizing bacteria on Meng Jina's agar, inorganic phosphorus-solubilizing bacteria on the culture medium of calcigenol simple and glucose, potassium-solubilizing

bacteria on potassium aluminium silicate agar (Xu and Zheng, 1986).

### Analysis of Functional Group Diversity in Rhizosphere Soil of *A. sinensis*

Enumeration of the functional group was determined with traditional plate-dilution frequency technique. The characteristic parameters including abundance, community diversity and evenness, and dominance concentration were calculated according to the type and amount of functional group (Magurran, 1988):

(1). Abundance ( $P_i$ ) was calculated by Berger-Parker method as  $P_i = N_i/N$ , where  $N_i$  represent the number of individuals in a cluster (or species) divided by the total number of isolates in the sample being analyzed. When  $P_i > 0.10$  as dominant groups,  $0.01 < P_i \leq 0.10$  as common groups,  $P_i \leq 0.01$  as scarce groups (Shen *et al.*, 2004).

(2). Community diversity (H) was calculated by Shannon-Wiener method (Klemedtsson *et al.*, 1987) as:

$H = - \sum_{i=1}^n P_i \ln P_i$ , where  $P_i$  represents the proportion of individuals of a given species.

(3). Community evenness (J) was calculated by Pielou method as  $J = H/\ln S$ . S stand for the species number in soil microbe communities.

(4). Dominance concentration (C) was calculate by Pielou method as  $C = \sum P_i^2$ .

### Statistical Analysis

Each treatment was replicated three times. Analysis of variance for the data was performed by the general linear procedure of the SPSS system. Means were compared by LSD at  $P < 0.05$  level.

## Results

### Plant Properties

**Plant height:** Mixed cropping had no effect on emergence time and rate of seedling emergence (data not shown). Analysis of plant height revealed a significant progressive increase in the intercropping treatment at each measured stage (Table 1). The root and shoot dry weight of *A. sinensis* were significantly increased (84.46 and 34.41%, respectively) when *A. sinensis* plants were grown with garlic as compared to the control (Fig. 1).

### Antioxidant Enzyme Activity and Lipid Peroxidation in Leaves

Compared with the control, the activity of SOD, POD and

**Table 1:** Effect of intercropping *A. sinensis* with garlic on plant height (cm) of *A. sinensis*

Treatment	Days after seedling		
	20 d	65 d	105 d
Monocropping	3.14±0.15 <sup>b</sup>	27.98±0.28 <sup>b</sup>	29.18±0.29 <sup>b</sup>
Intercropping	3.60±0.15 <sup>a</sup>	30.14±0.56 <sup>a</sup>	32.75±0.66 <sup>a</sup>

Lines in columns denoted by different letters are significantly different at  $P < 0.05$  according to least significant difference tests

**Table 2:** Effect of intercropping *A. sinensis* with garlic on antioxidant enzyme activity and lipid peroxidation of *A. sinensis* leave

Treatment	SOD (U·mg <sup>-1</sup> ·FM)	POD (U·min <sup>-1</sup> ·g <sup>-1</sup> ·FM)	CAT (U·min <sup>-1</sup> ·g <sup>-1</sup> ·FM)	MDA (μmol·g <sup>-1</sup> ·FM)
Monocropping	0.65±0.031 <sup>b</sup>	6.53±0.256 <sup>b</sup>	100.39±5.211 <sup>b</sup>	2.73±0.035 <sup>a</sup>
Intercropping	1.17±0.057 <sup>a</sup>	22.41±1.133 <sup>a</sup>	137.66±6.404 <sup>a</sup>	2.41±0.067 <sup>b</sup>

Lines in columns denoted by different letters are significantly different at  $P < 0.05$  according to least significant difference tests

**Table 3:** Effect of intercropping *A. sinensis* with garlic on yield and quality of *A. sinensis*

Treatment	Yield (g <sup>-1</sup> ·plant)	Essential oil content (%)	Extract content (%)
Monocropping	3.18	0.54	50.29
Intercropping	5.42	0.81	55.42

CAT in leaves of *A. sinensis* was significantly increased, but the content of MDA was significantly decreased in intercropping treatment (Table 2).

