Effects of Garlic/Cucumber Relay Intercropping on Soil Enzyme Activities and the Microbial Environment in Continuous Cropping

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Abstract. Soil sickness from the continuous cropping of cucumbers has become a major limiting factor for protected cucumber cultivation. The use of reasonable cropping systems and the employment of allelopathy between different crops are considered to be the major safe and effective measures for alleviating soil sickness. The objective of this study assessed the effects of garlic (*Allium sativum* L. cv. Yusuan No. 1)/cucumber (*Cucumis sativus* L. cv. Jinchun No. 4) relay intercropping on soil enzyme activities and the microbial environment in a continuous cropping regime. Cucumbers and garlic were selected and planted in plastic barrels. The following four treatments were included in the experiment: continuous cropping without crops (Cont), monoculture cucumbers (C), monoculture garlic (G), and the relay intercropping of garlic with cucumbers (CG). The results showed that relay intercropping with garlic promoted cucumber plant growth and attenuated damage caused by soil sickness. In comparison with the Cont treatment, the C treatment decreased soil urease, catalase, invertase, and phosphatase activities; by contrast, the CG treatment enhanced all soil enzyme activities. The C treatment resulted in lower numbers of soil bacteria and actinomycetes and a lower bacteria/fungi ratio, but there were a higher number of soil fungi than there were in the Cont treatment. However, the CG treatment increased the numbers of soil bacteria and actinomycetes as well as the bacteria/fungi ratio, and it decreased the number of soil fungi. In comparison with the Cont treatment, the C treatment reduced the microbial biomass carbon (MBC) and soil basal respiration (BSR) without affecting the metabolic quotient (qCO_2), whereas the CG treatment increased all three variables. A polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis revealed decreased bacterial community diversity and increased fungal community diversity in soil with the C treatment; the opposite trend was observed in the CG treatment. The results indicated that the relay intercropping of garlic with cucumbers improved soil enzyme activities and promoted the conversion of continuous cropping soil from a “fungal” type to a “bacterial” type. Additionally, relay intercropping altered the soil bacterial community structure, increased the bacterial diversity indices, and enriched the dominant bacterial populations in the soil. These mechanisms improved the soil microbial environment and effectively alleviated damage caused by soil sickness, thus promoting cucumber plant growth.

The continuous cropping of monoculture in a field can cause deteriorations in growth and yield, leading to a decline in crop yield and quality. This condition is also known as

soil sickness, and can occur in normal cultivation and management conditions (Ye et al., 2004). In China, the large-scale development and industrialization of protected agriculture have resulted in the presence of soil sickness over vast areas, which has limited the sustainable development of this system. Continuously growing crops, particularly the growth of horticultural crops in the same area, often causes soil sickness. This phenomenon is manifested as a weakening of the crop growth potential, a decline in the crop yield and quality, an enhancement of root autotoxicity, and a decrease in root activity. These traits are associated with the deterioration of soil physicochemical properties, soil acidification and secondary salinization, soil nutrient deficiency or imbalance, and an over-proliferation of

deleterious microbes. There is also a decrease in plant stress resistance and an exacerbation of soilborne diseases and pests (Nguyen and Ranamukhaarachchi, 2010). The primary causes leading to soil sickness include the deterioration of soil physicochemical properties, the accumulation of plant diseases and insect pests, variations in soil microbes, and autotoxicity (Hegde and Miller, 1990; Yao et al., 2006; Yu and Matsui, 1994). The management of soil microbes is critical for alleviating soil sickness. Li et al. (1996) found that the soil biological environment deteriorates in continuous cropping, which is primarily characterized by an imbalanced soil microbial population structure and also by the number, proportion, and exacerbated presence of soilborne diseases and pests. Singh et al. (1999) suggested that each crop exhibits unique microbial community characteristics in the rhizosphere. Long-term continuous cropping inevitably leads to the enrichment of soil microbes with a high parasitic ability (e.g., pathogenic bacteria, fungi, and nematodes), and the number of beneficial microbes decreases, thus affecting the normal growth of crops and causing yield reductions.

