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Influence of Alfalfa (*Medicago sativa* L.) at Various Growing Years on the Physico-chemical Properties and Microbiology of Irrigated Desert Soils

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ABSTRACT

Background: The arid area of northwest China is the main producing area of alfalfa (*Medicago sativa* L.) characterized by low rainfall, low vegetation coverage and vulnerable ecosystem. However, there are still very few reports about the influence of alfalfa at various growing years on the physico-chemical properties and microbial community structure in typical northwestern irrigated desert soil. This study has important theoretical and practical guiding significance for providing reference and scientific evidence for local alfalfa industry development and protection of soil ecosystem to maintain efficient alfalfa production.

Methods: The rhizosphere soils of "Sanditi" alfalfa and Longdong" alfalfa were collected to assess the influence of alfalfa varieties, growing years and growth stages on the physico-chemical properties, microbial biomass and microbial community structure. There were four treatments in this experiment: 1. "Sanditi" alfalfa planted in 2015 (S1); 2. "Sanditi" alfalfa planted in 2016 (S2); 3. "Sanditi" alfalfa planted in 2017 (S3); 4. "Longdong" alfalfa planted in 2017 (LD).

Result: Soil of all treatments had consistent pH at 8.11-8.45. The average soil organic matter content was 11.02 g. kg^{-1}, 11.43 g. kg^{-1}, 11.26 g. kg^{-1} and 11.20 g. kg^{-1} respectively for LD, S1, S2 and S3 (P>0.05). The total nitrogen content was 0.451 g. kg^{-1}, 0.468 g. kg^{-1}, 0.437 g. kg^{-1} and 0.428 g. kg^{-1}, respectively for growing seasons of LD, S1, S2 and S3 (P>0.05). The results suggested that alfalfa cropping did not significantly influence the soil organic matter content in 3 years. S1 significantly changed the ratio of fungal to bacterial typical fatty acids, which was significantly higher than S3 and LD, but insignificantly higher than S2. Continuous cropping of alfalfa in arid area affected the diversity of the fungi community and reduced soil bacterial community richness and diversity.

Key words: Alfalfa, Irrigated desert soil, Microbial community structure, Soil nutrients.

INTRODUCTION

Alfalfa (*Medicago sativa* L.), so-called "King of forages", has played a crucial role in animal husbandry and socio-economic development in western China with its wide adaptability, strong resistance and high feeding and economic value (Wang *et al* . 2021). The demand of alfalfa forage has been gradually increasing with the enlarged lack of high-quality forage. The current solution carried out by planting companies and farmers to improve yield is to significantly increase fertilization and irrigation (Albayrak *et al.* 2018). However, this ultimately resulted in reduced soil fertility, environmental contamination and destruction of soil ecosystem (Avci *et al.* 2017). The arid region of Northwest China is the main producing area of alfalfa hay which had obvious geographical characteristics such as drought, low vegetation coverage and fragile ecosystem (Zhang *et al.* 2021). In this case, the improper operation during land use is likely to result in saline-alkali land and inhibition of microbial activity in soil which further leads to nutritional deficit and soil degradation (Zhao *et al.* 2019).

The improvement of soil with planting alfalfa has been well studied worldwide, however, there are controversial academic opinions on the effects of alfalfa plantation on soil physico-chemical properties, fertility and microbiology. Many studies have shown that rhizobium and well-developed fibrous roots of alfalfa can effectively increase organic matter content in soil, improve soil pellets structure, and significantly enhance soil fertility as a result of nitrogen fixation (Herbert and Li 2002). Whilst other research suggested that long term plantation of alfalfa significantly reduces the soil fertility level due to nutrient removal by massive mowing and harvesting (Dong, 2008).

Therefore, systematic diagnosis on nutrient status of alfalfa plantation soil is important to reveal the influencing factors on soil nutrients. This study investigated the effects

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of alfalfa at various growing years on the physico-chemical properties and microbial community structure in typical northwestern irrigated desert soil, aiming to provide reference and scientific evidence for local alfalfa industry development and protection of soil ecosystem to maintain efficient alfalfa production while increasing soil fertility in the meantime.

