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## Article

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# Characteristics and influencing factors of soil microbial functional diversity in Shushan urban forest, Southeast China

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## ABSTRACT

The study of soil microbial functional diversity and its influencing factors in urban forests can provide important insights into maintaining a healthy material cycle within these ecosystems. In this study, we selected three plantation forests, including *Pinus massoniana* Lamb (PM), *Quercus acutissima* Carruth (QA), *Liquidambar formosana* Hance (LF), and secondary deciduous broadleaved forest (DB). We studied the metabolic characteristics of soil microbial carbon sources in the four kinds of forest vegetation in the growing season and dormant season and the 0-10cm, 10-20cm, 20-30cm soil layer, three soil layers (0-10, 10-20, 20-30cm) using the Biolog-ECO microplate method. Our study suggested that soil microorganisms with PM showed the lowest metabolic activity. Soil microorganisms in the four forest vegetation had the lowest carbon source utilization capacity for monosaccharide/ glycoside/ polymercan, and the highest utilization capacity for amines. The Shannon-Wiener ( $H'$ ) and Simpson ( $D$ ) diversity indices of the four kinds of forest vegetation were significantly different from 0-10cm in the soil layer, and the  $H'$  and  $D$  values of LF were higher than PM, QA and DB. Soil pH and total phosphorus (TP) are closely related to the microbial functional diversity and carbon source utilization of the four vegetation.

## Introduction

The urban population is rapidly growing globally, and more than half of the world's population inhabits urban and sub-urban areas. Currently, 55% of the global population lives in urban areas, and this proportion is estimated to increase to 68% by 2050<sup>1</sup>. Urbanization can change land-use type and soil lithology in urban areas, inevitably resulting in significant variations in soil ecosystem services and biogeochemical cycles<sup>2</sup>. Urban forests have been recognized to provide critical ecosystem services for human health and environmental quality, particularly with urbanization<sup>3</sup>. Therefore, urban forests play a crucial role in the process of urban development.

Soil microbes are crucial component of forest ecosystem and the most active part of forest soils<sup>3-4</sup>. They provide nutrients to soil ecosystem by breaking down animal and plant residues, participating in the material cycle and energy flow of forest ecosystems<sup>5</sup>. Studies have shown that different physical and chemical properties of soil in different habitats have an impact on the structure and function of soil microbial communities<sup>6</sup>. Soil microorganisms are highly sensitive to environmental changes and are influenced by varieties of factors<sup>7</sup>. Moreover, studies have found significant positive correlations existing between soil microorganisms and soil organic carbon (SOC), soil pH, total nitrogen (TN), total phosphorus (TP), and soil organic matter<sup>7-8</sup>. The physical and chemical properties of soil, as well as factors such as temperature and water availability, exhibit seasonal variations, thus causing soil microbial community structure and diversity to display clear seasonal dynamics<sup>8</sup>. Soil microorganisms interact with seasons, including their metabolic characteristics and community composition<sup>9</sup>. Different seasons affect soil microbial community structure by regulating forest microclimate and soil physical and chemical properties, there by affecting the decomposition of soil organic matter and biogeochemical cycles<sup>10</sup>. At the same time, plant and vegetable types regulated by seasons also have an influence on soil microbial community. For example, during the growing season, the root system activity of plants increases, and the turnover rate and secondary metabolite secretion of roots increase which accelerate the decomposition of recalcitrant substances and provide abundant nutrient and energy materials for soil microbial proliferation<sup>11-13</sup>. During the dormant season, the growth rate of plants slows down, and the turnover rate and vitality of roots decrease, limiting the growth of microorganisms and the transformation of soil elements<sup>14</sup>. The impact of seasonal changes on soil microbial diversity and activity is also influenced by external environmental factors<sup>15</sup>. However, some studies have found that seasons are not the primary factor affecting the structure of soil microbial communities<sup>16</sup>. Therefore, the influence of seasonal changes on soil microbial community structure and diversity remains controversial, and the relative importance of various environmental factors that results from seasonal changes on soil microbial community structure and diversity requires further study.

Recent decades of studies have shown that soil layer is a key factor affecting the community structure and diversity of soil microbes in the forest ecosystem<sup>17</sup>. In recent years, research progress indicates that differences in soil microbial community structure under different soil layers are closely related to seasonal dynamics<sup>18</sup>. Therefore, exploring the correlation between the changes in soil microbial community in different seasons and different soil layers is a crucial aspect of analyzing nutrient cycling and ecosystem functions in forest soil ecosystems<sup>19</sup>. We study is not only helpful for gaining a deeper understanding of the ecological functions of soil microbes in the forest ecosystem, but also provides a scientific foundation for ecological conservation and sustainable development.

The ability of microorganisms to use different carbon sources largely depends on the species and inherent nature of microorganisms, the Biolog method tests the ability of microorganisms to use a single carbon source, and reveals the variability of different soil microbial communities on carbon source utilization to reflect the functional diversity of the community<sup>20</sup>. In 1991, Garland et al. first proposed the Biolog microbial plate to detect the functional diversity of microbial communities<sup>21</sup>. Since then, Biolog technology, as a fast, simple method with high sensitivity and strong resolution has advantages of obtaining information about the overall activity and metabolic function of the microbial community, and retaining the original metabolic characteristics of the microbial community. It has been widely used in the functional diversity of different types of soil microbial communities<sup>22-23</sup>.

