

RESEARCH ARTICLE

Short-term impacts of soil enzyme activity in rhizosphere and bulk soils by forest gap size of a *Platycladus orientalis* plantation

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Abstract

Aim of study: We investigated how changes in forest gap size influence soil enzyme activities in both the rhizosphere and bulk soils, mediated by rhizosphere effects (RE).

Area of study: Xuzhou, Jiangsu Province, China.

Material and methods: The study was conducted in a 46-year-old *Platycladus orientalis* (L.) Franco, 1950 plantation in Xuzhou and its aim was accomplished by sampling soils, one year after forest gaps establishment, from three levels of forest gap sizes: small (S, with a radius of 4 m), medium (M, with a radius of 8 m), and large (L, with a radius of 12 m), as well as control plots (CK, without any gaps). Soil enzyme activities, including peroxidase (PER), dehydrogenase (DEH), urease (URE), and invertase (INV), were quantified.

Main results: Gap size and season strongly affected the RE on DEH and URE. The interaction of gap size, location and season significantly affected the RE on DEH, URE, and INV. Enzyme activities in bulk soils were more sensitive to changes in nutrient availability than in rhizosphere soils, and microbial biomass played a crucial role in modulating enzyme activity. Furthermore, both L and S forest gaps exerted an influence on the RE of enzyme activity, with L gaps exhibiting the most extensive impact.

Research highlights: This study observed that the RE of soil enzyme activity did not increase with the enlargement of forest gap size. However, in L forest gaps, its impact range was more extensive.

Keywords: enzyme activity, forest gap size, forest management, rhizosphere effect.

Impacto a corto plazo de la actividad enzimática del suelo en suelos rizosféricos y no rizosféricos según el tamaño de los claros en una plantación de *Platycladus orientalis*

Resumen

Objetivo del estudio: Investigamos cómo los cambios en el tamaño de los claros en el bosque influyen en las actividades enzimáticas del suelo, tanto en suelos rizosféricos como en los no rizosféricos, mediados por los efectos de la rizosfera (ER).

Área de estudio: Xuzhou, provincia de Jiangsu, China.

Material y métodos: El estudio se llevó a cabo en una plantación de *Platycladus orientalis* (L.) Franco, 1950 de 46 años en Xuzhou. Para ello, se tomaron muestras de suelo un año después de la apertura de claros en el bosque, considerando tres tamaños de claros: pequeños (S, con un radio de 4 m), medianos (M, con un radio de 8 m) y grandes (L, con un radio de 12 m), además de parcelas de control (CK, sin claros). Se cuantificaron las actividades de las enzimas del suelo, incluyendo peroxidasa (PER), deshidrogenasa (DEH), ureasa (URE) e invertasa (INV).

Principales resultados: El tamaño del claro y la estación del año afectaron significativamente en los ER sobre la DEH y la URE. La interacción entre el tamaño del claro, la ubicación y la estación influyó

significativamente en los ER de la DEH, URE e INV. Las actividades enzimáticas en los suelos no rizosféricos fueron más sensibles a los cambios en la disponibilidad de nutrientes que en los suelos rizosféricos, y la biomasa microbiana desempeñó un papel crucial en la modulación de la actividad enzimática. Además, los claros de bosque grandes (L) y pequeños (S) influyeron en los ER de la actividad enzimática, siendo los claros grandes (L) los que mostraron el efecto más amplio.

Conclusiones: Se observó que los ER de la actividad enzimática del suelo no aumentaron con la ampliación del tamaño del claro. Sin embargo, en los claros grandes (L), su impacto tuvo un alcance más extenso.

Palabras clave: actividad enzimática, efecto de la rizosfera, gestión forestal, tamaño del claro del bosque.

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Introduction

Soil enzyme activity serves as a vital interface for numerous biochemical reactions within soil ecosystems, playing a pivotal role in driving ecological processes. The regulation of enzyme activity is influenced by a complex interplay of physical, chemical and biological factors, which together elucidates the mechanisms governing nutrient cycling and biochemical transformations in soils (Cotrufo et al., 2015). Abiotic factors such as soil temperature and composition, alongside biotic factors like microbial community structure, collectively shape enzymes activity, making it a reliable indicator of changes within soil ecosystems (Utobo & Tewari, 2015).

