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Original article – Thematic Issue

Soil extract from an alfalfa (*Medicago sativa* L.) field improves potato rhizosphere enzyme activity and microbial quantity in a pot experiment

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Summary

The yield increase and nitrogen use efficiency improvement often recorded in crops following legumes have been a major attribute of crop rotation. However, the impact of crop rotation on soil enzyme activities and microbial community has rarely been reported particularly under potato-legume rotation system. A pot experiment was conducted in 2015 using soil extracts from legume (alfalfa) and potato fields under continuous cropping for years each. Potato cultivar 'Xindaping' and alfalfa (*Medicago sativa* L. 'Qingshui'), which is a legume, were used in this study. Soil extracts were prepared from these two separate fields and used as treatments in the pot experiment. The treatments were CK (soil extract from sole potato field) while T1-T5 were a mixture of extracts from the potato field and the legume field at ratios of 1:1, 1:2, 1:3, 1:4 and 1:5, respectively. The results indicated that T4 significantly improved catalase and alkaline phosphatase activity within the root zone of the potato in the pots. In addition, it also enhanced the activities of invertase, urease and increased the quantity of actinomycetes at the tuber formation stage. The application of T4 also improved urease activity at the tuber maturity stage. At the tuber growth stage, however, T3 greatly increased urease and invertase activity and enhanced bacteria and actinomycetes quantities but inhibited fungi quantity throughout the growth of the potato plants. Application of T3 maintained a high number of aerobic azotobacter, whereas the quantity of anaerobic azotobacter, nitrifying and denitrifying bacteria were highest in T4. Thus, our experiment has demonstrated that a potato-legume (alfalfa) rotation system could improve soil quality through the enhancement of enzyme activities and increase in the quantities of beneficial soil microbes.

Keywords

continuously grown potato, crop rotation, *Medicago sativa* L. 'Qingshui', soil extracts, soil quality, ratio

Significance of this study

What is already known on this subject?

- The complicated mechanism of crop continuous cropping obstacle.
- The crop-pasture rotation is an ancient and effective method in overcoming continuous cropping obstacle.

What are the new findings?

- A potato-legume (Alfalfa) rotation system could improve soil quality through the enhancement of enzyme activities and increase in the quantities of beneficial soil microbes.

What is the expected impact on horticulture?

- Our results contribute knowledge about crop and soil management to the scientific community regarding an agricultural scenario (crop rotation) that can be beneficial.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth major food crop after rice, wheat and corn in the world (Zhang and Tian, 2007). China is the world's largest producer of potato, and the potato industry plays an important role in the economic development and food security drive of China (Jansky et al., 2009). In recent years, with the development of China's potato industry, the continuous cropping of potato is very common, especially in semiarid area of China's Gansu central (Wang et al., 2008; Qin et al., 2011). Long-term continuous cropping can lead to decline of soil fertility, enhance toxic effect by root secretion, increase in quantity of pathogenic microorganism, and decline in crop yields (Lithourgidis et al., 2006; Li et al., 2006). Previous researches showed that the main reason for the continuous cropping obstacle is the associated decrease in soil bacteria and actinomycetes and rapid multiplication of soil-borne fungal pathogen (Sun et al., 2001). Continuous cropping also restricts soil enzyme activity including soil alkaline phosphatase, invertase and urease activities, which decrease with increasing years of continuous cropping (Yang et al., 2011). Soil enzyme activities are the direct expression of the soil community to metabolic requirements and available nutrients (Caldwell, 2005). Urease is involved in the N cycle and catalyzes the hydrolysis of urea into CO_2 and NH_3, which is of particular interest because urea is an important N fertilizer. Invertase participates in the C cycle by catalyzing the hydrolysis of sucrose into glucose and fructose. In the P cycle, phosphatase catalyzes the hydrolysis of both organic phosphate esters and phosphoric anhydrides into inorganic P, and catalase can promote the decomposition of hydrogen peroxide into water and oxygen (Zhou, 1987).