Despite some progress in studying the ecological environment of plant root microbiomes both domestically and abroad, there are still some shortcomings. Soil microbes are important components that soil converts soil organic matter into available nutrients<sup>24-25</sup>. As a link between plants and soil, soil microbes are closely related to the above ground productivity and the underground micro environment of plants<sup>26</sup>. Therefore, the structure and composition of soil microbial communities play a crucial role in this relationship<sup>25-27</sup>. Scholars around the world are continuously deepening their research on soil microbes at different stages. However, the current research on soil microbes mainly focuses on differences in population distribution and functional diversity in different regions, lacking comprehensive research on the ecological relationships between plants and soil, the integration effect of the soil layer and seasonal factors, and the dominant factors<sup>28</sup>. Currently, it is one of the shortcomings of soil microbial research. This study aimed to investigate soil microbial community structures and their ecological functions in different types of forests. The research objects included three plantation forests and a disturbed land (DB) within the Hefei Shushan Forest Park, China. Soil samples were collected from various seasons and soil layers, and the physical and chemical properties of each soil sample were analyzed. In addition, the carbon metabolic activities of the soil microbes were evaluated using the Biolog-ECO microplate method. The study aims to address the following issues: (1) explore the differences in the utilization of carbon source substrates by soil microbial communities in different seasons and different soil layers in various forest stands in Shushan; (2) analyze the impact of different forest stands and soil layers on the functional diversity of soil microbial communities in Shushan; (3) study the relationship between the metabolic activity and community diversity of soil microbes in different stands in Shushan urban forest and environmental factors. By studying the changes in soil microbial community structure with soil layer and season, and its influencing factors, this research will help to deepen the understanding of soil microbial ecology and its role in ecosystem services, and provide theoretical support for its conservation and sustainable utilization.

## Results

**The physicochemical characteristics of the soils from the four forest communities.** The TP, SOC, and TN levels of soils among three plantation forests and DB forest were significantly different during both the growing and dormant season (Table 1,2). There were substantial differences observed between PM plantation and the other three forest types (Table 1,2). However, the differences in the SOC, TP, and TN contents among the other three forest types are not significant (Table 1,2). In addition, the soil pH values of three plantation forests and DB forest also vary between the growing season and the dormant season, with pH values generally being slightly lower in the growth season, and gradually increasing with soil depth. In the growing season, there is a greater difference in soil pH values among three plantation forests and DB forest, while in the dormant season, the difference in pH values among three plantation forests and DB forest is less significant. In both the growth and dormant seasons, the soil in the DB plantation is weakly acidic, while the soil in the QA plantation is strongly acidic (Table 1,2).

**Two factors analysis of soil microbial community activity.** The interaction effects between three plantation forests and DB forest, soil layers, and seasons have no significant impact on soil microbial activity (Table S1). Among them, the effect of season on soil microbial activity is significantly different between QA and LF ( $p < 0.001$ ), and it has a significant difference between PM and DB forest ( $p < 0.05$ ) (Table S1). The effect of the soil layer on the microbial activity of LF is also significant ( $p < 0.05$ ), but it is not significant for the other three forest types (Table S1). In summary, the influence of season and plant type on soil microbial activity is more significant, and the differences in soil microbial activity among the growing and dormant seasons and three soil layers are significantly different for the four plantation types.

**The metabolic activity of the soil microbial communities.** Using the Biolog-ECO plate, the soil microbial activity was monitored for 7 consecutive days through the average well color development (AWCD), which was used to analyze the microbial utilization rate of carbon sources. The AWCD curve trends of the soil microbial population in the four different forest types were basically the same (Fig.2). As the cultivation time increased, AWCD gradually increased, indicating that soil microbial activity increased with cultivation time. Within 144 hours of cultivation, AWCD values increased rapidly with a high slope, indicating that microbial metabolism was most active during this period, and a large number of carbon sources in the Biolog-ECO plate were utilized (Fig.2). From 144h until the end of cultivation, the curve growth slowed down and tended to be stable (Fig.2). Therefore, in this study, the analysis of soil microbial activity was based on the AWCD value at 144h.

Overall, soil microbial activity in the same forest type was generally higher in the growing season than in the dormant season and gradually decreased with soil depth. Among the three plantation forests and DB forest, the microbial activity in PM soil showed two characteristics: low activity and slow growth rate. Although its AWCD value was not the lowest on the first day of 24h observation, it was gradually surpassed by the AWCD values of other forest types in the following days (Fig.2).

**Soil microbial carbon source utilization intensity.** The 31 carbon sources on the Biolog-ECO board can be divided into six categories: monosaccharide/ glycoside/ polymercan, amino acids, esters, alcohols, amines, and acids. The soil microorganisms of the three plantations and DB forest have low utilization of monosaccharide/ glycoside/ polymercan, but have high utilization of amines and esters (Fig.3,S1). Compared with the dormant season, during the growing season, the difference in the utilization of three plantations and DB forest to six carbon sources is greater than that of the dormant season (Fig.3,S1).