The use of reasonable cropping systems and the employment of allelopathy between different crops are considered to be the major safe and effective measures for alleviating soil sickness. In particular, appropriate crop rotations and intercropping, mixed intercropping, and relay intercropping are the most simple and effective ways to avoid soil sickness (Peterson and Harrison, 2002; Ren et al., 2008). Garlic (*A. sativum* L.) possesses a strong flavor that can repel certain pests and pathogens; garlic root exudates and stem volatiles contain a natural broad-spectrum antimicrobial substance (such as ajoene, garlicin, eruboside, etc.) that may inhibit the proliferation of pests and pathogens (Wei et al., 2011). Zhou et al. (2011) found that relay intercropping with onion (*Allium cepa* L.) and garlic increased the number and the diversity of the soil bacterial community in the cucumber rhizosphere, which remained stable until the next growing season. Additionally, the garlic roots secrete substances with bacteriostatic and fungistatic effects, and this agent can serve as an allelochemical that influences soil microbes. Garlic reportedly promoted the growth of soil bacteria, actinomycetes, and fungi and indirectly enhanced the soil urease, phosphatase, and catalase activities in the rhizosphere, thereby improving the turnover and recycling of soil nitrogen (N), phosphorus (P), and other nutrient elements in the garlic rhizosphere. This change created a sound environment in terms of rhizosphere nutrition, and it provided the desired microecological environment for the next crop rotation. Moreover, Zhou et al. (2013) found that the number of aphids significantly decreased and that their number of natural enemies increased, as did the wheat (*Triticum aestivum*) grain yield, because of the relay intercropping of wheat with garlic. Furthermore, Abdel-Monaim and Abo-Elyousr (2012) suggested that garlic

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intercropping markedly reduced the lentils (purple haricot) field incidence and disease index and that garlic root exudates substantially reduced the mycelial dry weight of the pathogens.

Cucumber (*C. sativus* L.) is a major vegetable that is produced by protected agriculture, and it is planted widely in China. However, the excessive pursuit of productivity and efficiency together with improper planting habits by farmers has led to soil sickness. Soil sickness includes the deterioration of the soil ecological and biological environments, the exacerbation of soilborne diseases and pests, and the decline of crop yields and quality, restricting the sustainable development of protected cucumber production. Yu and Matsui (1994) showed that cucumber roots secreted 11 phenolic acids including benzoic acid, 2,5-dihydroxybenzoic acid, p-hydroxy benzoic acid, and cinnamic acid, which affected the ion absorption in the next rotation of cucumber seedlings and inhibited cucumber plant growth. Lv et al. (2006) indicated that the soil microbial flora changed in continuous cucumber cropping and that the numbers of pathogenic fungi and root-knot nematodes increased in the rhizosphere, negatively affecting cucumber plant growth. In continuous cropping, cucumber root exudates facilitated the growth of soil fungi and bacteria, and suppressed the growth of soil actinomycetes (Wu and Wang, 2006). Therefore, soil sickness from the continuous cropping of cucumbers has become a major limiting factor for protected cucumber cultivation. The ability to control the damage caused by soil sickness in protected cucumber cultivation by reasonable rotation and intercropping, mixed intercropping, and the relay intercropping of allium crops with cucumbers has great implications for promoting the healthy and sustained development of protected cucumber cultivation. In this study, we used cucumber (cv. Jinchun No. 4) and garlic (cv. Yusuian No. 1) to assess the effects of relay intercropping on cucumber plant growth, soil enzyme activities and microbial diversity, and biomass and community diversity.

Materials and Methods

Experimental materials and treatments. The experiments were conducted with cucumber (cv. Jinchun No. 4) and garlic (cv. Yusuian No. 1). A 5-year continuously cropped greenhouse soil was obtained from the Wusong River Agricultural Park in Suzhou, Jiangsu Province, China. Surface (0–15 cm) soil samples were collected by using a five-point method between the cucumber harvest and the uprooting of the plants. The samples were kept moist and were transported to the experimental greenhouse for cultivating the cucumbers and garlics in plastic barrels. The basic soil chemical properties were as follows: organic matter (OM), 14.37 g·kg⁻¹; alkali-hydrolysable N, 167.3 mg·kg⁻¹; available P, 146.5 mg·kg⁻¹; available K, 213.4

mg·kg⁻¹; pH, 7.72; and electrical conductivity (EC), 0.83 mS·cm⁻¹.