MATERIALS AND METHODS

The experiment was conducted during March to October 2017 at High-quality forage farm in Xiazhongqi Village, Wunan Town, Liangzhou District, Wuwei City, Gansu Province. The soil in the test site is irrigated desert soil widely distributed in Hexi District. Multi-point sampling method was used to collect 500 g of plough layer (0~20 cm) mixed soil samples. The soil samples were subsequently well mixed, air dried and passed through 2 mm sieve. Soil routine analysis was performed on the samples using systematic approach for soil nutrient status evaluation. The test results showed that the control soil sample in the demonstration base had a pH of 8.0 and organic matter content of 0.98 g/kg.

"Longdong" and "Sanditi" alfalfa were used in this study. Unprocessed "Longdong" alfalfa seeds and coated "Sanditi" Alfalfa seeds were purchased from Beijing Bailv Seed Industry Co. Ltd. There were four treatments in this experiment: 1. "Sanditi" alfalfa planted in 2015 (S1); 2. "Sanditi" alfalfa planted in 2016 (S2); 3. "Sanditi" alfalfa planted in 2017 (S3); 4. "Longdong" alfalfa planted in 2017 (LD). The experiment groups were randomized and three replicates were conducted for each treatment with an experiment area of 150 m^2. Drill seeding was applied with seeding rate of 22.5 kg/hm^2.

Soil samples were collected on 1st May, 5th June, 15th July, 20th August, 15th September and 11th November 2017 based on the growth stages of local alfalfa, which were analyzed to evaluate the effects of alfalfa growing seasons on the physico-chemical properties and microbial community structure of Wuwei irrigated desert soil. According to diagonal five-point sampling method (Ren, 2000), soil drill was used to collect samples in 0-10 cm and 10-30 cm at each sampling point and mixed with quarter method. Soil samples for alfalfa rhizosphere microbial test were collected by removing the clods far away from alfalfa root system which was completely dug out and gently shaking to obtain the soils within 0-5 cm of the root system. The samples were stored in a bag at 4°C for phospholipid fatty acid test as soon as possible.

The physico-chemical properties of soil samples including pH, organic carbon, organic matter, total nitrogen, soil available phosphorus, soil available potassium were tested by pH meter (Zhen *et al.* 2012), combustion method (Vance *et al.* 1987), potassium dichromate volumetric method (Lauber, 2008), Kjeldahl method (Gurran *et al.* 2000), molybdenum antimony colorimetric method (Zumesteg and Pazrin 2012) and flame photometry respectively.

Soil microbial biomass and its biodiversity were tested using Phospho Lipid Fatty Acid (PLFA) method and the fatty acids for specific microbes are shown in Table 1 (Yan *et al.* 2006). GCMS (model Varian3800GC and MS2200) was used to test fatty acid methyl ester (FAME) with a column of 30.0 m × 0.25 mm × 0.25 um, injection volume of 1 μL, split ratio of 10:1, carrier gas (helium) flow rate of 1.0 mL/min.Initial temperature was kept at 50°C for 3 min and then increased to 260°C at 8°C/min. The samples were then tested by Electrospray Ionization Mass Spectrometry. The peak area was calculated automatically by computer integration with Supelcoe 37 Component FAME Mix as standard (Zhang *et al.* 2004).

RESULTS AND DISCUSSION

Effects of alfalfa at various growing years on soil physico-chemical properties

The physico-chemical properties of rhizosphere soil with various growing years of alfalfa are shown in Fig 1. Soil pH was relatively constant during the whole growing season at 8.11-8.45. However, there was a variance among 4 alfalfa treatment groups in total nitrogen analysis with the highest content of total nitrogen in August for LD soil while in June for the other groups. The organic matter content had a similar trend for all treatment groups reaching the highest in June, and decreased gradually to the lowest in November. There was no significant variance for organic matter content for soils of different alfalfa growing years. The average soil organic matter content was 11.02 g. kg^{-1}, 11.43 g. kg^{-1}, 11.26 g. kg^{-1} and 11.20 g. kg^{-1}respectively for LD, S1, S2 and S3 for the whole growing season. The results suggest that there is no significant influence of planting alfalfa on soil organic matter content in short terms. Fig 1 indicates that soil

Table 1: Effect of alfalfa planted in different years on rhizospheric soil PLFA pattern (Mol per cent of methyl ester).