In general, regardless of the growth or dormant season, the utilization rate of esters by soil microbes from the four plantation types was relatively high, while the utilization rate of monosaccharide/ glycoside/ polymercan was relatively low. During the growing season, there were significant changes in the utilization rates of various carbon sources by soil microbes from the four forest communities, while there were no significant changes in the dormant season (Fig.3,S1).

**Analysis of functional diversity of soil microorganisms.** Based on the AWCD values at 144 hours of cultivation, the soil microbial diversity indexes were calculated, including the  $H'$  and the  $D$ . The composition of soil microbial communities differed between the three plantation forests and DB in three soil layers (0-10, 10-20, 20-30cm) during the growing and dormant seasons (Table 3). The  $H'$  and  $D$  index in the 0-10cm layer is higher than that in the 10-20cm and 20-30cm layers (Table 3). By comparison of different seasons, the soil microbial diversity index is higher in the growing season than in the dormant season, indicating that the environmental conditions during the growing season are conducive to the survival and reproduction of soil microorganisms. There are significant differences in soil microbial diversity index among three plantation forests and DB. The  $H'$  and  $D$  index in the LF forest is significantly higher than that in the other three forests (Table 3).

**Principal component analysis of soil microbial carbon source utilization.** Principal component analysis (PCA) was performed on the AWCD values of the four forest systems at 144 hours to extract two principal component factors from 31 different factors, in order to analyze the metabolic functional characteristics of soil microbes in different forest systems. The two principal component factors during the growing season accounted for 22.2% and 50.3%, respectively, with a cumulative contribution of 72.5% (Fig.4). From the distribution of four communities on the coordinates, there were significant differences in the carbon source utilization characteristics between the PM forest and the DB forest in three soil layers. During the dormant season, the two principal component factors accounted for 6.6% and 86.4%, respectively, with a cumulative contribution of 93.0%. From the distribution of four communities on the coordinates, there were significant differences in the carbon source utilization characteristics of different forest systems in the same soil layer. LF and DB forests exhibited greater differences among three soil layers (Fig.4).

Comparing the distribution of four communities on the coordinates during the growing season and dormant season, it can be seen that the ability of the four forest systems to utilize carbon sources was greater during the growing season than during the dormant season, and there were greater differences in carbon source utilization among different forest systems during the dormant season than during the growing season. Among them, the utilization ability of carbon sources in the LF forest was relatively strong (Fig.4).

**Relationship between soil microbial metabolic activity, community diversity index and soil physicochemical properties.** Based on the correlation analysis between soil microbial diversity indices and soil physicochemical properties, from Figure 5, it can be seen from the figure that the diversity indices and carbon source metabolic activity of soil microbial communities in three plantations and secondary deciduous broadleaved forest types are positively correlated with soil carbon content. Since soil carbon content is the most active and easily variable part of soil organic matter, the greater the microbial carbon content in the soil, the more favorable it is for microbial growth and reproduction, and soil carbon content is an important factor affecting microbial metabolic activity and diversity. In addition, soil microbial indices are significantly positively correlated with plant species and soil pH ( $p < 0.05$ ), indicating that soil pH is also one of the important factors affecting soil

microbial community diversity (Fig.5). Soil pH can change the composition and diversity of soil microbes by affecting the composition, chemical properties, and utilization rate of the soil matrix.

## Discussion

Microorganisms are a vital component of the ecosystem, and their interaction with environmental factors is primarily realized through the community's metabolic characteristics and functional diversity<sup>29</sup>. The present study reveals that as the incubation time extends, microbial communities demonstrate an increasing ability in utilizing carbon sources. Furthermore, the AWCD values of soil microbial communities underwent the most significant changes between the 0-144h period. The peak of AWCD was reached at 144h, implying that the utilization of carbon sources by soil microbes had attained a high level. Moreover, AWCD remained constant after 144h, revealing the four plant's soil microbial communities' good adaptation and efficient utilization of environmental carbon sources, leading to maximal growth of soil microorganisms an observation consistent with findings from other studies<sup>30-31</sup>. The present research also highlights differences among the soil microbial communities of the four plants concerning their capacity to use carbon sources. The complex and diverse chemical structures of various carbon sources, coupled with differences in forest plants, soil types, and sampling seasons, may result in distinct patterns of carbon source utilization by soil microorganisms during different incubation periods<sup>30</sup>. Moreover, the AWCD values varied among the four forest plants due to these factors. Furthermore, the low AWCD value observed throughout the culture process for PM indicates that the soil microbial activity in PM is weak. The slow decomposition of needle litter in PM forests has been suggested as a possible reason for the slower release of woodland nutrients, which can affect soil microbial activity<sup>32</sup>. Our study also discovered that soil microbial activity decreased with increasing soil depth during both the growing and dormant seasons across all four vegetation types due to variations in soil constituents and nutrient content at various depths. As soil depth increases, soil organic matter and water and gas conditions deteriorate, diminishing microorganisms' capacity to utilize carbon source substrates<sup>33-34</sup>.