The experiment was performed in a greenhouse at the Pailou Experiment Base of Nanjing Agricultural University (Nanjing, China) between Sept. 2014 and July 2015. Cucumber seeds were immersed in 30 °C water for 6 h and then wrapped with wet gauze. The seeds were placed in an HZ-8811K incubator (Changzhou Weilai Instrument Manufacturing Co., Ltd., Jiangsu, China; 80% humidity and 28 °C) and pregerminated in the dark for 12 h. The germinated seeds were sown into 50-cell plug trays (length × width: 540 × 280 mm) made of black polystyrene that contained nursery substrate. The basic physiochemical properties of the substrate were as follows: total nutrients (N + P₂O₅ + K₂O), 2.8%; moisture (free water), 30%; OM, 28%; pH, 6.4; bulk density, 0.24 g·cm⁻³; total porosity, 65%; and EC, 1.6 mS·cm⁻¹. In accordance with the moisture of the substrate and the seedling growth, water was occasionally supplied to the substrate when growing the seedlings.

Peeled garlic bulbs were immersed in water for 2 d, and the water surface was kept just above the garlic bulbs. The following environmental conditions were used for pregermination: day/night temperature, 30/18 °C; humidity, 60% to 70%; and photoperiod, 11/13 h. Garlic seedlings with consistently germinating radicle buds and cucumber seedlings with two leaves and one bud were simultaneously transplanted into plastic barrels (diameter, 15 cm; height, 20 cm). Each barrel contained 5 kg of continuously cropped soil. One cucumber seedling and one garlic seedling were planted in each barrel at a spacing of 5 cm. Four treatments were included in the experiment as follows: no crops (Cont), monoculture cucumbers (C), monoculture garlic (G), and a relay intercropping of garlic with cucumbers (CG). Five barrels per treatment were replicated three times. The barrels were randomly arranged. The plants were irrigated with 1 × Hoagland nutrient solution once every 3 d during the experiment. The environmental conditions inside the greenhouse were as follows: day/night temperature, 28/18 °C; humidity, 60% to 70%; photoperiod, 10/14 h; and daytime average light intensity, 400 μmol·m⁻²·s⁻¹.

Thirty days after the garlic and cucumber seedlings were planted in the plastic barrels, we removed the surface soil from each barrel and collected 200 g of 0–15 cm soil samples per barrel. Soil samples from five barrels within the same replication were mixed to form a composite sample. Large debris was removed and the soil was then passed through a sieve (<2 mm). One portion of sieved soil was used to analyze the soil enzyme activity, soil microbial number, MBC, and BSR; the qCO₂ was then calculated. The other portion was frozen at -70 °C until it was used for microbial DNA extraction and PCR-DGGE analysis.

Determination of plant growth indicators. Thirty days after planting, we measured the

plant height, stem diameter, and fresh and dry weights of the cucumber plants, and we calculated the seedling index. The plant height (cm) was measured from the cotyledon node to the growing point with a ruler. Stem diameter (mm) was measured at the cotyledon node parallel to or below (0.5 cm) the direction of cotyledon expansion with vernier calipers. Cucumber plants were then removed from the barrels. The roots were washed with tap water to remove the soil and then dried with absorbent paper. Each plant was divided into two parts, that is, the shoots above the cotyledon node and the roots below the cotyledon node. Each part was weighed to obtain the fresh weight (g). Both shoots and roots were deactivated in an oven at 105 °C for 15 min and then dried at 75 °C to constant weight to obtain the dry weight (g). The seedling index = stem diameter/plant height × whole-plant fresh weight.

Determination of soil enzyme activities. The soil urease activity was determined by colorimetric assay by using sodium phenate–sodium hypochlorite (Hoffmann and Teicher, 1961). The soil catalase activity was determined by potassium permanganate titration (Johnson and Temple, 1964). The soil invertase activity was determined by colorimetric assay with 3,5-dinitrosalicylic acid (Frankenberger and Johanson, 1983). The soil phosphatase activity was determined by colorimetric assay with disodium phenyl phosphate (Tabatabai and Bremner, 1969).

