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Postprint: Analysis of Soil Microbial Metabolic Diversity Across Different Years Following Panax notoginseng Harvest

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Abstract

Using Biolog technology, an investigation was conducted on historical Panax notoginseng planting sites in three townships (Panlong, Ameng, and Ganhe) of Yanshan County, Yunnan Province, with soil never planted with Panax notoginseng as the control, to study the effects of different years (1–6 years) after Panax notoginseng harvest on soil nutrients, carbon source utilization by soil microorganisms, and soil microbial diversity. The results showed that the Average Well Color Development (AWCD), which reflects soil microbial activity, did not exhibit a clear trend with increasing years; microbial activity was vigorous after 96 h of incubation. Cluster analysis of absorbance values for 31 carbon sources on Biolog plates indicated that soil microbial carbon source utilization at 1 year and 6 years after Panax notoginseng harvest from the three townships could both be clustered into one group, with similar characteristics of soil microbial carbon source utilization, while clustering of carbon source utilization by soil microorganisms from other different years did not show a consistent pattern. Compared with soil never planted with Panax notoginseng, soil microorganisms at 1 year and 6 years after Panax notoginseng harvest showed 25.97%–55.59% and 53.14%–65.68% higher utilization of carbohydrates, polymers, carboxylic acids, and phenolic carbon sources, respectively. With increasing years after Panax notoginseng harvest, the utilization of carbohydrates, amino acids, and carboxylic acid carbon sources by soil microorganisms showed an increase at 2 years after harvest and a decreasing trend at 4 and 5 years. Compared with soil never planted with Panax notoginseng, soils at 1–6 years after Panax notoginseng harvest showed no significant differences in nitrogen, phosphorus, potassium, and organic matter contents. Except for Ganhe Township, the mean values of Shannon-Wiener index, richness index, and Simpson index of soil microorganisms at 1–6 years after Panax notoginseng harvest in Ameng and Panlong townships showed no significant overall differences compared with those of soil never planted with Panax notoginseng. The experiment indicates that Panax notoginseng rotation requires more than 6 years, and the preferential utilization of different carbon sources by soil microorganisms can reflect that the obstacle to continuous cropping of Panax notoginseng is closely related to differences in soil microbial community structure.

Full Text

Abstract

Continuous cropping obstacle represents a critical limiting factor in *Panax notoginseng* cultivation, yet the underlying mechanisms, particularly regarding changes in soil microorganisms under natural conditions, remain unclear. This study investigated soil nutrients and microbial community structure in fields after *P. notoginseng* harvest across three towns (Ganhe, A' meng, and Panlong) in Wenshan City, Yunnan Province, using Biolog-ECO technology to analyze average well color development (AWCD) and microbial community diversity. In each town, seven fields were selected as sampling sites: six fields at 1–6 years post-harvest and one control field never planted with *P. notoginseng*.

Results showed that AWCD values did not exhibit a consistent pattern with increasing years after harvest, though microbial activity peaked after 96 hours of incubation. Cluster analysis of 31 carbon sources revealed that soils at 1 and 6 years post-harvest grouped together across all three towns, indicating similar carbon utilization characteristics, while other years showed no clear pattern. Compared with control soils, microbial utilization of carbohydrates, polymers, carboxylic acids, and phenols was 25.97%–55.59% higher at 1 year and 53.14%–65.68% higher at 6 years post-harvest. With increasing time after harvest, utilization of carbohydrates, amino acids, and carboxylic acids increased at year 2 but declined at years 4 and 5. No significant differences were observed in nitrogen, phosphorus, potassium, or organic matter content between soils at 1–6 years post-harvest and control soils. Except for Ganhe Town, Shannon-Wiener, richness, and Simpson indices showed no significant overall differences between post-harvest soils and controls in A' meng and Panlong Towns. These findings suggest that *P. notoginseng* rotation requires at least six years, and that differential utilization of carbon sources by soil microorganisms reflects a close relationship between continuous cropping obstacles and shifts in microbial community structure.