While the diversity of soil organisms is important, the capacity of soil microbial communities to maintain functional

diversity of those critical soil processes through disturbance, stress or succession could ultimately be more important to ecosystem productivity (Franzluebbers, 2002; Caldwell, 2005). Therefore, soil enzyme and soil organisms are directly involved in soil organic matter cycling, energy conversion, biological cycles and the effective formation of soil fertility (Zhang et al., 2012). This reflects the transformation ability of soil nutrients and the activity of soil microbes. As a result, the soil enzyme activity is an important indicator to evaluate soil fertility and soil biological activity (Iyyemperumal and Shi, 2008; Böhme et al., 2005). Finding scientific way to reduce continuous cropping obstacles and autotoxicity is urgently required for increasing yield and quality of potato (Cao et al., 2013). Crop rotation which is conducive for crop growth and yield increase has been reported to reduce these obstacles (Wu and Wang, 2007). The crop-pasture rotation is an ancient and effective method in China's farming system (Kong et al., 2010). In recent years, crop-pasture rotation and grass planting for feeding livestock is increasing in northwest China with adjustment of rural industrial structure. While the semiarid area of Gansu Province belongs to the gully region of loess plateau, the region is severely affected by soil erosion. The grass-potato crop rotation has made a significant impact under the national western development grass policy (Xing et al., 2011). At the same time, a large number of studies have demonstrated that legume had a remarkable positive effect on the yield of spring wheat (Lu and Sun, 2003) and corn (Mallarino and Ortiz-Torres, 2006; Liu et al., 2007). Crop rotation could improve soil structure (Kar et al., 2006) and balance nutrient (Dai, 2003) through configuring the crop sequences. The introduction alfalfa into the crop rotation system is reported to have effectively improved soil fertility (Wang et al., 2007).

Research on crop rotation systems has focused mainly on soil physical properties (Song et al., 2015), crop yield (Yang et al., 2015), nutrient absorption and soil chemical properties (Qin et al., 2014). Studies on potato-legumes crop rotation is mainly on growth characteristics and analysis of potato and legume yields. At present, only a few studies have considered the impact of potato-legume cropping system on soil biological characteristics and enzyme activity under crop rotation system. We conducted our experiment with the hypothesis that long term potato-legume crop rotation could positively impact soil enzyme activities and microbial communities. We therefore conducted pot and laboratory experiments to determine the effect of soil extracts from four-year-old potato and alfalfa fields on soil enzyme activity and the quantity of microbial community using potato as a test crop. This study contributes knowledge about crop and soil management to the scientific community regarding an agricultural scenario (crop rotation) that can be beneficial.

Materials and methods

Experimental design and treatments

Pot culture experiments were conducted from April to November in 2015 at Gansu Agricultural University, Northwest China. Potato cultivar 'Xindaping' was used as test material and the tubers were sown on April 13th and harvested on September 8th.

The potato tubers were planted in large pots (32 × 28 cm) which were filled with vermiculite-perlite medium at 4:1 in a vinyl tunnel. Prior to planting, the medium was treated against fungi with carbendazim fungicide. Hoagland's nutrient solution was applied at 7-days interval to keep the medium moist after seedling emergence.

Soil extracts were obtained from a 4-year potato continuous cropping field and a 4-year old legume (alfalfa) cropping field. There were six treatments arranged in a completely randomized design with three replications. Each experimental unit consisted of 3 pots and a total of 9 pots for each treatment. The treatments were CK (extract from sole potato field) and T1, T2, T3, T4, and T5 which were a mixture of extracts from potato and alfalfa fields at the ratio of 1:1, 1:2, 1:3, 1:4, and 1:5 respectively. The extracts were applied to the growing medium at 600 mL per pot at 7-days intervals.

Preparation of soil extracts

Soil was collected from two separate fields which had been under sole legume (alfalfa) and sole potato cultivation for four years each. The legume field was at the Experimental Station (36°09'N, 103°70'E) of the College of Pratacultural Science, Gansu Agricultural University. The potato field was at the Academy of Agricultural Sciences Research Station, Dingxi (35°33'N, 104°35'E).

The chemical properties of the soils from the legume and potato fields are shown in Table 1. The initial pH was tested by acidimetric (PHS-3E) method. EC was tested by conductivity meter (DDSJ-308A). The CEC was tested by barium chloride-sulphate acid method. Available N was determined by the alkaline permanganate method alkali N-proliferation method, available P was determined by molybdenum blue colorimetric method and available K by the flame photometry method (Bao, 2007). Soil samples from the two fields were collected from 0–20 cm soil depth with soil auger (50 mm in diameter) within the root zone. The samples were put into ziplock bags and stored at -20°C temperature condition. Extracts were prepared by shaking 1:5 soil-water mixture at a speed of 150 r min^{-1} in 60 min on a large intelligent constant temperature shaking table, and the soil extract was filtered (70 mm in diameter). The vermiculite-perlite medium was fed with the extracts according to the treatments at 7-days interval.