The PCA analysis further revealed that microorganisms displaying high carbon source utilization in the 0-10 cm soil layer exhibited a seasonal distribution pattern, with higher activity concentrated during both the growing and dormant seasons. As such, studying the composition and activity characteristics of microbial communities in surface soil holds significant scientific value<sup>35</sup>. Additionally, our findings indicate that soil microbial activity is higher during the growing season compared to the dormant season. Differences in microbial activity level may be affected by alterations in environmental conditions and substrate availability between different seasons<sup>36</sup>. Research has shown that seasonal changes can significantly impact the composition of soil microbial communities. Our study examined the utilization intensity of different carbon sources across all four forest types, and we observed low utilization of monosaccharide/ glycoside/ polymer carbon sources by soil microorganisms. Some studies suggest that such carbon sources may not be conducive to improving soil microbial activity<sup>37</sup>. Our analysis revealed that ester carbon sources were more heavily utilized by soil microorganisms in all four forests. This attributed to the fact that esters are the primary carbon source for soil microorganisms and are easier to be directly utilized, thus improving microbial metabolic activity<sup>38</sup>.

Our study found that the soil microbial diversity index in the growing season is higher than that in the dormant season for all four vegetation types examined. This attributed to the fact that soil microorganisms in the growing season have higher carbon source metabolic activity, which provides a conducive environment for the growth of microorganisms, ultimately increasing soil microbial community diversity<sup>39</sup>. Furthermore, soil depth displayed significance between 0-10 cm for both the growing season and dormant season. This due to an elevated level of soil microbial activity among topsoil microorganisms. However, there existed no differences in soil thickness for depths between 10-20cm and 20-30cm, indicating that the community structure of soil microorganisms at these depths was largely consistent. according to the statement provided, the LF soil microorganisms exhibited higher *H* and *D* indices compared to the other three stands. The reason behind this could be due to LF higher catabolic activity throughout the culture process<sup>40-41</sup>. As a consequence, it led to significant changes in the population numbers of each species, which highlights that the diversity of the soil microbial community is influenced by vegetation types. The paragraph further suggests that the maple tree may have the highest diversity of soil microorganisms compared to the other vegetation types. Additionally, studies conducted by Yin et al. (2014) demonstrated that functional diversity of soil microbial communities varies among different vegetation types<sup>42</sup>. Overall, the diversity of soil microorganisms appears to have a strong correlation with vegetation type and can vary widely depending on the species of plants found in a particular region. Furthermore, factors such as catabolic activity and population dynamics can impact the overall soil microbial community diversity<sup>43</sup>.

As per the study described, the activity and diversity of soil microorganisms are interlinked with the physicochemical factors present in the soil<sup>44</sup>. Any alteration in the physicochemical properties can impact the microenvironment where microorganisms exist, thereby influencing the species and activities of soil microbial communities<sup>45</sup>. The results indicated that soil pH, TN, and TP play a crucial role in regulating the carbon source utilization and community diversity of soil microbial organisms. Based on these observations, it can be inferred that soil pH, TN, and TP act as controlling factors governing the soil microbial community's carbon source utilization. In summary, it highlights the importance of understanding the physicochemical soil properties' effects on soil microbes and how they can impact the broader ecosystem functions<sup>46-47</sup>. PH, SOC, and TN exhibit a positive correlation

with soil microbial activity and community diversity, which may be attributable to the heightened level of human perturbation in urban forests<sup>48</sup>. Huang et al. discovered that as urbanization intensifies and atmospheric nitrogen precipitation surges, excessive release of nitrate ions within soil will escalate soil acidification, culminating in lowered soil pH<sup>49</sup>. Furthermore, soil acidification can heighten the solubility of toxic soil microorganisms such as aluminum<sup>50</sup>. Soil microbial community structure and activity can be impacted by pH via alterations in the composition, chemical characteristics, and accessibility of soil apparatus<sup>51</sup>. Notably, both the activity of soil microorganisms and the diversity of communities exhibit a marked negative correlation with TP. Although the input of phosphorus into terrestrial ecosystems is rapidly rising, there exist limited investigations on soil microbes, with inconsistent findings reported.

The results indicated that soil microbial activity and community diversity varied across both seasonal shifts and alterations in soil thickness. Via two-way ANOVA, we discovered that seasonal dynamics exerted a more pronounced effect on microbial activity. Furthermore, certain research findings also demonstrated that seasonal changes had a notable impact on the composition of soil microbial communities.

## Conclusion

Based on sampling analysis, we have drawn the following conclusions about the soil microbial community characteristics and influencing factors of four typical forest stands in the Shushan area of Hefei: The metabolic activity of soil microorganisms in the three plantations and secondary deciduous broadleaved forest increased with the culture time. The  $H'$  and  $D$  indices differ significantly from 0-10cm in the soil layer. The  $H'$  and  $D$  of LF were higher than the other three stands. The three plantations and secondary deciduous broadleaved forest showed lower utilization of sugars and higher utilization of amines. The soil microbial activity and diversity index of the three plantation and secondary deciduous broadleaved forest were positively correlated with pH and TN and negatively with TP.