Keywords: *Panax notoginseng*; continuous cropping obstacle; microbial metabolism; Biolog-ECO; functional diversity; diversity index

Introduction

Soil microorganisms constitute a vital component of soil ecosystems and participate in the decomposition of organic matter and nutrient transformation processes [1]. Microbial diversity reflects the structure and function of soil ecosystems, particularly in terms of microbial carbon source utilization patterns [2]. Research indicates that soil microorganisms are sensitive to changes in tillage and fertilization practices, and their characteristics can serve as biological indicators of soil quality and fertility [3]. In 1991, Garland and Mills [4] first applied the Biolog method to study soil microbial communities. This approach quantitatively describes functional diversity of microbial communities through sole-carbon-source utilization, enabling evaluation of how different crop cultivation practices affect soil quality [5-6] and has become widely used in soil microecology research.

Continuous cropping obstacle refers to the phenomenon where continuous cultivation of the same or closely related crops leads to reduced yield, increased pest and disease pressure, deteriorating quality, and poor growth even under normal management conditions [7]. *Panax notoginseng* (Burk) F. H. Chen, a valuable traditional Chinese medicinal herb cultivated in Wenshan City, Yunnan Province, is a perennial herbaceous plant [8-9] requiring three or more years from sowing to harvest [10]. Due to its specific growth and cultivation characteristics, *P. notoginseng* has particularly stringent soil requirements, and continuous cropping problems are especially prominent in production, typically addressed through crop rotation or fallow periods of eight or more years [11]. Huang et al. [12] found that total saponin content in *P. notoginseng* increased with rotation duration, and that fields should not be replanted within five years. Using Biolog technology, this study analyzed soil microbial communities in historical *P. notoginseng* cultivation areas in Yanshan County, Yunnan Province, examining changes in soil microorganisms at different intervals after harvest to elucidate temporal patterns in microbial community dynamics and explain potential causes of continuous cropping obstacles.

1. Materials and Methods

1.1 Sampling Sites

To characterize overall changes in *P. notoginseng* cultivation soils in Yanshan County, soil samples were collected from July 20-22, 2012, in three towns: Ganhe Township (104°22 50.4 E, 23°41 48.9 N), A' meng Township (104°35 32.6 E, 23°45 23.6 N), and Panlong Township (104°22 48.9 E, 23°31 13.7 N). Each sample consisted of a composite from five points within the same field (666.67 m^2 area). Detailed sampling site information is provided in .

1.2 Sample Collection

At each sampling point, four 20 m × 20 m quadrats were established as replicates. After removing surface debris, five soil cores (0–20 cm depth) were collected in an S-shaped pattern within each quadrat and mixed. Samples were placed in sealed bags, stored in ice-filled coolers, transported to the laboratory, passed through a 2 mm sieve, and stored at 4 °C for Biolog analysis and physicochemical property determination.

1.3 Soil Physicochemical Property Determination

Soil pH was measured using potentiometry, organic matter by potassium dichromate volumetry, available phosphorus by molybdenum-antimony colorimetry, alkali-hydrolyzable nitrogen by alkaline diffusion, and available potassium by NH OAc extraction-flame photometry [13–15].

1.4 Soil Microbial Functional Diversity Analysis

Soil microbial metabolic functional diversity was analyzed using the Biolog method. Within three days of sampling, Biolog™ ECO Plates (Biolog Inc., Hayward, CA, USA) were used to determine soil microbial community metabolic function. Soil equivalent to 10 g dry weight was added to a flask containing 100 mL sterile water, shaken at 260 r • min ^{1} for 30 min, and diluted 1,000-fold with 0.85% NaCl solution. Aliquots (150 L) were inoculated into each well of Biolog ECO microplates and incubated at 25 °C. Data were read at 24-hour intervals for six days using a Biolog reader [16].

1.5 Data Processing and Analysis

1) **Average Well Color Development (AWCD):** AWCD describes soil microbial metabolic activity, which increases with incubation time [5]. The calculation formula is as follows [17–18]:

Where: C is the absorbance value of each substrate-containing well; R is the absorbance value of control wells; n is the number of substrate wells (n = 31 for Biolog Eco plates).

2) **Shannon-Wiener Diversity Index (H):** This index represents the diversity of carbon source utilization capabilities of soil microbial communities, expressed as:

Where: P is the ratio of the relative absorbance value of well i to the sum of relative absorbance values of all wells on the microplate.

3) **Evenness Index (E):** The evenness index is expressed as:

Where: H is the Shannon-Wiener index; S is the number of carbon sources utilized by microorganisms (i.e., wells showing color change), calculated from the Shannon index.

4) **Simpson Dominance Index:** This index evaluates the dominance of the most common species, expressed as:

The Simpson index is represented by the 1/D value.