Collection of medium samples for analysis

Samples of the medium in each pot were collected from within the root zone with hand trowel during tuber formation, tuber growth and at tuber maturity stages in July, August and September respectively. The samples were put into separate ziplock bags and taken to the laboratory in an ice box. Part of the samples were immediately used to determine soil microorganism, and the other part was air-dried, ground and passed through a 2-mm mesh and kept at room temperature for determination of enzyme activities.

Table 1. Chemical properties of experiment soils from legume and potato fields.

Experiment soil	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	pH	EC (mS m^{-1})	CEC (cmol kg^{-1})
Legume field	9.78	15.11	41.44	7.92	147.43	204.14
Potato field	11.78	13.71	142.42	7.9	186.13	211.02

Enzyme activity determination

Enzyme activities were determined as described by Zhou (1987). The activity of invertase was measured through 3, 5-dinitrosalicylic acid colorimetry using sucrose as a substrate and it was expressed in mg glucose g^{-1} dry soil. The activity of urease was assayed through sodium hypochlorite colorimetry using urea as a substrate and it was expressed in mg NH_3-N g^{-1} dry soil. The activity of alkaline phosphatase was measured through disodium phenyl phosphate colorimetry using p-nitrophenyl phosphate as a substrate and it was expressed in mg phenol g^{-1} dry soil. Catalase was assayed using titration method and it was expressed in consumptive mL $KMnO_4$ g^{-1} dry soil.

Soil micro-organisms determination

Spread plate method is used for quantification of bacteria, fungi, actinomycetes and aerobic azotobacter under sterile conditions, besides beef extract peptone agar medium (pH: 7.0–7.2), Rose-Bengal agar medium, actinomycetes culture medium, and Ashby nitrogen-free agar medium were collected to cultivate these organisms respectively (Xu, 1986).

Anaerobic azotobacter, nitrifying and denitrifying bacteria were detected according to most probable number (MPN) method. The corn meal medium, improved Stephen culture medium and defined medium were used to culture them (Lin, 2010).

Statistical analysis

The Origin 8.5 software was used to process data and SPSS 19.0 statistical software was used for analysis of variance (ANOVA), and the Duncan Multiple Range Test was used for separation of means at $P < 0.05$.