## Methods

**Study site.** The selected plant populations for this study are located in the Shushan Forest Park in Hefei, Anhui Province, China (31°50' 44" N, 117°10' 37" E; Fig. 1). This area is located in the northern subtropical zone and is one of the typical monsoon climate zones in China. The annual average temperature in the forest park is 14-17°C, the annual average precipitation is 969.5 mm, and the annual average relative humidity is 74%-78%. The soil in this area is relatively deep and mainly consists of erosion yellow-brown soil or local red loam. The total area of the forest park is approximately 437 square kilometers, with mixed needle and broad-leaved forests, pure broad-leaved forests, and mixed broad-leaved forests being the main types of forest vegetation. Currently, the area of mixed needle and broad-leaved forests is approximately 200 square kilometers, with a coverage rate of 0.85 and a canopy density of 0.5; the area of pure broad-leaved forests is approximately 237 square kilometers, including approximately 7.6 square kilometers of QA forest and approximately 3 square kilometers of LF forest, with a coverage rate of 0.82 and a canopy density of 0.5. The main forest vegetation is the subtropical deciduous and evergreen broad-leaved mixed forest, with PM forests covering over 200 square kilometers, 90% of which are artificial forests, accounting for 65% of the entire forest area.

**Analysis of soil physical and chemical properties.** The soil samples are mixed with dry soil and distilled water in a ratio of 1:2.5 (W/V)<sup>52</sup>. The mixture is shaken and left to settle for 30 minutes before the soil pH is measured using a HORIBA-212 pH meter. Soil organic carbon and total nitrogen are determined using an EA3000 elemental analyzer<sup>53</sup>. The soil samples are digested using a wet digestion method with a 3:1 mixture of concentrated nitric and perchloric acid ( $\text{HNO}_3\text{-HClO}_4$ ), and total phosphorus is measured using a flow injection analyzer<sup>54</sup>.

**Determination of soil microbial carbon source metabolism.** Soil microbial carbon source metabolism characteristics were determined using the Biolog-ECO microplate method. The Biolog-ECO Plate contains a total of 96 micropores, arranged in an 8×12 matrix, with every 32 micropores replicated three times in parallel<sup>55</sup>. Each micropore, except the control, contained a different organic carbon source and the same amount of tetrazolium redox dye, reflecting the utilization ability of microorganisms for corresponding carbon sources under certain temperature conditions. Specifically, 10g of fresh soil was weighed and added to a 90mL triangle flask containing sterilized physiological saline (0.85%). The mixture was shaken for 30 min at 250 r/min and then allowed to settle for 10 min. The resulting bacterial suspension was then diluted 1,000 times, and 150  $\mu\text{L}$  of the diluted solution was added to each micropore of the Biolog-ECO plate. The plate was then incubated at 28°C, and data were automatically read by a Biolog reader every 24h for a total of seven days<sup>56</sup>.

**Data analysis.** The Biolog-ECO 96 microplates contained three sets of repeat units, with each set containing 6 categories of 31 different carbon sources, including 10 types of sugars, 7 types of carboxylic acids, 6 types of amino acids, 4 types of polymers, and 2 types of both amines and phenols, as well as a blank control micropore.

The AWCD of each micropore in the Biolog-ECO plate, which is calculated using the formula  $AWCD = [\Sigma(C - C_0)]/31$ , is used to represent the overall ability of the microbial community to utilize carbon sources, where  $C$  is the absorbance value of the 31 carbon source micropores and  $C_0$  is the absorbance value of the control micropore.

The functional diversity index of soil microbial communities was calculated following methods commonly used in plant ecology, including the  $H'$  value (Shanon-Wiener index) and  $D$  value (Simpson index). The  $H'$  value reflects the level of diversity in carbon source utilization and species richness within the microbial community. The  $D$  value assesses the dominance of the most commonly found species.

Shannon-Wiener index :  $H' = -\sum (p_i \times \log p_i)$

Simpson index :  $1/D = [(\sum n_i(n_i - 1)) / N(N - 1)]$

The calculation of the soil microbial community functional diversity index involves several parameters, including  $p_i$ ,  $n_i$ , and  $N$ .  $p_i$  represents the ratio of the relative absorbance value ( $C - C_0$ ) of the  $i$ th micropore to the total relative absorbance value of the entire microplate.  $n_i$  represents the relative absorbance value ( $C - C_0$ ) of the  $i$  micropore, and  $N$  represents the total absorbance value.

**Data Processing.** In this study, Spass 19.0 software (IBM SPSS Inc., Chicago, USA) was used to analyze the differences in soil physical and chemical properties and functional diversity index among different forest types. The relationship between soil microbial activity and diversity and soil physicochemical factors using R language. Analysis of the utilization and principal components of different carbon sources by soil microorganisms was performed using Origin software (OriginLab Corporation, North Andover, Massachusetts, USA).

**Data availability.** The authors confirm that the data supporting the findings of this study are available within the supplementary materials.