Treatment	Fungal fatty acid	Fungal fatty acid/ bacterial fatty acid	17:0 cyclopropyl fatty acid	19:0 cyclopropyl fatty acid
LD	1.62c	0.034b	3.64a	7.22b
S1	2.14b	0.048a	2.77b	8.68a
S2	1.71c	0.041b	3.59a	7.36b
S3	2.35a	0.057a	2.65b	8.81a

Note: Analysis of variance used the different rice breed soil for LSD comparison; Different letter indicated the significant difference at P<0.05. 18:2w6, 9c indicated fungal fatty acid; 15:0i+15:0a+16:0i+16:1w5c+17:0i+17:0a+17:0cy+17:0+18:1w7c+19:0cy indicated bacterial fatty acid.

available phosphorus had an identical trend decreasing with the alfalfa growing and harvesting for all treatment groups in the alfalfa growth period. The soil available potassium content was higher in growing seasons than non-growing seasons of alfalfa. Specifically, LD rhizosphere soil had a high soil available potassium content in rejuvenation period and maturity period and low in other stages. However, there was no significant variance of soil available potassium content for all treatments and between different alfalfa types.

Alfalfa plantation did not significantly affect soil organic matter in the irrigated desert soil in arid areas, which likely caused by the short term of alfalfa plantation. Tai *et al.* (2009) suggested long-term plantation of alfalfa reduced soil bulk density and increased organic matter with growing years. Hu *et al.* (2019) found alfalfa plantation in vineyards increased organic matter content after continuously planting alfalfa for a few years. The enhanced accumulation of organic matter and soil nutrients was also reported in Tephrosia candida plantations (Manpoong *et al.* 2020). Wu *et al.* (2021) reported that planting pastures in orchard increased organic matter content but at an insignificant level. In this research we found that alfalfa plantation increased nitrogen content in the soil with insignificance variance between alfalfa types. This is in line with some research results, *e.g* Wang *et al.* (2006) indicated that nitrogen fixed by alfalfa planted in the same year was approximately 35-305 kg/hm^2 which was higher than other crops and grasses. Su *et al.* (2009) found that available phosphorus content significantly dropped after harvesting alfalfa and gradually increased during alfalfa dormant period. Similar trend observed for potassium content in soil. This possibly caused by alfalfa absorption of phosphorus and potassium during growth period resulting in reduced available phosphorus and potassium in soil (Kong, 2020). Growing years do not significantly affect the rhizosphere soil phosphorus content in non-growth period while significantly affect that in growing seasons (Lv *et al.* 2006).

Effect of alfalfa at various growing years on types of soil microorganisms

Fig 2 shows 37 PLFA from C_{12} to C_{20} for rhizosphere soil microorganisms of alfalfa in different growing years. The results suggest that the content of typical fatty acids 14:0i, 15:0a, 16:1w7c, 16:0, 17:0 and 18:1w7c for rhizosphere soil