Results

Soil enzyme activity

In this study, enzyme activities varied widely between

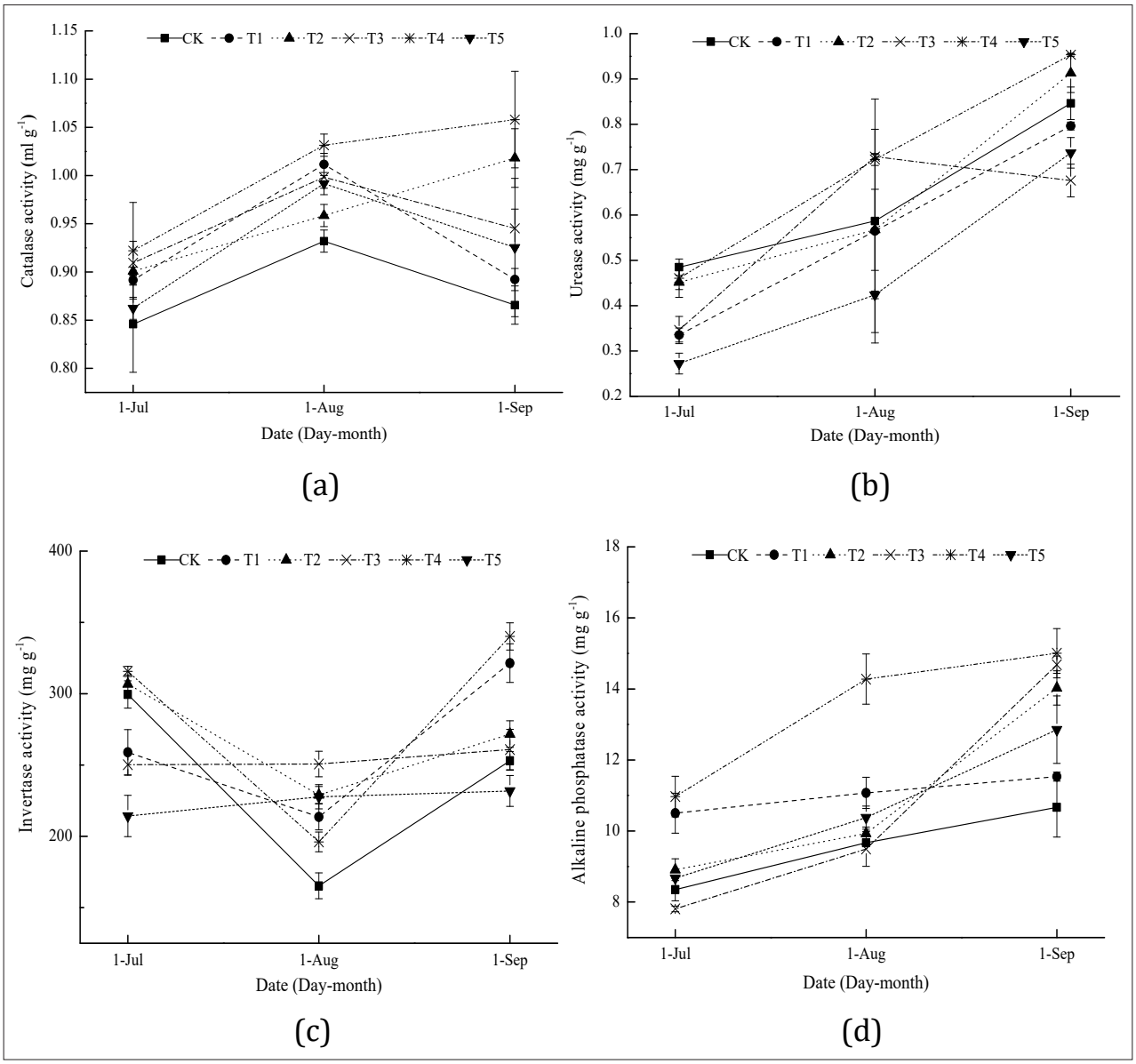


Figure 1. Effects of the leguminous soil extract on soil enzyme activities of (a) catalase, (b) urease, (c) invertase, and (d) alkaline phosphatase in soil samples collected on 1 July, 1 Aug. and 1 Sep. CK is irrigated with sole soil extract of potato continuous cropping field; T1, T2, T3, T4, T5 were irrigated with potato soil extract mixed with legumes soil extract at the ratio of 1:1, 1:2, 1:3, 1:4, 1:5, respectively.

treatments and growth stages (Figure 1). Catalase activity was higher in the treatments which had a mixture of soil extracts than in the extract from sole potato continuous cropping field. Catalase activity consistently increased throughout the growth period of potato in T2 and T4. The T4 medium had the greatest catalase activity which was 8%, 11% and 22% more than the CK at tuber formation, tuber growth and tuber maturity stages respectively (Figure 1a). Urease activity also was increased with time in T1, T2, T4, and T5. In addition, urease activities were highest in T3 and T4 at the tuber growth stage and these were 24% and 22% more than the CK. Urease activities in the other treatments were, however, lower than the CK (Figure 1b). Invertase activity declined drastically in T1, T2, and T4 at the tuber growth stage but showed a flattened trend in T3 and T5 throughout the growth stages. Besides, invertase activity was significantly higher in all the treatments compared to the CK. Application of T1 and T4 had beneficial effect on invertase activity compared to CK at the tuber maturity stage, with an increase of 34% more in T4 relative to CK (Figure 1c). Alkaline phosphatase activity of each treatment also increased to different extent at different growth stages. In particular, alkaline phosphatase activity in T4 increased rapidly at the tuber formation and tuber growth stages compared with all the other treatments (Figure 1d). Nevertheless, the activity of alkaline phosphatase in T2, T3, and T5 had a higher trend in at the tuber growth and maturity stages.