The 31 carbon substrates on Biolog ECO plates can be divided into six categories [19]: carbohydrates, amino acids, polymers, carboxylic acids, amines, and phenols. Diversity indices evaluate microbial community diversity in different soils; higher values indicate greater microbial community diversity [2].

2. Results

2.1 Soil Physicochemical Characteristics

presents mean values of available potassium, available phosphorus, alkali-hydrolyzable nitrogen, organic matter, and pH in soils from the three towns at different years post-harvest. Results showed that compared with the control, soils at 1-6 years post-harvest exhibited no significant differences in pH, organic matter, alkali-hydrolyzable nitrogen, or available potassium ($P > 0.05$), except for significant differences in available phosphorus content at years 3 and 4 ($P < 0.05$).

2.2 Soil Microbial Metabolic Characteristics

2.2.1 Dynamics of Average Well Color Development (AWCD) AWCD reflects the overall level of carbon source utilization by soil microbial communities. AWCD values were measured at 24-hour intervals to generate dynamic change curves ([Figure 1: see original paper]).

In A' meng Township ([Figure 1a: see original paper]), AWCD showed minimal change during the first 24 hours of incubation, increased rapidly from 24–72 hours (indicating vigorous microbial activity), and gradually stabilized after 96 hours. At 96 hours, the highest mean AWCD occurred at year 3 post-harvest, while the lowest occurred at year 1. The control showed higher AWCD than years 6, 2, 4, and 5.

In Ganhe Township ([Figure 1b: see original paper]), microbial activity was low within 24 hours, peaked at 72 hours, and stabilized after 96 hours. The highest mean AWCD occurred at year 5 post-harvest, while the control showed the lowest. Year 2 exhibited higher AWCD than years 3, 1, 6, and 4.

In Panlong Township ([Figure 1c: see original paper]), microbial activity was low within 48 hours, entered exponential phase from 48–72 hours, and plateaued after 96 hours. The highest mean AWCD occurred at year 4 post-harvest, while the lowest occurred at year 3. Year 6 showed higher AWCD than years 1, control, 2, 5, and 3.

2.2.2 Carbon Source Metabolic Characteristics Cluster analysis of 31 carbon sources on ECO plates is presented in [Figure 2: see original paper]. In Panlong Township ([Figure 2a: see original paper]), carbon source utilization patterns first divided into two major groups: year 4 post-harvest versus all other years, with years 5, 6, and 1 forming a sub-cluster. In Ganhe Township ([Figure 2b: see original paper]), patterns separated into two groups: years 2, 4, and 5 versus control, year 3, year 1, and year 6. In A' meng Township ([Figure 2c: see original paper]), patterns divided into control versus all post-harvest years, with years 3, 1, 6, and 2 clustering together.

Across all three sites, soils at 1 and 6 years post-harvest clustered together for carbon source utilization. Except in Ganhe Township, these did not cluster with the control. Soils at year 4 post-harvest showed distinct patterns from years 1 and 6.

The 31 carbon sources were categorized into six chemical groups: carboxylic acids, amino acids, carbohydrates, polymers, amines, and phenols. Analysis of mean optical density differences (C-R) at 96 hours revealed:

In Panlong Township, utilization of carbohydrates, amino acids, carboxylic acids, and polymers decreased at years 2, 3, and 5 but increased at years 1, 4, and 6. Amine utilization decreased at years 1, 3, and 5 but increased at years 2, 4, and 6. Phenol utilization decreased at years 1, 2, and 4 but increased at years 3, 5, and 6.

In Ganhe Township, carbohydrate and amino acid utilization decreased at years 3, 4, and 6 but increased at years 1, 2, and 5. Carboxylic acid utilization decreased at years 4 and 6 but increased at years 1, 2, 3, and 5. Polymer utilization decreased at years 3 and 6 but increased at years 1, 2, 4, and 5. Amine utilization decreased at years 2, 4, and 6 but increased at years 1, 3, and 5. Phenol utilization decreased at years 2 and 4 but increased at years 1, 3, 5, and 6.

In A’ meng Township, carbohydrate utilization decreased at years 1, 4, and 5 but increased at years 2, 3, and 6. Amino acid utilization decreased at years 1, 4, 5, and 6 but increased at years 2 and 3. Carboxylic acid utilization decreased at years 4 and 5 but increased at years 1, 2, 3, and 6. Polymer utilization decreased at years 1, 4, and 6 but increased at years 2, 3, and 5. Amine utilization decreased at years 1, 2, 5, and 6 but increased at years 3 and 4. Phenol utilization decreased at years 4 and 5 but increased at years 1, 2, 3, and 6.