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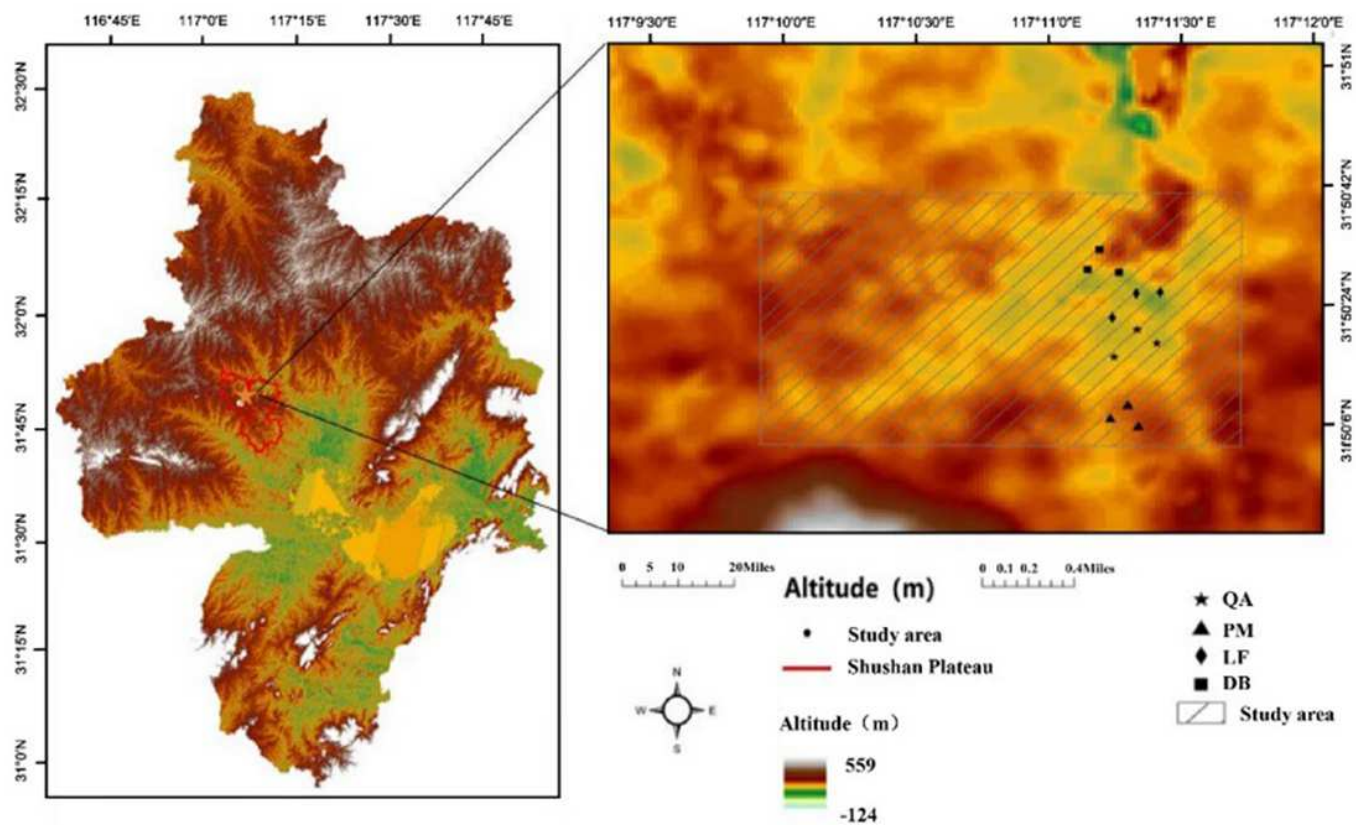
## Author contributions

Conceptualization, methodology, X.X.N.(Xiaoniu Xu); software, validation, formal analysis, investigation, L.H.Y. (Haiyang Liu); writing original draft preparation, W.M.M.(Mimi Wang), L.H.Y.; supervision, L.G. (Gang Li); writing review, editing, visualization, supervision, project administration, funding acquisition, X.X.N.,W.M.M. All authors have read and agreed to the published version of the manuscript.

## Competing interests

The authors declare no competing interests.

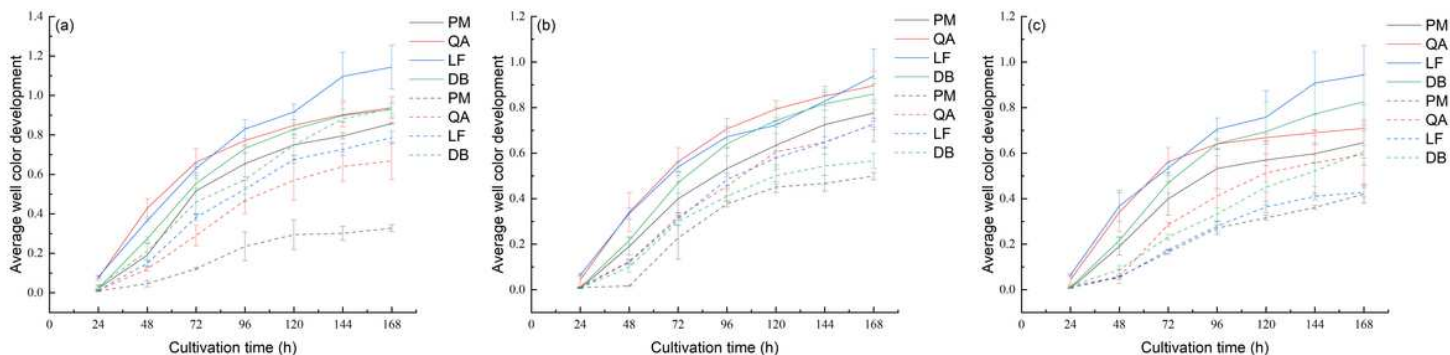
Figures



**Figure 1.** A schematic representation of the study area in Shushan urban forest park and location of field sites. The sampling sites were *Pinus massoniana* Lamb (PM), *Quercus acutissima* Carruth (QA), *Liquidambar formosana* Hance (LF), and secondary deciduous broadleaved forest (DB).