Soil microbial quantity

1. Bacteria, fungi and actinomycetes. The quantity of soil bacteria, fungi and actinomycetes were affected significantly among different ratios of soil extracts compared with CK (Figure 2). The differences in the quantity of soil bacteria, fungi and actinomycetes also varied at the different growth stages. The population of bacteria, fungi and actinomycetes increased from the tuber formation stage but decreased towards the tuber maturity stage. Bacteria quantity was higher in T4 than in all the other treatments at the tuber formation

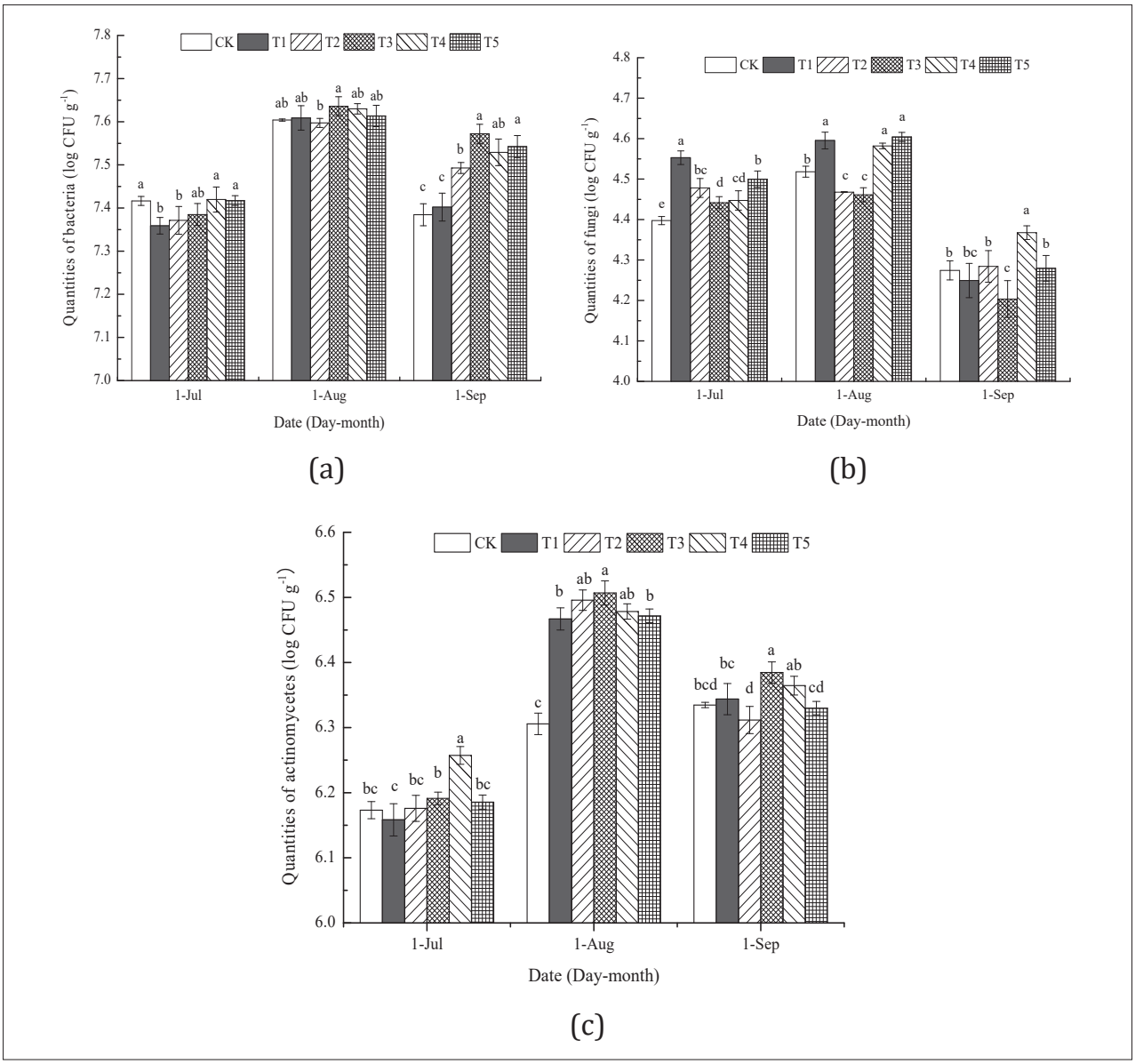


Figure 2. Total soil microbial populations of (a) bacteria, (b) fungi, and (c) actinomycetes in soil samples collected on 1 July, 1 Aug. and 1 Sep. CK is irrigated with sole soil extract of potato continuous cropping field; T1, T2, T3, T4, T5 were irrigated with potato soil extract mixed with legumes soil extract at the ratio of 1:1, 1:2, 1:3, 1:4, 1:5 respectively. Data in a column followed by the same letter are not significantly different at $P = 0.05$.