Key enzymes responsible for carbon and nitrogen cycling, including peroxidase (PER), dehydrogenase (DEH), urease (URE), and invertase (INV), exhibit high sensitivity to environmental fluctuations (Feng et al., 2019). As an extracellular enzyme, PER is mainly involved in the degradation of complex organic compounds and it plays a key role in the carbon cycle, an activity that supports microbial metabolism and helps to stabilise organic carbon in the soil, while being influenced by the soil microenvironment (Nakayama & Tateno, 2022). Dehydrogenase, as an indicator of microbial oxidative activity, reflects the metabolic potential of soil microbial communities and is often used as a proxy for overall microbial activity, whereas extremes like drought or waterlogging inhibit its activity (Makoi & Ndakidemi, 2008; Filipović et al., 2020). Urease plays an important role in the nitrogen cycle, and soil organic matter content and the availability of urea or other nitrogenous substrates greatly affect urease activity (Sun et al., 2021a). Invertase provides a source of energy for soil microorganisms, is an important participant in the carbon cycle, and is closely linked to soil organic matter dynamics, with its activity influenced by root exudates, organic carbon availability and soil microbial biomass (Ma et al., 2023).

Despite significant progress in understanding the individual roles of these enzymes, the intricate relationships between nutrient availability and enzyme activity within ecosystems remain inadequately explored. Gaps persist in our knowledge of how environmental factors, particularly forest management practices like gap creation, affect enzyme dynamics. Addressing these gaps is essential for advancing forest management strategies. Specifically, understanding the impact of forest gap size on soil enzyme activity will provide valuable insights into optimizing forest ecosystems for biodiversity conservation, nutrient cycling, and overall ecological stability.

Within the rhizosphere, the phenomenon known as the rhizosphere effect (RE) encompasses alterations in nutrient cycling, enzyme activities, and microbial communities (Finzi et al., 2015; Chen et al., 2018). In soil ecosystems, enzyme and microbial activities are heightened in the rhizosphere compared to bulk soils devoid of roots (Pausch & Kuzyakov, 2011). Numerous studies have consistently demonstrated

that rhizosphere soils exhibit distinctive microbial behaviors and physicochemical properties compared to bulk soils without roots (Philippot et al., 2013). The discrepancy is attributed to the substantial influx of plant photosynthetic products into the rhizosphere, which subsequently alters soil properties and enhances nutrient assimilation (Nakayama & Taten, 2022). Concurrently, labile organic compounds, known as root exudates, are continuously released into the rhizosphere by the fine roots of plants (Kuzakov & Razavi, 2019). These root exudates serve as a primary reservoir of energy and substrate for microbial activities, thereby establishing the rhizosphere as a hotspot for heightened microbial abundance and activity (Wang et al., 2019a). Consequently, microbial and enzyme activities are significantly augmented within the rhizosphere soils (Kuzakov & Razavi, 2019).

Forest gaps play significant roles in forest succession and nutrient cycling (Gray et al., 2012; Yang et al., 2017). Upon the formation of forest gaps, environmental conditions such as light, temperature, and humidity undergo changes due to the sudden reduction of the forest canopy and a decrease in the degree of shading (d'Oliveira & Ribas, 2011). This alteration triggers the resurgence of understory vegetation, modifies soil nutrient cycling, and subsequently induces shifts in microbial and enzyme activities (Gray et al., 2012; Fei et al., 2023). In recent decades, there has been a growing emphasis on establishing silvicultural forest gaps that mimic natural disturbances, as they are more conducive to ecologically restoring forests (Muscolo et al., 2014). Creating the silvicultural forest gaps aims to conserve biodiversity and ecosystem functioning while optimising harvesting practices (Vajari et al., 2012). The size of the forest gap predicts the degree of the environmental heterogeneity it introduces (Muscolo et al., 2014). Forest gaps of different sizes also vary in the intensity of their impacts on environmental conditions and soils within and surrounding them (Nakayama & Taten, 2022).

Therefore, different sizes of forest gaps introduce varying degrees of environmental heterogeneity to forest ecosystems, resulting in alterations in understory environmental conditions. These environmental changes affect rhizosphere and bulk soils, prompting inquiries into how microbial communities, enzyme activities, and nutrient cycling respond to such shifts. This aspect necessitates further exploration and a deeper understanding and clarification. To investigate the effects of forest gap sizes on the RE of four soil enzymes in *Platycladus orientalis* (L.) Franco, 1950 plantation, three different sizes forest gaps were established in Xuzhou, China. A range of soil biological and physicochemical variables characterizing the soil, including microbial biomass nitrogen and carbon (MBN and MBC, respectively), total nitrogen and carbon (TN and TC, respectively), soil soluble organic carbon (SOC), NO_3^- -N, and NH_4^+ -N, were determined.