Determination of soil microbial numbers. The soil microbial numbers were determined by a conventional plate count method (Li et al., 2012). Bacteria were cultured in beef extract peptone medium (containing beef extract, peptone, and sodium chloride, etc.); the plates were inverted and cultured at 28 °C for 24–48 h before the observation and counting of colonies. Fungi were cultured in Martin medium (containing potassium dihydrogen phosphate, anhydrous magnesium sulfate, and peptone, etc.); the plates were inverted and cultured at 28 °C for 48–72 h before the observation and counting of colonies. Actinomycetes were cultured with Gauze's Medium No. 1 (containing potassium nitrate, dipotassium hydrogen phosphate, and anhydrous magnesium sulfate, etc.); the plates were inverted and cultured at 28 °C for 72–96 h before the observation and counting of colonies. The soil microbial number per gram of dry soil (colony-forming units) = average number of colonies × dilution factor/dry weight of soil sample.

Determination of MBC. The soil MBC was determined by chloroform fumigation–K₂SO₄ extraction (Lin et al., 1999). Thirty grams of freshly sieved soil samples were weighed into a vacuum desiccator and fumigated with chloroform vapor for 24 h. The residual chloroform was removed by repeated vacuuming. An equal amount of soil samples was taken without fumigation. Each soil sample was extracted with 100 mL of 0.15 mM K₂SO₄ solution and immediately filtered after 30 min of oscillation. The filtered extracts were frozen at -15 °C until

Table 1. Effect of relay intercropping with garlic on cucumber plant growth after planting of 30 d.

Treatment	Plant ht (cm/plant)	Stem diam (mm/plant)	Shoot fresh wt (g/plant)	Shoot dry wt (g/plant)	Root fresh wt (g/plant)	Root dry wt (g/plant)	Seedling index
C	26.03 ± 0.64 b	3.54 ± 0.16 b	23.13 ± 1.58 b	2.03 ± 0.75 b	3.34 ± 0.23 ab	0.25 ± 0.06 ab	3.59 ± 0.26 b
CG	31.30 ± 1.62 a	4.32 ± 0.15 a	37.53 ± 2.64 a	3.55 ± 0.25 a	4.36 ± 0.47 a	0.33 ± 0.12 a	4.90 ± 0.30 a

C = monoculture cucumbers; CG = relay intercropping of garlic with cucumbers.

Each value is the mean ± SE of 15 plants. Different letters indicate significant differences at $P < 0.05$ according to least significant difference-Duncan's multiple comparison test.

Fig. 1. Effects of garlic relay intercropping with cucumbers on the soil urease activity (A), catalase activity (B), invertase activity (C), and phosphatase activity (D) in continuously cultivated soils. Each value is the mean of three replicates. Soil samples from five barrels within the same replication were mixed to form a composite sample. Cont = control, C = monoculture cucumbers, G = monoculture garlic, CG = garlic relay intercropping with cucumbers. Different letters indicate significant differences at $P < 0.05$ according to least significant difference-Duncan's multiple comparison test.

analysis. The organic carbon (OC) content in the extracts was determined by potassium dichromate oxidation. The soil MBC was calculated as $\text{MBC} = \Delta\text{OC}/\text{KC}$, where ΔOC indicates the OC difference in extracts between fumigated and non-fumigated soil samples, and KC indicates the conversion coefficient, $\text{KC} = 0.38$.

Determination of BSR and $q\text{CO}_2$. The BSR determination was performed in accordance with the amount of CO_2 that was released by microbial respiration during incubation for 3 d (Isermayer, 1952). Fifty-gram moist soil samples were incubated in sealed glass bottles at 25°C , and the CO_2 production within each bottle was quantified at the end of the incubation. To measure the respiration rate, the CO_2 was dissolved in a NaOH solution, followed by the addition of a BaCl_2 solution to form BaCO_3 precipitate. The residual NaOH solution was neutralized by HCl titration, and the results were

expressed as $\text{mg CO}_2\text{-C/kg soil/h}$. The BSR/MBC ratio was used to calculate the $q\text{CO}_2$.