Compared with control soils, microbial utilization of carbohydrates, polymers, carboxylic acids, and phenols in soils at 1 and 6 years post-harvest was 49.61%, 55.59%, 44.89%, and 25.97% higher at year 1, and 53.14%, 65.27%, 65.68%, and 63.70% higher at year 6, respectively. With increasing time after harvest, utilization of carbohydrates, amino acids, and carboxylic acids increased at year 2 but declined at years 4 and 5. Microbial community composition at year 6 post-harvest was similar to that at year 1 and distinct from the control, indicating that rotation periods must exceed a certain duration (at least >6 years) before replanting ([Figure 2: see original paper]).

2.2.3 Soil Microbial Community Diversity Index Characteristics Diversity indices calculated from 96-hour optical density values are presented in . The Shannon-Wiener index (H) reflects microbial community richness. In Ganhe Township, the highest Shannon-Wiener index occurred at year 5 post-harvest, while the control showed the lowest, with significant differences between them ($P < 0.05$). No significant differences were observed among years 1, 2, 3, and 6, though all differed significantly from year 4 ($P < 0.05$). In A’ meng Township, the control showed the highest diversity index, while year 6 showed the lowest, with no significant differences among years 1-5 ($P > 0.05$). In Panlong Township, the highest index occurred at year 5 and the lowest at year 3, with significant differences between them ($P < 0.05$), while years 1, 2, 4, and 6 showed no significant differences ($P > 0.05$).

The evenness index (E) reflects the uniformity of species distribution. In Ganhe Township, evenness indices were highest at years 5 and 6, lowest in the control (significantly different, $P < 0.05$), and significantly lower at years 2 and 4 compared with years 1, 3, 5, and 6 ($P < 0.05$). In A' meng and Panlong Townships, no significant differences in evenness were observed between the control and post-harvest years ($P > 0.05$), with values exceeding 0.9 and 0.8, respectively.

The Simpson index (D) reflects microbial diversity. In Ganhe Township, the control showed the lowest Simpson index, significantly different from all post-harvest years, while year 4 differed significantly from other years ($P < 0.05$). In A' meng Township, the control showed no significant differences from other years ($P > 0.05$), though year 2 differed significantly from year 6 ($P < 0.05$).

3. Discussion and Conclusion

Biolog-ECO technology enables investigation of soil microbial carbon source utilization capacity, providing insights into functional differences among microbial communities [20]. The 31 sole carbon sources on Biolog ECO plates are generally categorized into six groups: polymers, carbohydrates, amino acids, carboxylic acids, amines, and phenols [20]. Cluster analysis and principal component analysis of these carbon sources can further reveal metabolic preferences of environmental microbial communities [21]. Zhang et al. [22] demonstrated that crop rotation improved microbial community structure and physicochemical properties in *P. notoginseng* continuous cropping soils, effectively mitigating continuous cropping obstacles.

Our Biolog data cluster analysis revealed that across all three sites, soils at 1 and 6 years post-harvest clustered together for carbon source utilization. Except in Ganhe Township, these did not cluster with the control, and year 4 soils showed distinct patterns from years 1 and 6. These differences arise from variations in microbial community metabolic preferences, indicating that soil microbial community composition differs among post-harvest years. The distinct carbon source metabolism in soils at 1 and 6 years post-harvest compared with control soils suggests that *P. notoginseng* cannot be replanted even after 6 years. Lin et al. [23] investigated microbial regulation mechanisms in *Salvia miltiorrhiza* rotation cultivation, finding that soil microorganisms adjust community structure and physiological bacterial groups to establish a new ecosystem balance, requiring at least 2 years for natural recovery. Our finding that soils at 1 and 6 years post-harvest cluster separately from the control indicates that *P. notoginseng* fields require longer recovery periods than previously reported.