Figure 1

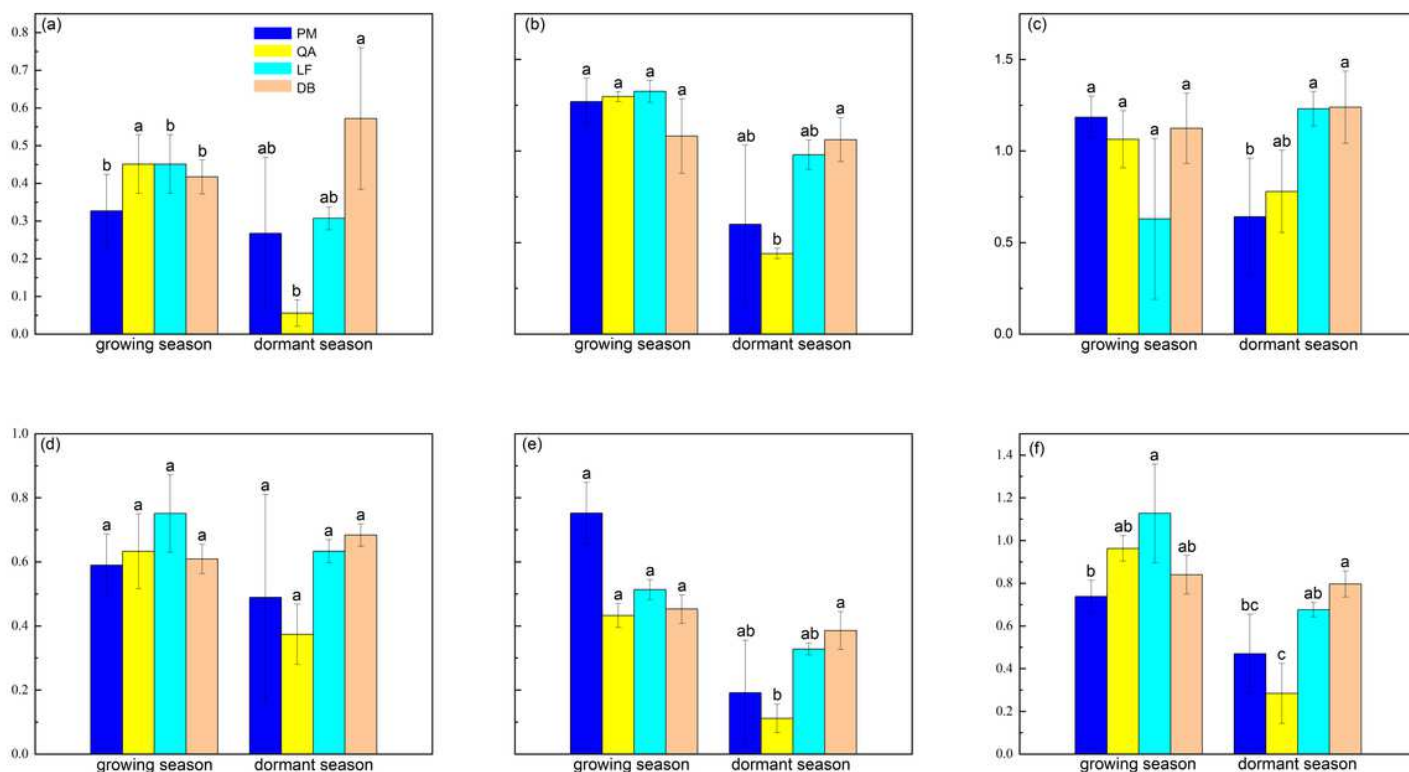
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**Figure 2.** Changes of soil microbial average well color development (AWCD) in three plantation forests and secondary deciduous broadleaved forest (DB). The solid line represents the growing season, and the dotted line represents the dormant season. The solid represents the growing season and the hollow represents the dormant season (a) 0-10cm, (b) 10-20cm, (c) 20-30cm.