We hypothesized that (1) The heterogeneity of the understory environment increases with the expansion of forest gap size, leading to a corresponding rise in the RE of soil enzyme activities, and (2) the larger the forest gap size, the broader its effect on the RE of activity extends. This study provides valuable insights into the intricate interactions between forest gap size and the RE of soil enzyme activity. Consequently, this study may contribute to enhancing forest management strategies and practices.

Material and methods

Study region

The study region was selected in Xuzhou City (34°12' N, 117°30' E), Jiangsu Province, China (Figure 1a), with altitudes ranging from 50 to 60 m above sea level. Geographically situated within a warm-temperate zone, this region experiences a climatic pattern characterized by monsoons. Over the period from 2002 to 2013, the mean annual air temperature was 14.0 °C, the average monthly lowest and highest air temperature was -3.3 °C and 23.5 °C respectively, and the annual precipitation amounted to 872 mm. The dominant parent materials in the study region are primarily clay and loess. The main soil groups in Xuzhou are classified as silty clay loam and cinnamon soils. The fundamental characteristics of study region are summarized in Table 1.

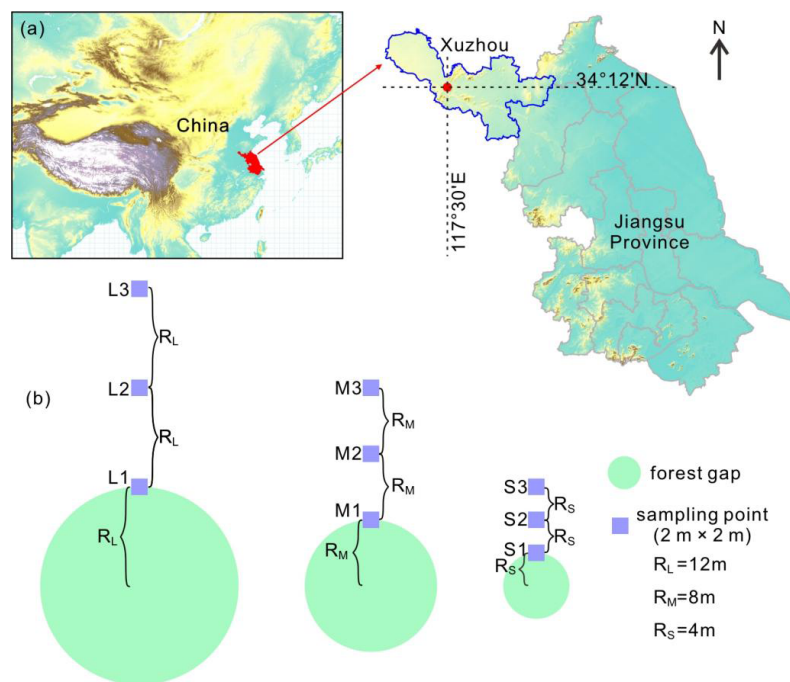


Figure 1. (a) The experimental sample plots were located in Xuzhou City, in the northwestern part of Jiangsu Province, China. (b) In this study, three circular forest gaps of different sizes were established, with radius of R_L , R_M , and R_S , respectively ($R_L=12\text{m}$, $R_M=8\text{m}$, and $R_S=4\text{m}$). From the center of each forest gap, proceeding due north, three 2 m×2 m sampling points were established at radius intervals. The sampling points were designated as L1, L2, L3, M1, M2, M3, S1, S2, and S3.

Table 1. Stand survey of *Platycladus orientalis* plantation in Zhaotuan Forest Farm of Xuzhou City, China.

Slope Aspect	Slope Gradient/(°)	Stand Age/(a)	Stand Density/(Tree·hm ⁻²)	Canopy Density/(%)	Height/(m)	Diameter at Breast Height/(cm)
Southeast	5	46	4728	85	8.7	9.5

The values are mean ± standard deviations. WC: water content; TC: total carbon; TN: total nitrogen; SOC: soluble organic carbon. (n=120)