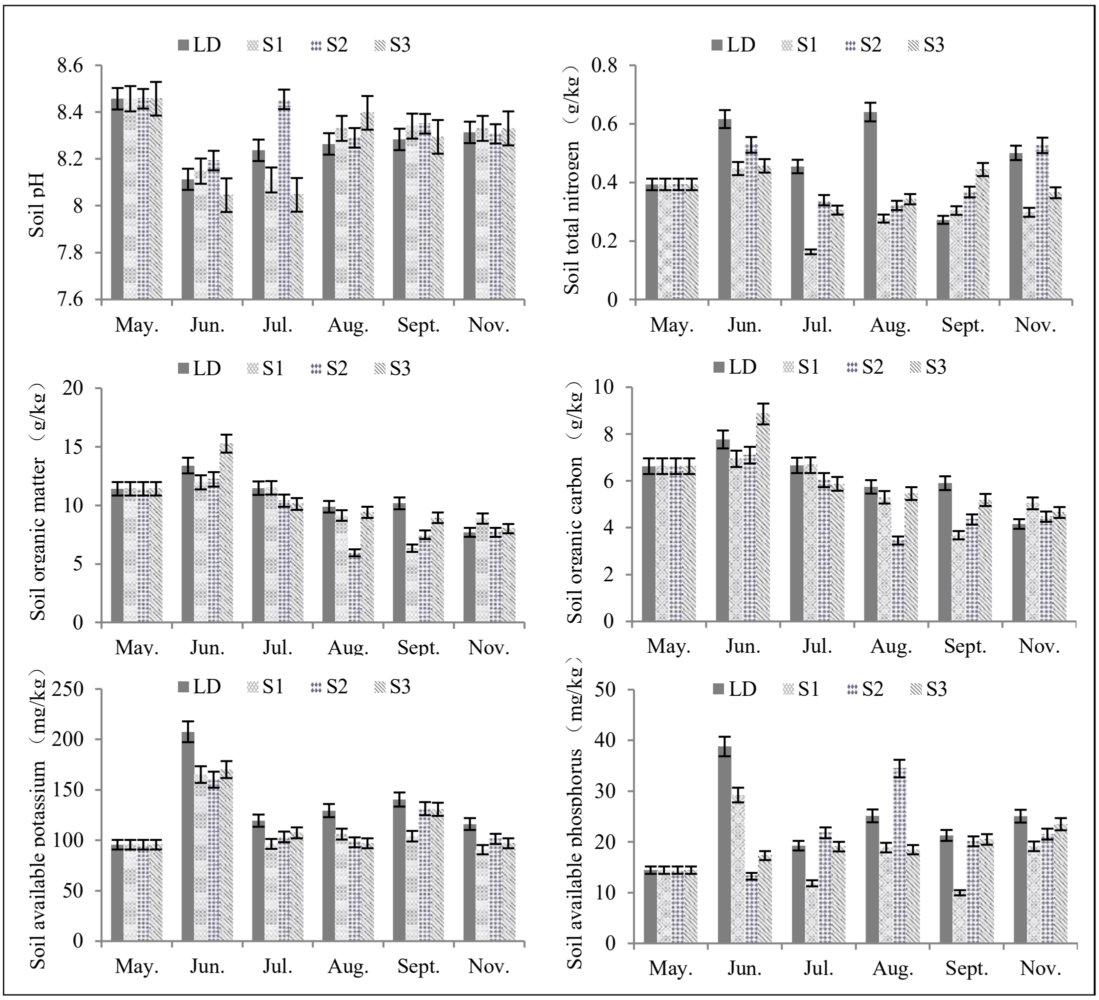

Fig 1: Physicochemical property in rhizospheric soil of different alfalfa during May to November 2017.

microorganisms of “Sanditi” alfalfa in different growing years was higher than that of LD alfalfa while the content of 16:0i,16:0 (10Me), 17:0i, 17:0a, 17:0cy, 16:1 2OH, 17:0 (10Me), 18:2w6,9c, 18:0 (10Me) and 19:1w11c was lower. Table 1 shows the results of cluster analysis for rhizosphere soil microorganisms PLFA in various treatment alfalfa groups. It indicates that the three replicates for different treatments clustered for many distance scales. LD and S1 clustered at distance scale 14 which suggests that there are some similarities in the soil microorganism community structure between the two groups while S2 is similar to S3. Different treatments had identical dynamic trends for amount of bacteria, fungi and actinomycetes with the highest amount observed in July and August and slowly reduced to the lowest