Soil microbial DNA extraction and PCR-DGGE analysis. Microbial DNA was extracted from the soil samples and purified with a PowerSoil DNA Isolation Kit (12888; Mobio, Solana Beach, CA). DNA extracts were dissolved in 30 mL of sterile deionized water and checked by 1% agarose gel electrophoresis. The 16S ribosomal DNA (rDNA) sequences of the bacteria were PCR-amplified with primers 357F-GC/518R (Muyzer et al., 1993). The PCR reactions (Bio-Rad Laboratory, LA) were performed at 94°C for 2 min, followed by 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The 18S rDNA fungal sequences were PCR-amplified with primers EukA516r-GC/Euk1A (Smit et al., 1999). The PCR conditions were as follows: 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30

s, and a final extension at 72°C for 5 min. The PCR products of the bacteria and fungi were subject to DGGE on 8% (w/v) acrylamide gels with denaturing gradients of 47% to 63% and 32% to 45%, respectively. Electrophoresis was performed with $1 \times \text{TAE}$ in a Bio-Rad water bath system (Bio-Rad Laboratory) at 70 V and 60°C for 16.5 h. After electrophoresis, the gels were stained with 400 mL of Biolinker DNA Red, and they were stained for 40 min. The DGGE profiles were photographed under an ultraviolet lamp with the Bio-Rad gel imaging system (Bio-Rad Laboratory).

Data analysis. The data were plotted in Excel 2007 (version 5.0; Microsoft Corp., Redmond, WA). A least significant difference-Duncan's multiple comparison test was performed in SPSS Statistics (version 20.0; IBM Corp., Armonk, NY) ($P < 0.05$). The DGGE profiles were analyzed with Quantity One (version 4.5; Bio-Rad Laboratory), and the position and intensity of each DNA

Fig. 2. Effects of garlic relay intercropping with cucumbers on the numbers of bacteria (A), the numbers of fungi (B), the numbers of actinomycetes (C), and the bacteria-to-fungi ratio (D) in continuously cultivated soils. Each value is the mean of three replicates. Soil samples from five barrels within the same replication were mixed to form a composite sample. Cont = control, C = monoculture cucumbers, G = monoculture garlic, and CG = garlic relay intercropping with cucumbers. Different letters indicate significant differences at $P < 0.05$ according to least significant difference-Duncan's multiple comparison test.

band were automatically determined. The Shannon-Wiener diversity index (H) was calculated as follows: $H = -\sum(p_i)(\log_2 p_i)$, where p_i represents the ratio of the i -th species. The evenness index (E) was calculated as $E = H/H_{\max}$, where $H_{\max} = \ln S$ (S is the total number of species) (Shannon and Weaver, 1963). The standard data were subjected to principal component analysis (PCA) with Windows 4.5 (Joynt et al., 2006).

Results

Cucumber plant growth. Table 1 shows that the CG treatment increased the cucumber plant height, stem diameter, shoot fresh weight, shoot dry weight, and seedling index by 20%, 22%, 62%, 75%, and 36%, respectively, in comparison with those of the C treatment. However, the root fresh weight and dry root weight were not significantly affected by the CG treatment relative to the C treatment. The results indicate that relay intercropping with garlic strongly promoted cucumber plant growth and had a positive effect in preventing soil sickness.

Soil enzyme activities. The C treatment resulted in lower soil urease, catalase, invertase, and phosphatase activities in comparison with the Cont treatment. The G

treatment resulted in higher soil catalase and invertase activities than the Cont treatment (Fig. 1), but it did not significantly change the soil urease or phosphatase activity. Soil catalase and invertase activities were higher in the CG relative to the Cont and C treatments. In terms of soil urease and phosphatase activities, the CG treatment had higher activities than the C treatment, and it exhibited no significant difference in comparison with the Cont treatment. These results indicate that continuously cropping the cucumbers reduced soil urease, catalase, invertase, and phosphatase activities. By contrast, relay intercropping of garlic with cucumbers enhanced soil enzyme activities.

Soil microbial numbers. Compared with Cont treatment, the numbers of soil bacteria (Fig. 2A) and actinomycetes (Fig. 2C) was decreased by C treatment, but the number of soil fungi (Fig. 2B) was increased, and the bacteria/fungi ratio (Fig. 2D) exhibited a decreasing with C treatment. The G treatment resulted in higher numbers of soil bacteria and actinomycetes but a lower number of soil fungi and a higher ratio of bacteria/fungi in comparison with the Cont treatment. The numbers of soil bacteria and actinomycetes and the bacteria/fungi ratio were higher in the CG in comparison with the Cont and other

treatments. With respect to the number of soil fungi, the CG treatment had lower numbers than those of the Cont and C treatments, and it exhibited no significant difference in comparison with the G treatment. These results indicate that the continuous C decreased the numbers of soil bacteria and actinomycetes but increased the number of soil fungi, thus lowering the bacteria/fungi ratio and promoting the conversion of soil from a bacterial to a fungal type. By contrast, relay intercropping CG increased the numbers of soil bacteria and actinomycetes but decreased the number of soil fungi, thus increasing the bacteria/fungi ratio and leading to the conversion of this soil from a fungal to a bacterial type.