## Figure 2

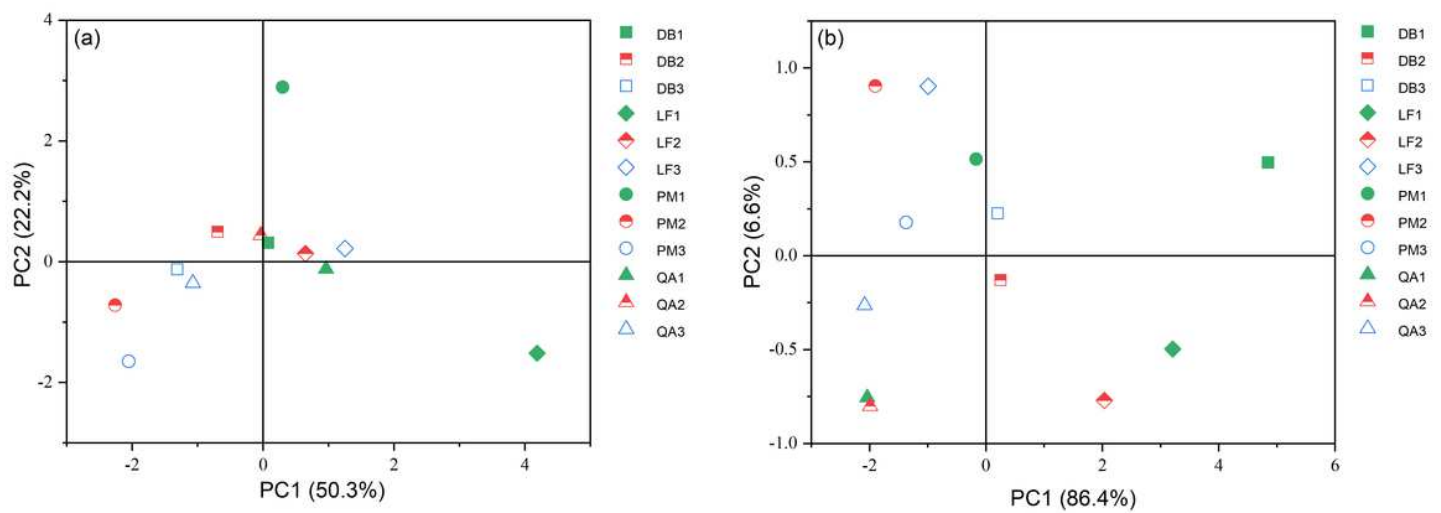
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**Figure 3.** Utilization of different carbon sources by soil microorganisms of three plantations and secondary deciduous broadleaved forest species. (a) Monosaccharide / Glycoside / Polymercan; (b) Amino acids; (c) Esters; (d) Alcohols; (e) Amines; (f) Acids. (soil layer 0-10cm).

## Figure 3

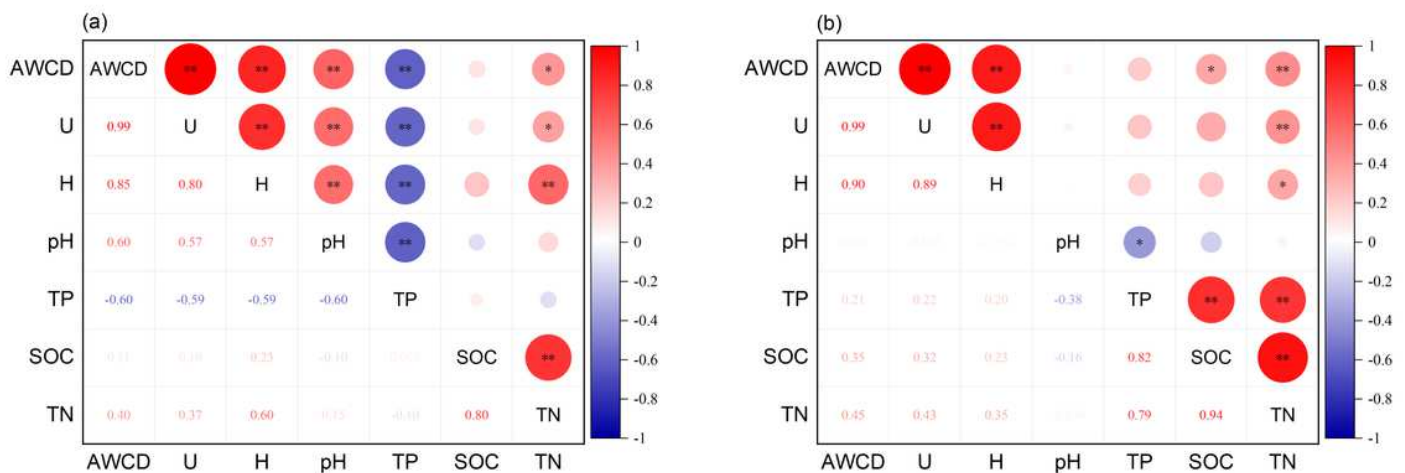
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**Figure 4.** Principal component analysis (PCA) of soil microbial communities during the growing and dormant seasons based on the average color change rate of carbon source (AWCD). (a) Growing season; (b) Dormant season. The number after the capital abbreviation letter represents the plot number in each forest type.

**Figure 4**

See image above for figure legend



**Figure 5.** Correlation analysis of soil microbial community diversity indices and metabolic activity indices with soil physicochemical properties in three plantation and a secondary deciduous broadleaved forest. Circles of different colors and sizes in the plot represent the size of the correlation coefficients. The number indicates the correlation coefficient, \*Significant at the 0.05 probability level. \*\*Significant at the 0.01 probability level. AWCD Average well color development; H Shannon-Wiener index; U Simpson index; C carbon; TN total nitrogen; TP total phosphorus. (a) Growing season; (b) Dormant season.

**Figure 5**

See image above for figure legend

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