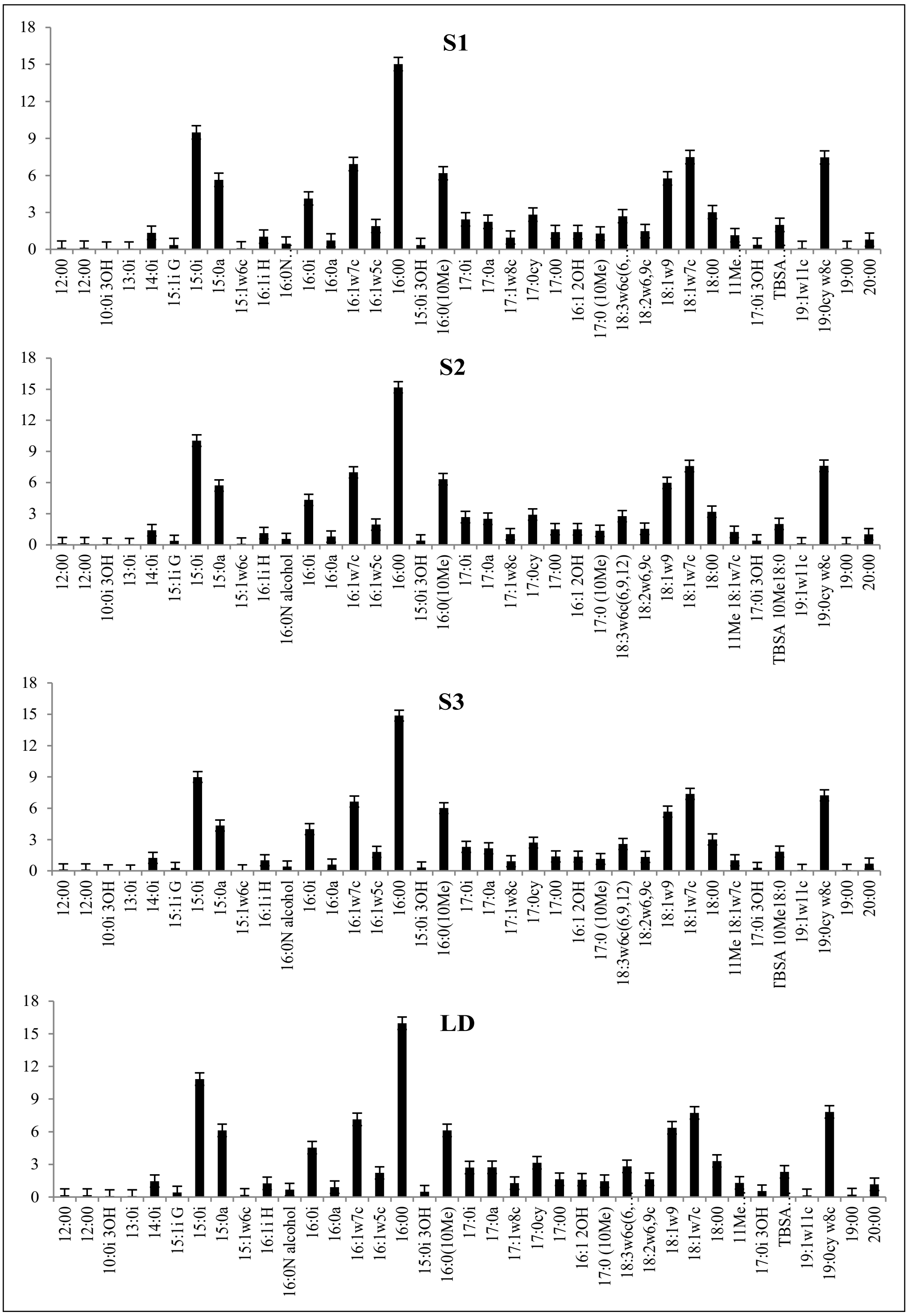

Fig 2: Mol composition of PLFA in paddy soils of Alfalfa at various planting years.

in November (Fig 3, Fig 4 and Fig 5). The total amount of bacteria in soil planted with alfalfa had no significant change until markedly declining in November. In the meanwhile, the effect on amount of fungi and actinomycetes was obvious with both rapidly increased from the lowest in May to the highest in August and then significantly dropped. No significant variance was observed in the amount of fungi and actinomycetes (P>0.05).

Soil microorganisms interact with soil environment and are closely related which depend on carbon resources supplied by plant litter and root exudates and they can be influenced by changes in plant-derived organic matter (Wardle *et al*. 2004). Perennials influence soil microbial community structure and distribution by secreting root exudates, thereby altering microbial richness and diversity (Dennis *et al.* 2010). Geng *et al.* (2020) found continuous cropping of legumes reduced soil microbial diversity levels, reduced the number of soil bacteria and actinomycetes and lead to the transformation of soil from bacterial type to fungal type. Long-term cultivation of alfalfa in the rainfed area of the loess Plateau significantly affected the diversity of the fungi community in the loessial soil, but did not significantly affect the richness and diversity of the soil bacterial community (Zhang *et al*. 2021).