MBC, BSR, and qCO_2 . The C treatment decreased soil MBC (Fig. 3A) and BSR (Fig. 3B) by 16% and 26%, respectively, in comparison with the Cont treatment; however, no significant changes were observed in qCO_2 (Fig. 3C). By contrast, the G treatment increased the soil BSR and qCO_2 , and no significant change was observed in the MBC. The CG treatment resulted in higher soil MBC, BSR, and qCO_2 than those of the Cont and C treatment, but the differences were not significant in comparison with the G treatment. These results indicate that the continuous cropping of cucumbers resulted in lower

Fig. 3. Effects of garlic relay intercropping with cucumbers on MBC (A), BSR (B), and qCO₂ (C) in continuous soils. Each value is the mean of three replicates. Soil samples from five barrels within the same replication were mixed to form a composite sample. Cont = control, C = monoculture cucumber, G = monoculture garlic, and CG = garlic relay intercropping with cucumber. MBC = microbial biomass C; BSR = basal soil respiration; and qCO₂ = the ratio of basal soil respiration to microbial biomass C. Different letters indicate significant differences at $P < 0.05$ according to least significant difference-Duncan's multiple comparison test.

soil fertility and microbial activity, whereas relay intercropping garlic with cucumbers improved soil fertility, microbial activity, and qCO₂.

DGGE profiles of bacterial and fungal communities. The PCR-DGGE analysis revealed that the CG treatment altered the community structure of soil bacteria (Fig. 4A) and fungi (Fig. 4B). The bacterial DGGE profiles showed no significant difference between the C and Cont treatments. In comparison with the Cont and C treatments, the G treatment resulted in distinct characteristic bands for a and b, which were not obvious in the previous two treatments. A comparison of the fungal DGGE profiles showed that the number of bands exhibited a relative increase with C treatment, and it decreased with CG treatment, in comparison with the Cont treatment.

PCA analysis can simply and clearly elucidate the relative relations between treatments; the shorter the distance is on the PCA plot, the higher the similarity of the samples.

The PCA analysis of the DGGE banding patterns for soil bacteria (Fig. 4C) and fungi (Fig. 4D) showed that the Cont and C treatments clustered together for the bacterial community and that the CG treatment sample was clearly separated from the Cont and C treatments. This result indicates high similarity between the Cont and C treatments and low similarity between the CG treatment and the other two. For the fungal community, the G and CG treatments were clustered together, and the C treatment was clearly separated from the Cont treatment. This finding indicates a high similarity between the G and CG treatments and a low similarity between the C and Cont treatments.

Diversity indices. To determine the diversity of the soil microbial community structure, we calculated the richness index (S), the Shannon-Wiener index (H), and the evenness index (E) on the basis of the number of relatively clear DGGE bands (Table 2). The diversity indices bacteria community in the soil did not change significantly when comparing the C and Cont treatments. However, the S , H , and E values for the soil bacteria community were higher for the CG treatment relative to the Cont and C treatments. The diversity indices of the soil fungal community showed the opposite trend. The H and E values for the fungal community were higher in the C treatment relative to the Cont and CG treatment; the S value for the fungal community were higher in the C treatment in comparison with the Cont treatment, but it exhibited no significant difference in comparison with the CG treatment.

Discussion

Numerous studies show that intercropping and relay intercropping can be employed to take full advantage of the land, light, heat, and water resources, improve crop productivity, and obtain economic and ecological benefits. In addition, intercropping and relay intercropping can improve the ecological microclimate, prevent adverse environmental impacts, increase soil biodiversity, and improve soil quality (Olasantan et al., 1996). There is a certain allelopathic effect between different crops. Making the most of the promoting effects between crops can effectively prevent and alleviate soil sickness. The results of the present study show that relay intercropping with garlic can significantly increase cucumber plant height, stem diameter, shoot fresh weight, shoot dry weight, and seedling index in comparison with monoculture cucumbers (Table 1), thereby promoting cucumber plant growth and reducing the damage caused by soil sickness.

