Acta Ecologica Sinica 37 (2017) 10–17

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

Effect of stand age on soil microbial community structure in wolfberry (*Lycium barbarum* L.) fields

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ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form 29 October 2015
Accepted 3 December 2015

Keywords:
Lycium barbarum L.
Stand age
Soil physicochemistry
Phospholipid fatty acid
Microbial community structure

ABSTRACT

Soil physicochemical properties and microbes are essential in terrestrial ecosystems through their role in cycling mineral compounds and decomposing organic matter. This study examined the effect of stand age on soil physicochemical properties and microbial community structure in wolfberry (*Lycium barbarum* L.) fields, in order to reveal the mechanism of soil degradation due to long-term stand of *L. barbarum*. The objective of the study was achieved by phospholipid fatty acid (PLFA) biomarker analysis of soil samples from *L. barbarum* fields in Zhongning County, Ningxia Province—the origin of *L. barbarum*. Five stand ages of *L. barbarum* were selected, <1, 3, 6, 9, and 12 years (three plots each). The results showed that soil bulk density increased slightly with increasing stand age, while no clear trend was observed in soil pH or total salinity. As the stand age increased, soil organic matter and nutrients first increased before decreasing, with the highest levels being found in year 9. There was an amazing variety of PLFA biomarkers in soil samples at different stand ages. The average concentrations of total, bacterial, fungal, and actinomycete PLFAs in the surface soil initially decreased and then increased, before decreasing with the stand age in summer. The PLFA concentrations of major microbial groups were highest in year 9, with the total PLFA concentrations being 32.97% and 10.67% higher than those in years <1 and 12, respectively. Higher microbial PLFA concentrations were detected in summer relative to autumn and in the surface relative to the subsurface soil. The highest ratios of Gram-positive to Gram-negative bacterial (G^-/G^+) and fungal to bacterial (F/B) PLFAs were obtained in year 6, on average 76.09% higher than those at the other four stand ages. The soil environment was most stable in year 6, with no differences between other stand ages. Therefore, soil microbial community structure was strongly influenced by the stand age in year 6 only. The effect of stand age on soil G^-/G^+ and microbial community structure varied with season and depth; there was little effect for F/B in the 20–40 cm soil layer. Principal component analysis revealed no correlations between microbial PLFA concentrations and total salinity in the soil; negative correlations were noted between soil pH and F/B in summer ($P < 0.01$), as well as between soil pH and fungal PLFA in autumn ($P < 0.05$). Moreover, microbial PLFA concentrations were correlated with soil organic matter (mean $R = 0.7725$), total nitrogen (mean $R = 0.8296$), total phosphorus (mean $R = 0.8175$), available nitrogen (mean $R = 0.7458$), and available phosphorus (mean $R = 0.7795$) ($P < 0.01$). On the whole, the soil ecosystem was most stable in year 6, while soil organic matter, nutrients, and microbial PLFA concentrations were maximal in year 9; thereafter, soil fertility indices and microbial concentrations decreased and soil quality declined gradually as the stand age increased. Therefore, farmers should reduce the application rate of fertilizers, especially compound or mixed fertilizers, in *L. barbarum* fields; organic or bacterial manure can be applied increasingly to improve the soil environment and prolong the economic life of *L. barbarum*.

1. Introduction

Long-term tree stand can result in changes in soil physicochemical properties and microbial characteristics [1]. Soil organic matter, alkali-hydrolyzable nitrogen (N), available phosphorus (P), available potassium (K), and enzyme activities tend to increase before decreasing as the tree age increases [2–4]. However, Fu and Huang [3] have pointed out that the microbial community diversity decreases with an increase in stand age, while the soil quality and certain key activities decline substantially in the rhizosphere soil of Nanfeng Tangerine (*Citrus reticulata* Blanco). With increasing tree age, soil bacterial and fungal populations and microbial biomass carbon (C) initially decrease and then increase, while microbial biomass N constantly decreases in pure and mixed stands of Masson's pine (*Pinus massoniana*) and camphor tree (*Cinnamomum camphora*), as well as in *Sonneratia* forests [1,4]. As the

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http://dx.doi.org/10.1016/j.chnaes.2016.12.003
1872-2032/

stand age of kiwi increases, soil bacterial population, microbial biomass C, and microbial biomass N initially increase before decreasing, while soil fungal population increase constantly [5]. Moreover, Bauhus et al. [6] found that soil microbial C and N, microbial C to organic C ratio, microbial N to total N ratio, and the proportion of fungi increase with tree age in trembling aspen and paper birch stands aged 50–124 years.

The above findings indicate that soil physicochemical properties and microbial characteristics change in different trends with increasing age of various tree species. Different conclusions have been drawn even from the same tree species. A few studies have indicated that as the age of tea tree increases, there are decreases in soil bacterial and fungal populations and microbial functional diversity index, with little change in soil actinomycetes [7–8]. However, other researchers have reported that soil total organic C, active organic C, and microbial biomass C contents first increase and then decrease, while soil fungal population increases constantly with increasing stand age of tea trees [9]. In addition to tree species, soil physicochemical properties and microbial characteristics are influenced by other factors including climate, season, and soil type [10]. Therefore, it is crucial to study soil properties at different stand ages of key tree species in specific areas, in order to reveal the trend of soil quality.