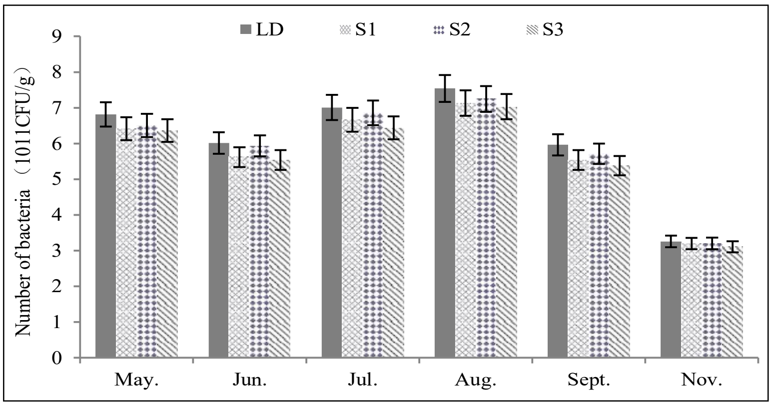

Fig 3: Changes of bacteria number in rhizospheric soil of different alfalfa during May to November 2017.

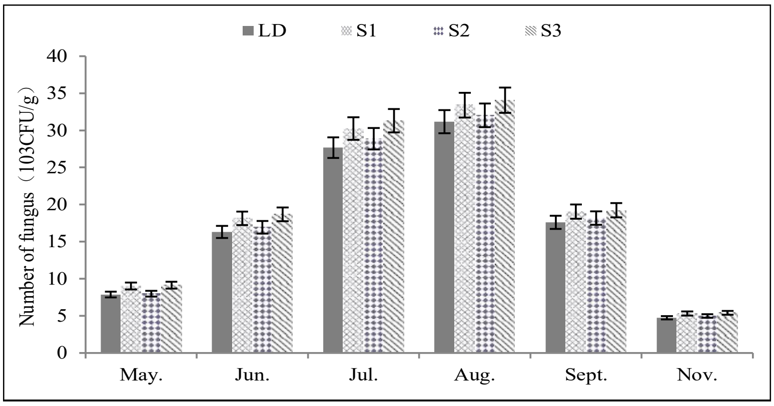

Fig 4: Changes of fungus number in rhizosperic soil of different alfala during May to November 2017.

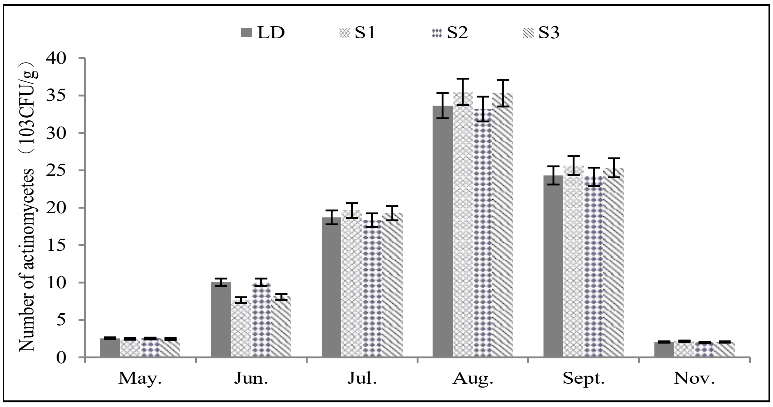

Fig 5: Changes of actinomyce number in rhizospheric soil of different alfalfa during May to November 2017.

Effect of alfalfa at various growing years on soil microbial biomass in various soil layers

It suggests that soil microbial biomass decreased with deepened soil layers with biomass in 0-5 cm significantly higher than that in 15 cm and 30 cm (Fig 6, Fig 7 and Fig 8). In addition, microbial biomass in different soil layers experienced inverted "U" shape change with the growing of alfalfa. Affected by seasons, microbial biomass dropped to the lowest in winter and showed obvious increasing trend from May since temperature increased and alfalfa rejuvenated, finally arriving at the highest in July and August.

Huge microbial difference in and outside rhizosphere soil is due to the suitable growing conditions in rhizosphere soil (Pietri and Brookes 2008). Microbial biomass is often affected by soil temperature, humidity, and nutrients. Meanwhile, alfalfa showed a significant effect on microbial biomass (Zhao *et al*. 2020). There are a few possible mechanisms: 1) Alfalfa root secretions and shedding provide sufficient nutrients for microbial propagation (Su *et al.* 2021). 2) Root secreted organic acids during alfalfa growing promotes mineral dissolve and breakdown for microbial growth and propagation (Li *et al*. 2018). 3) Alfalfa growth improves soil environment including loosing soil, improve