stage but from the tuber growth stage towards the maturity stage, soil bacteria quantity was greatest in T3 (Figure 2a). T4 and T5 maintained the highest fungi quantities at the tuber growth and maturity stages (Figure 2b). Actinomycetes quantity was highest in T4 at the tuber formation stage and it increased slightly by 1% compared with CK. In addition, actinomycetes quantity increased in T3 at the tuber growth and maturity stage, resulting in an increase of 3% and 1% respectively (Figure 2c).

2. Physiological function groups of nitrogen. Aerobic azotobacter quantity in T3 was 1%, 2% and 2% more than the CK at the tuber formation, tuber growth and tuber maturity stages respectively (Figure 3a). In addition, the quantity of anaerobic azotobacter in T4 was higher than the CK at all the growth stages (Figure 3b). The specific dynamic changes of aerobic azotobacter and anaerobic azotobacter differed among the growth stages and in treatments. In contrast with aerobic azotobacter, anaerobic azotobacter quantity did not differ among treatments ($P<0.05$) at the tuber formation stage. At the tuber growth stage, aerobic azotobacter benefitted from T1, T3, T4, and T5 (Figure 3a), but for anaerobic azotobacter, T3, T4, and T5 increased its quantity (Figure 3b). From the tuber growth to maturity stage, the average aerobic azotobacter and anaerobic azotobacter quantity decreased by 2% each. The quantity of anaerobic azotobacter improved in T4 compared to aerobic azotobacter.

The quantity of nitrifying bacteria decreased at the tuber growth stage but increased at the tuber maturity stage (Figure 3c). However, the quantity of denitrifying bacteria was highest at the tuber growth stage. T4 had the greatest stimulating effect on the quantity of nitrifying and denitrifying bacteria and it maintained their quantities high throughout the growth period of the potato (Figure 3c, d).