#### Yield and Quality

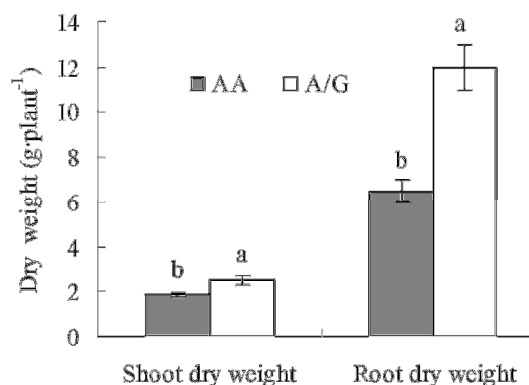
Intercropping treatment significantly increased the yield, content of essential oils and alcohol-soluble extract by 70.13, 50.00 and 10.20%, respectively as compared to monocropped control (Table 3).

#### Enumeration of Culturable Microbial Populations in Rhizosphere Soil of *A. sinensis*

As shown in Table 4, the number of culturable bacteria in the intercropping treatment increased significantly, as compared with the control at both seedling and rootstock thickening stages, while decreased significantly at harvest stage. For actinomycete and fungi, no significant difference was found at both seedling and rootstock thickening stages between intercropping treatment and the control, while have significant decrease at the harvest stage.

#### Enumeration of Bacteria Functional Groups in Rhizosphere Soil of *A. sinensis*

This study indicated that intercropping treatment changed the number of bacteria functional groups in rhizosphere soil of *A. sinensis*. The number of AB was significantly lower than that of the control at all determined stages, decreasing by 17.25, 47.26 and 22.01%, respectively (Table 5). On the contrary, the number of IPSB in intercropping soil was increased by 20.48, 49.46 and 20.05%, respectively compared with the control. The number of ACDB and OPSB was not significantly different between intercropping and the control at the seedling and harvest stages, but a

**Fig. 1:** Biomass (dry weight, g) of shoot and root of *A. sinensis* at rootstock thickening stage at different treatment. Within each plant component, vertical bars (s.e.) with the same letter are not significantly different ( $P < 0.05$ )

significant difference was recorded at the rootstock thickening stage, with treatment increasing by 23.19 and 103.44%, respectively compared with the control. Nevertheless, the number of KSB was not significantly different between intercropping and the control at the rootstock thicken stage, but significantly increased (19.06 and 75.59%, respectively) at both seedling and harvest stage in treatment, as compared with the control.

#### Analysis of Functional Group Diversity in Rhizosphere Soil of *A. sinensis*

As shown in Table 6, *A. sinensis* intercropping with garlic decreased slightly the abundance of AB in rhizosphere soil of *A. sinensis* at all growth stages compared with the control, while the abundance of ACDB, OPSB, IPSB and KSB was increased markedly.

**Table 4:** Population of microorganisms in rhizosphere soil at different growth stage of *A. sinensis*

Treatment	Seedling stage			Rootstock thickening stage			Harvest stage		
	Bacteria ( $\times 10^5 \text{cfu} \cdot \text{g}^{-1}$ )	Actinomycete ( $\times 10^3 \text{cfu} \cdot \text{g}^{-1}$ )	Fungi ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )	Bacteria ( $\times 10^5 \text{cfu} \cdot \text{g}^{-1}$ )	Actinomycete ( $\times 10^3 \text{cfu} \cdot \text{g}^{-1}$ )	Fungi ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )	Bacteria ( $\times 10^5 \text{cfu} \cdot \text{g}^{-1}$ )	Actinomycete ( $\times 10^3 \text{cfu} \cdot \text{g}^{-1}$ )	Fungi ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )
Monocropping	43.96 $\pm$ 2.74 <sup>b</sup>	26.10 $\pm$ 2.48 <sup>a</sup>	31.61 $\pm$ 2.86 <sup>a</sup>	58.32 $\pm$ 5.44 <sup>b</sup>	17.76 $\pm$ 0.74 <sup>a</sup>	20.88 $\pm$ 2.36 <sup>a</sup>	35.41 $\pm$ 1.46 <sup>a</sup>	2.90 $\pm$ 0.15 <sup>a</sup>	17.19 $\pm$ 1.33 <sup>a</sup>
Intercropping	72.25 $\pm$ 3.90 <sup>a</sup>	24.95 $\pm$ 1.17 <sup>a</sup>	35.78 $\pm$ 2.63 <sup>a</sup>	89.53 $\pm$ 4.09 <sup>a</sup>	17.94 $\pm$ 1.22 <sup>a</sup>	20.95 $\pm$ 1.59 <sup>a</sup>	24.18 $\pm$ 0.66 <sup>b</sup>	2.19 $\pm$ 0.15 <sup>b</sup>	13.40 $\pm$ 0.68 <sup>b</sup>