Experimental design

In May 2016, various sizes forest gaps were established in a *P. orientalis* plantations at Zhaotuan Forest Farm in Xuzhou city in a randomized manner. The forest gaps were categorized based on their radius as 4 m (R_S), 8 m (R_M), and 12 m (R_L). Each gap size was represented by three replicates, and three control plots (CK) were also established within a contiguous closed-canopy forest. The distance between the CK plots and the forest gaps was ensured to be no less than 100 m, with all adjacent forest gaps and CK plots also being at least 100 m apart. From the center of each forest gap, proceeding due north, three 2 m×2 m sampling points were established at radius intervals (Figure 1b). Locations 1, 2, and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively. Depending on the distance of the sampling point from the centre of the forest gap, the sampling points were labeled as L1, L2, and L3 in the L forest gap, M1, M2, and M3 in the M forest gap, and S1, S2, and S3 in the S forest gap (Figure 1b). Following the falling of trees to create the forest gap, all plant debris in the area surrounding the forest gap was meticulously removed.

Samples were collected from three control plots and nine forest gaps. Within each sampling point, five soil samples were randomly selected, mixed, and labeled. This process entailed removing an undecomposed layer

of litter, followed by the extraction of soil samples from a depth of 0 to 20 cm. Fine roots (<2 mm) were delicately removed, and the soil within 2 mm of the fine roots was separated and labeled as rhizosphere soil. The remaining soil was classified as bulk soil. Subsequently, these soil samples underwent sieving using a 2 mm mesh, were appropriately labeled, and promptly transported for thorough analysis. Soil sampling was conducted at four time points in 2017 (April, June, August and October). The initial fundamental physical and chemical attributes of the sample plots are outlined in Table 2.

Table 2. Initial fundamental soil physical and chemical properties of the sample plots in the *Platycladus orientalis* plantation in Zhaotuan Forest Farm of Xuzhou City, China.

	WC (%)	TC (g·kg ⁻¹)	TN (g·kg ⁻¹)	NH ₄ ⁺ -N (mg·kg ⁻¹)	NO ₃ ⁻ -N (mg·kg ⁻¹)	SOC (mg·kg ⁻¹)
Bulk soil	21.58±0.75%	30.79±1.35	2.27±0.09	1.16±0.06	1.68±0.08	116.77±4.96
Rhizosphere soil	24.06±0.94%	33.56±1.59	2.61±0.11	1.35±0.09	1.87±0.10	129.40±6.11

The values are mean ± standard deviations. WC: water content; TC: total carbon; TN: total nitrogen; SOC: soluble organic carbon. (n=120)

Laboratory analysis

We measured the photosynthetically active radiation (PAR) at every sampling point and every distance point using the SKP 215 photosynthetically active radiation sensor (SKYE, UK), which was observed at 6:00-18:00 every 2 h on 6 days per month. Soil temperature (ST) was ascertained in situ employing DS1921-F5# temperature sensor (Maxim, USA), configured to record data at 30-minute intervals. Soil water content (WC) was obtained by calculating the loosed weight of fresh soil with the weight of approximately 10 g after 105 °C oven-drying for a duration of 24 h. Quantifications of TC and TN were executed utilizing the Vario EL III elemental analyzer (Elementar, Germany).

The concentrations of NO₃⁻-N and NH₄⁺-N were measured by extracted a soil sample in 2 mol/L KCl. Subsequently, the concentrations of NO₃⁻-N and NH₄⁺-N were evaluated with colorimetric method, employing the indophenol blue and the Griess assay method (Miranda et al., 2001) respectively. These solutions were conducted using Synergy HXT microplate reader (BioTek, USA) with the optical density readings taken at wavelengths of 636 nm and 540 nm, correspondingly.

We use Shimadzu TOC-VCPN carbon analyzer (Japan) to determinate the concentration of SOC. The chloroform fumigation extraction method was using to ascertain the MBN and MBC in the soil were (Brookes et al., 1985; Vance et al., 1987). Concisely, underwent fumigate 20 g of fresh soil with anhydrous CHCl₃ within a desiccator at 25 °C for 24 h. Post CHCl₃ removal, both unfumigated and fumigated soil samples were subjected to extraction with 80 ml of 0.5 mol/L K₂SO₄ for 30 min. We analyzed the organic nitrogen and carbon present in the solution using Shimadzu TOC-VCPN carbon analyzer (Japan). Applying a conversion factor of $k_{EN} = 0.68$: MBN = EN/ k_{EN} and $k_{EC} = 0.45$: MBC = EC/ k_{EC} the differential between the unfumigated and fumigated samples facilitated the computation of MBN and MBC was computed (Brookes et al., 1985; Joergensen & Mueller, 1996).