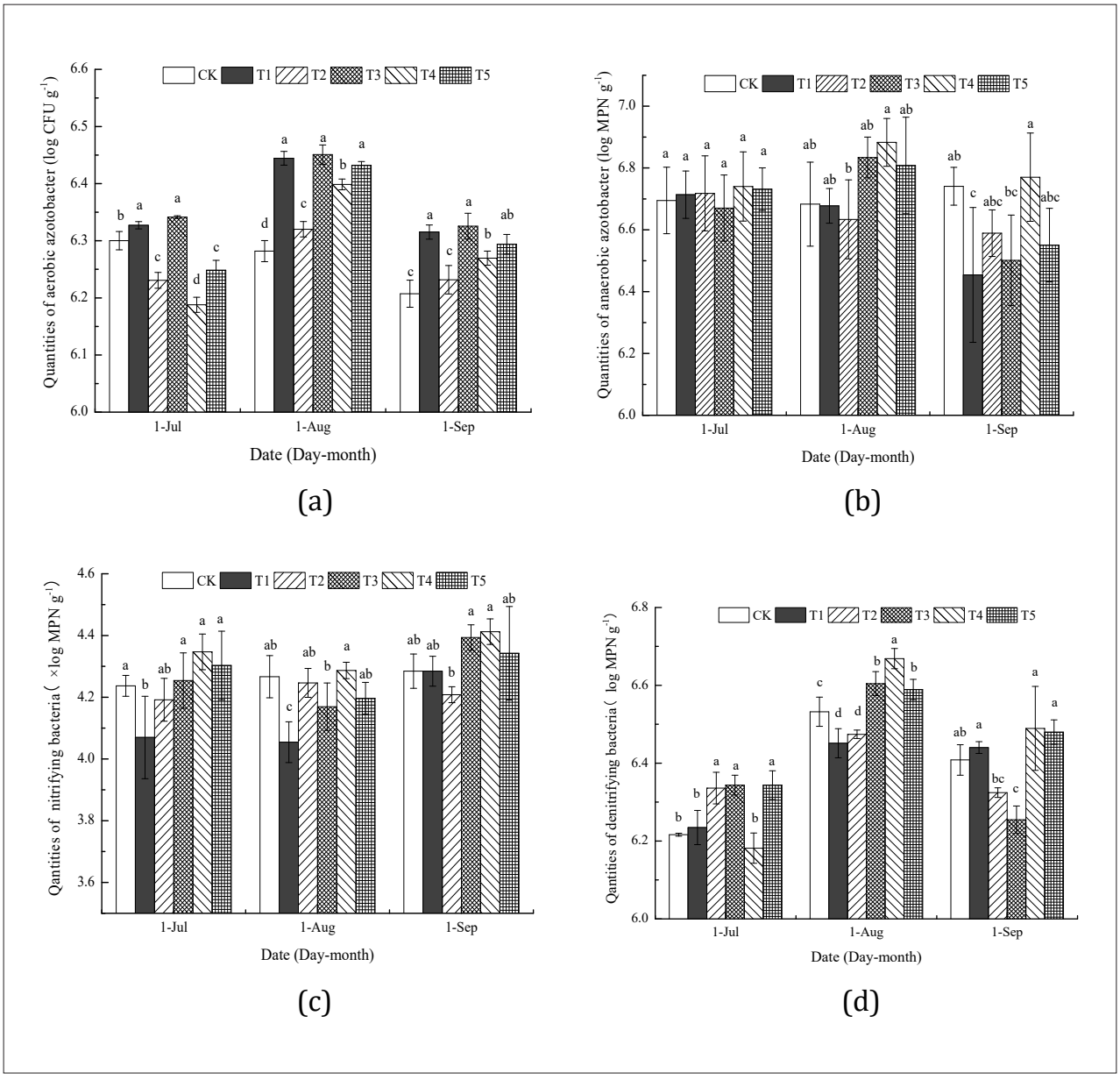


Figure 3. Total soil microbial populations of (a) aerobic azotobacter, (b) anaerobic azotobacter, (c) nitrifying bacteria, and (d) denitrifying bacteria in soil samples collected on 1 July, 1 Aug. and 1 Sep. CK is irrigated with sole soil extract of potato continuous cropping field; T1, T2, T3, T4, T5 were irrigated with potato soil extract mixed with legumes soil extract at the ratio of 1:1, 1:2, 1:3, 1:4, 1:5 respectively. Data in a column followed by the same letter are not significantly different at $P=0.05$.

Discussion

Soil enzyme activities are life indexes of soil quality that are closely connected with soil biota, quantity and biodiversity to promote metabolic processes (Taylor et al., 2002; Glenn and Gold, 1985). The soil extracts from potato continuous cropping field and legumes in different proportions had a positive effect on catalase activity in potato matrix, and this was in agreement with findings of Gao et al. (2014), who reported that catalase reached the highest activity under rotation at flowering period in buckwheat continuous cropping. T4 significantly improved catalase and alkaline phosphatase activity of the potato matrix, and also promoted the invertase and urease activity at potato tuber formation and tuber mature stages. T3 greatly enhanced urease and invertase activity at potato tuber growth period. T3 and T4 improved invertase activity, which showed clearly that the biological activity and quality of soil were improved (Zhang et al., 2004). This indicates that the legume had the potential to ameliorate the adverse effects of continuous cropping of potato. But the trends of the enzyme activity were clearly inconsistent with an earlier research finding which reported that the dynamic changes of alkaline phosphatase enzyme activity were probably the same with the principle of crop growth (Jiang et al., 2009). That may be because high temperature caused the damage of potato leaf and root recession during potato tuber growth period. The change of enzyme activity was therefore inconsistent with regular potato growth and fertilizer requirement. In this study, soil enzyme activities were stimulated in different proportions at different growth stages, which is consistent with previous studies. The authors confirmed that soil enzyme activity of potato significantly improved after rotation (Cao et al., 2013; Qin et al., 2014; Moulin et al., 2015).