Soil enzymes participate in various metabolic processes and in soil energy conversion. They are important indicators of soil properties and ecological stability (Tian et al., 2010). Enhanced invertase activity is conducive to the conversion of OM in soil. Urease is directly involved in the conversion of N-containing organic compounds in soil,

and enhanced urease activity can improve the N supply levels. Neutral phosphatase can promote the hydrolysis of organic phosphorus compounds to produce inorganic P that is in turn available for crops (Yang et al., 2005). In this study, activities of soil urease, catalase, invertase, and phosphatase activities significantly decreased in monoculture cucumbers and increased with relay intercropping of garlic with cucumbers relative to the control (Fig. 1). These findings indicate that continuously cropping cucumbers can lower soil enzyme activities, weaken oxidation in soil, and inhibit hydrogen peroxide decomposition, thus aggravating root autotoxicity and leading to soil sickness. By contrast, relay intercropping with garlic can improve soil enzyme activities and facilitate soil OM conversion, thus improving the soil quality and helping to overcome soil sickness.

The imbalance of the soil microbial population structure is a major factor in crop yield losses and soil quality decline (Behera and Sahani, 2003). Studies have shown that an increasing planting age will promote soil conversion from a bacterial to a fungal type and will lead to eventual reduced soil fertility (Yin et al., 2004). The higher the fungal number is, the more reduced soil fertility is. Our results showed that the numbers of soil bacteria (Fig. 2A) and actinomycetes (Fig. 2C) increased and the number of soil fungi (Fig. 2B) decreased in the rhizosphere soil with monoculture cucumbers in comparison with the control. These changes resulted in a significantly lower ratio of bacteria/fungi (Fig. 2D) and promoted the soil's conversion from the "bacterial" type to the "fungal" type. "Fungal-type" results associated with serious sickness in continuously cropped soil, primarily because continuous cropping enables the enrichment of specific microbial populations, particularly plant pathogenic fungi. These fungi are adverse to the balance of soil microbial populations and conducive to the occurrence of plant root diseases. Therefore, the growth of cucumber plants is inhibited in continuously cropped fields, and the soil sickness is aggravated. There were significantly increased numbers of soil bacteria and fungi but decreased numbers of fungi under the relay intercropping of garlic and cucumbers, which led to a higher ratio of bacteria/fungi and the conversion of the soil from the "bacterial" type to the "fungal" type. "Bacterial-type" soil contains numerous beneficial microbes. For example, nitrifying bacteria can convert ammonia that is produced by organic fertilizer breakdown into nitrates that are available to plants. Phosphorus and K bacteria can convert P and K that cannot be used directly by plants into bioavailable forms. These traits could facilitate nutrient uptake by plants and enhance plant resistance to diseases, thereby preventing soil sickness.

Soil MBC is an easy-to-use nutrient pool and it drives OM decomposition and N mineralization. MBC is closely associated with the cycling of carbon (C), N, P, and sulfur (S), among various soil nutrient

Fig. 4. DGGE profile (A) and PCA analysis (C) of the soil bacterial community, and DGGE profile (B) and the PCA analysis (D) of the soil fungal community among different treatments. Cont = control, C = monoculture cucumbers, G = monoculture garlic, and CG = garlic relay intercropping with cucumbers.

Table 2. Diversity indices of soil bacterial and fungal communities based on denaturing gradient gel electrophoresis analysis.

Treatment	Bacterial community			Fungal community		
	<i>S</i>	<i>H</i>	<i>E</i>	<i>S</i>	<i>H</i>	<i>E</i>
Cont	57 b	3.46 b	0.86 b	26 c	2.67 c	0.82 b
C	54 b	3.34 b	0.84 b	36 a	3.07 a	0.86 a
G	50 c	3.35 b	0.86 b	30 b	2.83 b	0.83 b
CG	60 a	3.64 a	0.89 a	36 a	2.92 b	0.81 b

S = richness index; *H* = Shannon-Wiener index; *E* = evenness index; Cont = control; C = monoculture cucumbers; G = monoculture garlic; CG = garlic relay intercropping with cucumbers.