Richness, Shannon index, and evenness parameters are widely used to characterize soil microbial community diversity [24]. Lu et al. [25] found that besides pathogen-induced soil-borne diseases, soil physicochemical properties and microbial community structure (including dominant population ratios) contribute to *P. notoginseng* continuous cropping obstacles. In Ganhe and A' meng Townships, Shannon-Wiener indices at year 6 post-harvest were lower than other years, though evenness indices exceeded 0.9 and showed no significant differences from other years. The lack of significant differences in Shannon-Wiener indices among different post-harvest years, combined with lower evenness in control soils compared with years 1 and 6 (except in A' meng), suggests that while total microbial biomass remains relatively stable, the proportion of dominant populations decreases, leading to increased evenness—consistent with previous research.

Soil microorganisms serve as the driving force for soil organic matter and nutrient transformation, directly affecting nutrient availability [26]. Microorganisms act as both nutrient pools and degradation catalysts during crop residue decomposition, driving material decomposition, transformation, and nutrient cycling [27], with soil fertility and microbial activity influencing each other [28]. Crop rotation facilitates nutrient uptake while regulating microbial communities and altering their environment, enabling full nutrient utilization [29]. Without timely nutrient supplementation during subsequent cultivation, continuous cropping may deplete soil nutrients and impair plant growth. For example, root splitting in *Salvia miltiorrhiza* is widely considered a result of micronutrient deficiency [30]. Hao and Wu [31] found that improving soil nutrient supply conditions favors high-quality, high-yield ginseng production [32]. Research indicates that longer rotation intervals significantly reduce disease incidence in *P. notoginseng* [33], possibly due to differential nutrient uptake by rotation crops, suggesting that timely, appropriate fertilization is crucial for minimizing continuous cropping losses [34]. Liu et al. [35] found that available micronutrients in *P. notoginseng* soils varied inconsistently across different rotation intervals, though generally equivalent between new soils and those with 7-year intervals. Wu et al. [36] reported no regular patterns in five heavy metal elements across different rotation years, while Zhang et al. [37] found that rotation improved soil physicochemical properties, indirectly affecting microbial diversity and activity. By planting different crops, rotation systems modify soil properties, alter microbial communities, and change community composition, thereby increasing diversity, enhancing enzyme activity, and improving crop yield and quality.

Our study found no significant differences in macronutrients (N, P, K) or organic matter among different post-harvest years, indicating that macronutrient variation is not the primary factor limiting *P. notoginseng* continuous cropping, and that appropriate rotation intervals can mitigate soil nutrient imbalance. Previous studies on micronutrients and heavy metals also showed no significant differences after 7-year rotations, though those measurements were taken after *P. notoginseng* planting rather than after subsequent harvest. Compared with other post-harvest years, Ganhe Township soils at year 4 showed significantly lower microbial diversity, while Panlong Township soils at years 2 and 3 showed relatively low diversity indices, possibly related to root exudates from rotation crops affecting microbial community structure, composition, and abundance—consistent with previous research [38].

This study suggests that land use patterns, soil types, and climatic conditions are the main factors influencing microbial carbon source utilization. Yang et al. [39] confirmed that *P. notoginseng* emergence rate increased, root rot decreased, and yield and quality improved with longer rotation periods, with soils under >5-year rotation supporting better performance. Our results show that compared with the control, soils at 1 and 6 years post-harvest exhibited higher utilization of carbohydrates, carboxylic acids, and phenols, suggesting that microorganisms in these soils may improve the microecological environment through differential carbon source utilization. The improved yield and quality observed in >5-year rotations may be associated with these changes in the soil microbial environment, warranting further investigation.

Unlike previous studies on *P. notoginseng* continuous or rotation cropping soils, this research examined historical cultivation sites using Biolog technology. By comparing with never-planted soils and excluding the influence of active *P. notoginseng* cultivation, we found no significant differences in macronutrients or microbial diversity indices among post-harvest years, though carbon source utilization differed significantly. Shannon-Wiener indices were lower than the control at years 1 and 6, while evenness indices were higher, suggesting that the most significant change in post-harvest soils is altered microbial community structure. Even after 6 years, the proportion of dominant microbial populations remained lower than in never-planted soils, which may explain why *P. notoginseng* requires at least a 6-year interval before replanting. Our samples originated from historical cultivation sites with different current crops and complex planting histories. While rotation interval is an important consideration, we did not analyze the relationship between current crops and soil microorganisms, representing a limitation of this study. For high-value, high-input medicinal crops like *P. notoginseng*, excessively long rotation intervals restrict land use, limiting industrial development and quality assurance. Therefore, modern engineering solutions are needed to address continuous cropping obstacles.

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