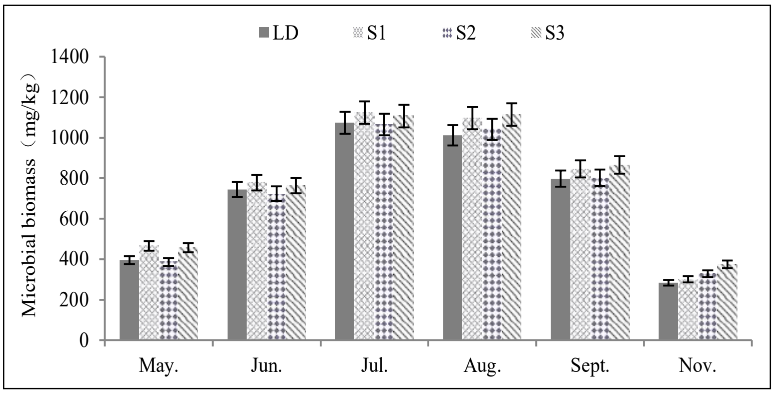

Fig 6: Changes of microbial biomass in 0-5 cm deep soil of different alfalfa during May to November 2017.

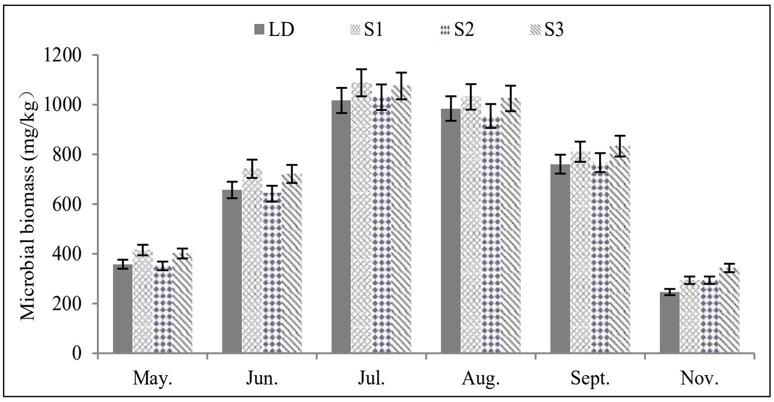

Fig 7: Changes of microbial biomass in 15 cm deep soil of different alfalfa during May to November 2017.

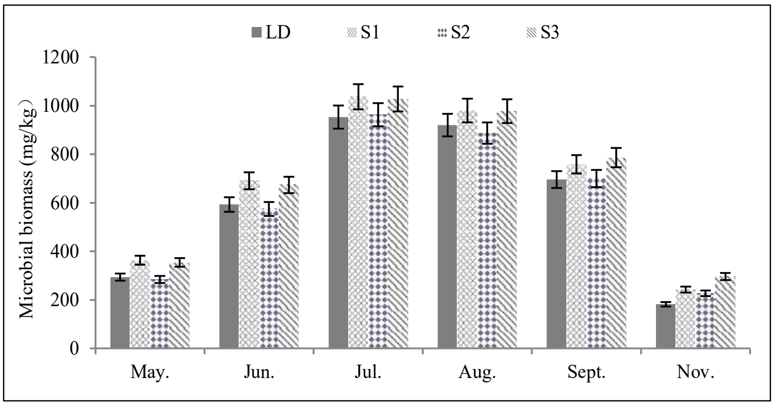

Fig 8: Changes of microbial biomass in 30 cm deep soil of different alfalfa during May to November 2017.

soil pellets structure and improve water retention by adsorption for microbial growth (Wang *et al.* 2020).

CONCLUSION

Alfalfa planted in different years did not significantly influence the soil organic matter and soil nutrients content in 3 years. Nutrients such as soil available phosphorus and available potassium decreased to varying degrees with the massive harvest of alfalfa. Continuous cropping of alfalfa in arid area affected the diversity of the fungi community and reduced soil bacterial community richness and diversity. The seasonal variation of soil microbial biomass and quantity was obvious, but the difference between different planting years was not significant. The microbial composition had no significant difference for all groups. The microbial biomass decreased with the increase in soil depth. The microbial biomass in surface layer (0-5 cm) was significantly higher than that in 15 cm and 30 cm.

Conflict of interest: None.

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