Each value is the mean of three replicates. Soil samples from five barrels within the same replication were mixed to form a composite sample. Different letters indicate significant differences at $P < 0.05$ according to least significant difference-Duncan's multiple comparison test.

elements. The MBC change can reflect changes in cropping systems and soil fertility as well as the soil pollution level. The BSR generally indicates the intensity of soil CO₂ release or the absorption of O₂. The measurement of the soil respiration rate is regarded as an indicator of total soil microbial activity or one of the indicators that can be used to evaluate soil fertility (Coleman et al., 2002). In comparison with the Cont and the C, the CG markedly increased the soil MBC and

BSR (Figs. 3A and B). This finding indicated that relay intercropping with garlic improved soil fertility and microbial activity, facilitated OM decomposition, and promoted plant uptake of nutrient elements. This effect might be related to the increased number of bacterial communities and the decreased number of fungal communities in the soil after relay intercropping with garlic. Because the vast majority of soil bacteria can degrade soil OM, these bacterial communities are conducive

to enhancing soil microbial activity and thus contribute to increased microbial biomass. qCO₂ is considered to be an index for evaluating the efficiency of soil microbial communities. The more efficient the microbial function is, the higher the proportion of basal C incorporated into biomass and the less the respiration loss of biomass C per unit (Behera and Sahani, 2003). Our results (Fig. 3C) showed that the qCO₂ markedly increased under the CG and that it decreased in C (i.e., continuous cropping), in comparison with the Cont. This result showed that C resulted in lower soil microbial respiration activity and that the CG increased the soil microbial respiration activity. The low qCO₂ might be related to the presence of fungi-dominated microbial biomass, and fungal populations have a relatively high utilization efficiency for basal C (Sakamoto and Oba, 1994). Our results suggest that the CG can improve soil fertility and the microecological environment, thereby improving plant resistance and effectively easing cucumber soil sickness.

Extensive research has shown that soil microbial communities are affected by many factors (e.g., the soil type, management practices, soil pH, and plant species) (Romaniuk et al., 2011). Cropping patterns can affect soil microbial communities, and the abundance and community structure of soil bacteria and fungi change with the cropping patterns (Acosta-Martínez et al., 2010; Larkin and Honeycutt, 2006). Wu et al. (2008a) found that soil type strongly influenced soil microbial community diversity. Zhou et al. (2011) reported that the relay intercropping of tilled onions with cucumbers significantly changed the soil microbial community structure during the first, second, and third rotations. Wu and Wang (2007) found that the crop rotation of cucumbers with wheat and soybeans markedly increased soil microbial diversity and richness and evenness indices. In the present study, the soil fungal community appeared to be more sensitive than the bacterial community was to the continuous cropping of cucumbers. Continuous cropping strongly reduced the fungal diversity (Fig. 4B) and thus altered the balance of dominant fungal populations in the soil. This finding may be an important reason for soil sickness in cucumbers. The CG resulted in distinct bands in the bacterial DGGE profile and in markedly increased bacterial diversity indices (Table 2). The soil bacterial diversity also increased (Fig. 4A), showing its strong adaptability to environmental changes. The PCA analysis (Figs. 4C and D) also revealed that C had a significant effect on the fungal community and that the CG strongly influenced the bacterial community. The crop rotation or companion planting of garlic and cucumbers has been reported to alter soil microbial diversity and to increase bacterial diversity indices (Wu et al., 2008b). These findings are consistent with our results in the present study, possibly because the unique antibacterial components of garlic have inhibitory effects on a variety of pathogenic bacteria and fungi. Taken together, these results indicate

that C can significantly alter the soil fungal community structure and increase fungal diversity, thereby breaking the balance of microbial populations and exerting adverse effects on the microbial environment of the soil. By contrast, the CG can markedly change the bacterial community structure and increase bacterial diversity indices; it therefore increases the dominant soil bacterial populations and improves the soil microbial environment, preventing soil sickness in cucumbers.

In conclusion, The relay intercropping of CG enhanced soil enzyme activities and promoted the soil's conversion from a "fungal" to a "bacterial" type, and increased the soil MBC, BSR, and qCO_2 ; it also altered the soil bacterial community structure and increased the bacterial diversity indices. Consequently, the dominant bacterial populations were increased and the soil microbial environment was improved, ultimately preventing soil sickness in cucumbers.

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