Where CFU: Colony forming units

Lines in columns denoted by different letters are significantly different at  $P < 0.05$  according to least significant difference tests**Table 5:** Populations of bacteria functional groups in rhizosphere soil at different growth stages of *A. sinensis*

Growth stages	Treatment	AB ( $\times 10^4 \text{cfu} \cdot \text{g}^{-1}$ )	ACDB ( $\times 10^3 \text{cfu} \cdot \text{g}^{-1}$ )	OPSB ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )	IPSB ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )	KSB ( $\times 10^2 \text{cfu} \cdot \text{g}^{-1}$ )
Seedling stage	Monocropping	36.52 $\pm$ 3.16 <sup>a</sup>	21.55 $\pm$ 1.04 <sup>a</sup>	14.67 $\pm$ 1.91 <sup>a</sup>	26.90 $\pm$ 0.43 <sup>b</sup>	21.30 $\pm$ 1.14 <sup>b</sup>
	Intercropping	30.22 $\pm$ 2.79 <sup>b</sup>	21.22 $\pm$ 1.91 <sup>a</sup>	12.65 $\pm$ 0.49 <sup>a</sup>	32.41 $\pm$ 0.35 <sup>a</sup>	25.36 $\pm$ 2.03 <sup>a</sup>
Rootstock thickening stage	Monocropping	21.20 $\pm$ 1.79 <sup>a</sup>	12.85 $\pm$ 0.62 <sup>b</sup>	6.68 $\pm$ 1.33 <sup>b</sup>	8.31 $\pm$ 0.33 <sup>b</sup>	14.16 $\pm$ 1.56 <sup>a</sup>
	Intercropping	11.18 $\pm$ 1.72 <sup>b</sup>	15.83 $\pm$ 0.57 <sup>a</sup>	13.59 $\pm$ 1.23 <sup>a</sup>	12.42 $\pm$ 0.92 <sup>a</sup>	15.61 $\pm$ 0.65 <sup>a</sup>
Harvest stage	Monocropping	26.76 $\pm$ 0.58 <sup>a</sup>	3.42 $\pm$ 0.06 <sup>a</sup>	43.21 $\pm$ 2.19 <sup>a</sup>	8.53 $\pm$ 1.23 <sup>b</sup>	48.06 $\pm$ 1.34 <sup>b</sup>
	Intercropping	20.87 $\pm$ 0.99 <sup>b</sup>	3.38 $\pm$ 0.10 <sup>a</sup>	46.87 $\pm$ 2.07 <sup>a</sup>	10.24 $\pm$ 0.40 <sup>a</sup>	84.39 $\pm$ 0.73 <sup>a</sup>

Where CFU: Colony forming units; AB: Ammonifying bacteria; ACDB: Aerobic cellulose-decomposing bacteria; OPSB: organic phosphorus-solubilizing bacteria; IPSB: Inorganic phosphorus-solubilizing bacteria; KSB: Potassium-solubilizing bacteria

Lines in columns at the same stage denoted by different letters are significantly different at  $P < 0.05$  according to least significant difference tests**Table 6:** Parameters of bacteria functional groups diversity in rhizosphere soil at different growth stages of *A. sinensis*

Growth stages	Treat.	Abundance (Pi)					Total individual No.(N) $\times 10^4$	Community diversity (H)	Evenness (J)	Concentration(C)
		AB	ACDB	OPSB	IPSB	KSB				
Seedling stage	Monocropping	0.9238	0.0602	0.0037	0.0068	0.0054	39.53	0.3254	0.2022	0.8572
	Intercropping	0.9145	0.0642	0.0038	0.0098	0.0077	33.05	0.3620	0.2249	0.8406
Rootstock thickening stage	Monocropping	0.9308	0.0564	0.0029	0.0036	0.0062	22.78	0.2981	0.1852	0.8696
	Intercropping	0.8481	0.1201	0.0103	0.0097	0.0118	13.18	0.5390	0.3349	0.7340
Harvest stage	Monocropping	0.9523	0.0122	0.0154	0.0030	0.0171	28.10	0.2515	0.1563	0.9076
	Intercropping	0.9225	0.0149	0.0207	0.0045	0.0373	22.62	0.3646	0.2265	0.8531

Where AB: Ammonifying bacteria; ACDB: Aerobic cellulose-decomposing bacteria; OPSB: organic phosphorus-solubilizing bacteria; IPSB: Inorganic phosphorus-solubilizing bacteria; KSB: Potassium-solubilizing bacteria

Although the total population of functional groups in rhizosphere soils of *A. sinensis* intercropping garlic was lower than in the control, community diversity and evenness were increased by 11.25-80.81 and 11.22-80.83%, respectively at all growth stages, whereas dominance concentration was decreased by 1.94-15.60% (Table 6).