Soil microbial quantity is the huge driving force of nutrients source for soil ecosystem which is one of the most sensitive indicators for soil biological activity. Under the conditions of this study, our results showed that different ratios of soil extracts can increase the quantity of soil microbial. The result is in agreement with conclusions of Li et al. (2015), who considered that long-term continuous cropping of cotton decreased the activities of soil microbial diversity in oasis farmland, while crop rotation alleviated the negative influence. T4 significantly increased the quantity of actinomycetes at the tuber formation stage, T3 significantly promoted bacteria and actinomycetes quantity, while markedly inhibited the quantity of fungi within the root zone throughout the growth period. This indicates that leguminous soil extracts have the potential to significantly increase the quantity of bacteria and actinomycetes in potato's matrix, and significantly reduce the number of fungi. The result may be ascribed to the appropriate ratio (T3 and T4) of soil extracts, which stimulated the soil microorganism, and the two soil extracts were effective to ameliorate soil fatigue partly by the activity of microorganism. Soil microbial quantity could be change at different growth stages and nutritional status in same crop (Zantua and Bremner, 1975). In the present study, soil microbial quantity showed change pattern; it increased initially and then dropped at potato tuber mature stage. That is, the quantity of microorganisms was greater at the tuber growth stage because the potato grew rapidly from the tuber formation stage. The rapid growth of potato during this period caused fast transformation of soil nutrient, resulting in increased quantity of soil microbial quantity. However, from the tuber growth stage to the maturity stage, the potato plants grew slowly and slow soil metabolism leads to a decrease in the number of soil microbes. The results were consistent with earlier reports which showed that total number of soil microorganism firstly increased and then decreased at different growth stages, and different grass-crop rotation patterns of soil microbial quantity showed differences during the same period. These suggest that crop rotation changes the environmental factors of microbes in comparison with continuous cropping (Hu et al., 2014). Our study demonstrated that the alfalfa soil extract is conducive to activate soil enzyme activity, bacteria and actinomycetes, while inhibiting fungi. It thus confirms that legumes in rotation could improve the quantities of beneficial soil microbes for the benefit of potato crop.

Aerobic azotobacter and anaerobic azotobacter are mainly involved in the nitrogen cycle (Wang, 2012). In this study, aerobic azotobacter increased initially and then decreased towards the maturity stage of the potato plants. The number of aerobic azotobacter was higher than the control at tuber growth and tuber maturity stages. The findings of this study showed that soil extract from the alfalfa field increased the number of aerobic azotobacter in potato soil. For anaerobic azotobacter, the changes were inconsistent under tuber maturity stage, anaerobic azotobacter quantity was lower than the control with the application of T1, T2, T3, and T5. This was probably because anaerobic azotobacter required strict anaerobic environment in the growth process. In addition, it is inevitable to avoid air in the process of sampling and vaccination. Nitrifying bacteria in soil plays an important role in the conversion of NH_4^+-N to NO_3^--N, and the number of nitrifying bacteria reflects the soil's ability in supplying nitrogen to plants. Denitrification is a process that reduces nitrate and releases N_2 and N_2O under anoxic conditions and the changes of denitrifying bacteria quantity may reflect the intensity of denitrification on suitable conditions. In this research, the number of nitrifying bacteria at the potato tuber maturity stage was higher than at potato tuber growth period. Nitrifying bacteria are extremely sensitive to changes in soil temperature, and the increase of temperature will stimulate the growth of nitrifying bacteria in soil within a certain temperature range. However, temperatures exceeding this range may lead to acceleration of soil organic matter decomposition and inadequate soil oxygen supply resulting in increased inhibition of nitrifying bacteria quantity in soil. The suitable living temperature of soil nitrifying bacteria is 28°C (Zhang et al., 2002). In our study, bacteria quantity decreased at the potato tuber growth stage, possibly because the temperature in the greenhouse exceeded 30°C from July to August, which resulted in the inhibition of nitrifying bacteria. However, the lower temperature observed in September was suitable for the growth of nitrifying bacteria. As a result, nitrifying bacteria quantity at the potato tuber maturity stage was higher than at tuber growth stage. The number of denitrifying bacteria in the potato growing period showed an increasing trend after the initial decrease. In this study, soil extract played a significant role in promoting the growth of denitrifying bacteria under T4. The contrasting results of nitrifying bacteria and denitrifying bacteria may be due to a wide temperature range of 5–65°C for denitrification, as such numbers of denitrifying bacteria were not affected during high temperatures in summer.

The results of our experiment have demonstrated that a potato-legume (alfalfa) rotation system could improve soil quality through the enhancement of enzyme activities and increase the quantities of beneficial soil microbes. Further research into the mechanism behind the effect of soil ex-

tracts on soil amelioration in potato fields is needed, particularly with regard to microbial diversity under potato-alfalfa rotation system.

Acknowledgments

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