Wolfberry (*Lycium barbarum* L.) is a perennial deciduous shrub of the genus *Lycium* in the family Solanaceae. Modern medicine, nutrition and pharmacology studies have found that the fruits, leaves, and roots of *L. barbarum* contain polysaccharides, amino acids, vitamins, and trace elements required by the human body, with high medicinal and nutritional value [11]. Owing to its geographical and climatic conditions, Ningxia, an autonomous region of the People's Republic of China, has been the origin and optimal habitat of *L. barbarum*. The government of the Ningxia Hui Autonomous Region has included *L. barbarum* as a pillar industry. The yield of *L. barbarum* can be sustained for 30 to 40 years under natural conditions. However, there have been growing problems with increased excessive fertilization, frequent picking, and pesticide spraying over the recent years, which lead to a marked decline in the yield and quality of *L. barbarum* after the stand age of ~10 years. This situation has forced the farmers to cut down the *L. barbarum* shrubs and thus caused huge economic losses.

Research on *L. barbarum* has mainly focused on medicinal ingredients [12–13] and fruit quality [14]. Thus far, few studies have investigated soil physicochemical properties and microbial characteristics at different stand ages of *L. barbarum* [15]. Phospholipid fatty acids (PLFA) analysis can provide information on microbial biomass, physiological stress, flora structure and diversity. The PLFA technique has been extensively used to study soil microbial community diversity [16–17]. In the present study, PLFA analysis was used to examine the effect of stand age on soil physicochemical properties and microbial community structure in *L. barbarum* fields. Further, we discussed the mechanism of soil degradation due to long-term stand of *L. barbarum*. This study provides reference data for improving soil quality in *L. barbarum* fields, prolonging the economic life of *L. barbarum*, and promoting the sustainable development of *L. barbarum* industry.

2. Materials and methods

2.1. Study area description

The study area is located in Zhongning County, Zhongwei City, Ningxia Hui Autonomous Region, China (105°15′–106°05′ E, 36°49′–37°47′ N). Zhongning is known as the *L. barbarum* production base county and origin of *L. barbarum* in China. It is also the main producing area of *L. barbarum*. The annual average temperature is 9.5 °C and the annual average precipitation is 202.1 mm. The number of days with an average daily temperature ≥ 10 °C is ~170 days, and the ≥0 °C accumulated temperature is 3200–3300 °C.

L. barbarum field plots at different stand ages (<1, 3, 6, 9, and 12 years) were selected in the Nanqiao Village, Ning'an Town, Zhongning County. Three plots were selected for each stand age (15 plots in total) and the plot area was >667 m^2 each. The major soil type was anthropogenic-alluvial soil and the soil texture was sandy loam [18]. The soil pH was 7.57–8.57 in the 0–20 cm surface soil and 7.90–8.66 in the 20–40 cm subsurface soil; the total salinity was 0.76–1.62 g/kg in the surface soil and 1.02–1.61 g/kg in the subsurface soil.

In the study area, farmers planted *L. barbarum* individually, other than collectively. The plots of different stand ages belonged to different households, and the management measures including fertilization and irrigation were individual behaviors. Therefore, the fertilization rate, weeding, and plowing were not identical. According to our field survey, local farmers performed three to four times of irrigation, once to twice of plowing, four to five times of weeding, and eight to ten times of picking in each year. Chemical fertilizers were applied twice a year on average (mostly in spring and autumn), generally including diammonium, Stanley (N–P_2O_5–K_2O = 18–18–18), compound fertilizer (N–P_2O_5–K_2O = 26–20–21 or N–P_2O_5–K_2O = 26–0–21), mixed fertilizer (N–P_2O_5–K_2O = 20–5–23), and urea and special fertilizer for *L. barbarum* ($N + P_2O_5 + K_2O \geq 12\%$, organic matter ≥ 10%). Organic fertilizers comprised the manure of poultry (mainly chicken) or livestock (mainly sheep and pigs) raised by the households.

Owing to different households and stand ages of *L. barbarum*, the application rates of chemical fertilizers ranged from 3000 to 8400 kg/hm^2, with an average rate of 6327 kg/hm^2. Almost half of the households applied chemical fertilizers only, and the average application rate of organic manure in other plots was 15,000 kg/hm^2. Pesticides were sprayed collectively, eight to ten times a year. The pesticides commonly used were acetamiprid, pyridaben, psylla killer, and chlorpyrifos. For each application, one or multiple pesticides were diluted as required and sprayed to the crown at the rate of 1500 ml/hm^2 each.

2.2. Sample collection

Soil samples were taken in summer (full-fruit stage, July 14) and autumn (leaf-fall stage, October 25). Prior to each sampling, surface litter and leaves were first removed out of the projection range of the crown. A soil auger was used to collect the surface (0–20 cm) and subsurface (20–40 cm) soil samples. Nine points were selected in each plot to collect soil samples from different layers. The samples of the same plot were thoroughly mixed for different layers. Fresh samples were divided into two portions. One portion was stored at low temperature for delivery to the laboratory and then frozen at −20 °C before soil microbial PLFA extraction and analysis. The other portion was delivered to the laboratory and dried in air before the analysis of soil texture, organic matter, nutrients, pH, and total salinity. For bulk density analysis, soil samples were collected near each sample point using a cutting ring.

2.3. Soil analysis

2.3.1. Physicochemical analysis

Soil physicochemical properties were analyzed as follows: bulk density – cutting ring method; texture – pipette method; pH – pH meter measurement; total salinity – mass determination; organic matter – external heating method; total N – Kjeldahl method; available N – alkaline hydrolysis diffusion method; total P – acid dissolution–Mo-Sb colorimetry; available P – Oslen method; total K – NaOH melt–flame photometry; and available K – ammonium acetate extraction–flame photometry [19].

2.3.2. PLFA analysis

Lipid extraction was performed using the modified Bligh–Dyer [20]. Fresh or frozen soil samples (4 g each) were weighed into 23 ml of chloroform–methanol–phosphoric acid buffer and oscillated in the dark for 2 h. The suspensions were then centrifuged at 2500 rpm and the supernatants were transferred into separatory funnels. The extraction was repeated once following the same procedure and the second supernatant