## Discussion

Long-term continuous cropping changed soil microbial community structure and diversity, and soil physicochemical property, which result in the negative effect on crop growth, yield and quality (Ryszkowski *et al.*, 1998; Sun *et al.*, 2001; Monneveux *et al.*, 2006). However, diverse intercropping system can manage crop disease and improve crop production (Govaerts *et al.*, 2006). In this study, the root and shoot dry weight of *A. sinensis* were significantly increased when *A. sinensis* plants were grown with garlic as compared to the control. This indicated that intercropping garlic could increase primary product of *A. sinensis* under continuous cropping condition. Similar trends have also been found in mustard (Sarker *et al.*, 2007), Chinese cabbage (Cai *et al.*, 2011) and geraium (Singh *et al.*, 2011).

Soil microorganisms play an important role in the soil

ecosystem, and to a certain extent, soil microbial community play an important role in plant growth and metabolic functions of soil (Hagn *et al.*, 2003). Commonly, continuous cropping result in increase in fungi and decrease in bacteria as well as decrease of bacteria to fungi ratio, and thereby accelerate abundance of soil borne pathogens around plant roots (Ryszkowski *et al.*, 1998; Dai *et al.*, 2009). The above mentioned changes in types or amounts of soil microorganisms have wide impact on soil quality, and consequently on crop production. This study revealed that the number of bacteria increased significantly by intercropping together with decrease in the number of fungi, and the ratio of bacteria to fungi was higher than that of the control. This result indicated that garlic plants might change the status of soil microorganisms, which were beneficial to a healthy soil environment for *A. sinensis* plants, and this corresponded with improved growth of *A. sinensis* (Table 1, 2 and 3; Fig. 1).

Plants generate more reactive oxygen species (ROS) and stimulate resistance responses of plants when exposed to the stressful conditions (Thoma *et al.*, 2003). These ROS are either toxic by-products of aerobic metabolism or regulators of growth, development and the defense pathways (Ding *et al.*, 2007; Zhang *et al.*, 2013). Toxic ROS

can affect membrane permeability, induce lipid peroxidation, and ultimately lead to programmed cell death. In fact, plants possess efficient systems for scavenging ROS that protect them from destructive oxidative reactions (Olmos *et al.*, 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. The activity of antioxidant enzymes has been reported to decrease under continuous cropping of cucumber (Zhang *et al.*, 2007) and grape (Guo *et al.*, 2010), but increases in pepper (Hou *et al.*, 2006). In our previous study, the activity of antioxidant enzymes (SOD, POD and CAT) decreased under continuous cropping condition, which is in agreement with results obtained from cucumber (Zhang *et al.*, 2007) and grape (Guo *et al.*, 2010). In present studies, activities of SOD, POD and CAT of *A. sinensis* were increased in the intercropping system along with the improved of plant growth. MDA, an indicator of stress damage, is produced during the peroxidation of membrane lipids (Ohkawa *et al.*, 1979). In our study, intercropping decreased the MDA content, and this was consistent with the results of Su *et al.* (2008). This suggested enhanced growth of *A. sinensis* by intercropping over continuous cropping condition might be due to increased antioxidant enzyme activity, which further improve the ability of plant for scavenging ROS.

## Conclusion

The microflora in rhizosphere soil of *A. sinensis* was significantly improved in intercropping system through changed microbial community structure and increased community diversity and evenness of some functional groups. These alleviated the *A. sinensis* soil sickness by restraining the disease and regulating the protective enzymes system. The intercropping has some advantages on state of soil microorganisms and *A. sinensis* growth under continuous cropping condition. Further studies are needed to understand the implications of intercropping of *A. sinensis* with a variety of plants in field condition and long-term effect of the intercropping system.

## Acknowledgements

This work is supported by Natural Science Foundation of China (31060182) and Agricultural Biotechnology Research and Application Development of Gansu province (GNSW-2010-18).

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(Received 20 August 2013; Accepted 12 June 2014)