Soil enzyme activity was determined by adding substrates specific to different enzymes to air-dried soil and measuring the concentration of their products after a unit of time which was assessed using the colorimetric technique with spectrophotometric quantification. The activity of PER was ascertained and denoted as µg purple gallic acid g⁻¹ soil h⁻¹ (Markkola et al. 1990). The activity of DEH was estimated and denoted as µg TPF g⁻¹ soil h⁻¹ (Casida et al. 1964). Urease activity was determined and denoted as mg NH₄-N g⁻¹ soil d⁻¹ (Kandeler & Gerber, 1988). Invertase activity was obtained and denoted as mg glucose g⁻¹ soil d⁻¹ (Frankeberger & Johanson, 1983).

The RE was obtained as the differential between bulk soil and rhizosphere soil:

$$RE = \frac{C_{rhizosphere_soil} - C_{bulk_soil}}{C_{bulk_soil}}$$

$C_{rhizosphere_soil}$ denoted microbial biomass, soil properties, and enzyme activities in the rhizosphere soils, while C_{bulk_soil} denoted attributes in the bulk soils.

Statistical analysis

Repeated-measures analysis of variance (RMANOVA) of the effect of gap size, location, season and their interaction and graphical representations were conducted in the R Studio software program (version 4.2.0). One-way analysis of variance (ANOVA) in SPSS 26.0 was conducted to scrutinize the variances among disparate gap sizes, with subsequent utilizing the method of Least Significant Difference (LSD) ($p \leq 0.05$) to multiple comparisons. The correlations among the pertinent explanatory factors and soil enzyme activities were discerned by deployed Pearson correlation analysis in SPSS.

Results

Environmental factors in forest gaps

Forest gaps exerted significant effects on PAR in all seasons and across all sizes of forest gaps (Figure 2a). As depicted in Figure 2a, PAR was maximal at location 1 in every season and all forest gap sizes, decreasing proportionally with the distance from the center of the forest gap. The PAR at location 3 was the lowest and did not significantly different with CK (Figure 2a).

The size of the forest gaps had a more pronounced impact on ST in August (Figure 2b). As depicted in Figure 2b, ST at location 1 was the highest in August ($>35^\circ\text{C}$) across all sizes of forest gaps. In comparison to CK, ST showed no significant difference in June and October (Figure 2b). However, a decrease in ST was observed under all sizes of the forest gaps in October (22°C) (Figure 2b).

Soil physical and chemical properties and microbial biomass

In Figure 3a, it is evident that the WC was lower at location 1 than in the CK ($p < 0.05$) in June, specifically in the bulk soil. Similarly, in August, the WC was significantly lower than CK in all locations of the M and S gaps, and in October, it was notably lower in all locations of the M gaps ($p < 0.05$) (Figure 3a). Conversely, in October, the soil water content in all locations of the L gaps was significantly higher than CK ($p < 0.05$) (Figure 3a). From Figure 3b, it is apparent that in the rhizosphere soil, the WC was lower than CK at all locations except location L3 in August. However, in October, it was higher than CK at location L1 and L2, and lower than CK at all locations in M gaps and location S1 and S2 ($p < 0.05$) (Figure 3b).

As depicted in Figure 4a, in the bulk soils, TC was lower at M3 and S2 than CK in April ($p < 0.05$). In June and October, TC was significantly lower at all locations in the L gaps than CK ($p < 0.05$) (Figure 4a). Conversely, in June, TC was noticeably higher at all locations in the M gaps, and the opposite trend was observed in October ($p < 0.05$) (Figure 4a). Additionally, in October, TC was significantly higher than CK at all locations in the S gaps ($p < 0.05$) (Figure 4a). From Figure 4b, it is apparent that in the rhizosphere soils, TC was lower than CK in all seasons in the L gaps, while it was higher than CK in most locations and seasons in the M and S gaps ($p < 0.05$). Furthermore, as depicted in Figure 4c, in the bulk soils, TN was lower than CK in all locations in the L gaps in June, and higher than CK in April and June in the M gaps and location S2 ($p < 0.05$). Lastly, from Figure 4d, it can be observed that in rhizosphere soils, TN was lower in April, June, and October for all locations in the L gaps compared to CK, and lower in October for the M gaps and the locations S1 and S3 compared to CK ($p < 0.05$).

In Figure 5a, it is observed that in bulk soils, SOC levels at locations L1, L2 and S1 were lower than CK in August, while all locations in the M gaps exhibited significantly higher SOC levels than CK ($p < 0.05$).

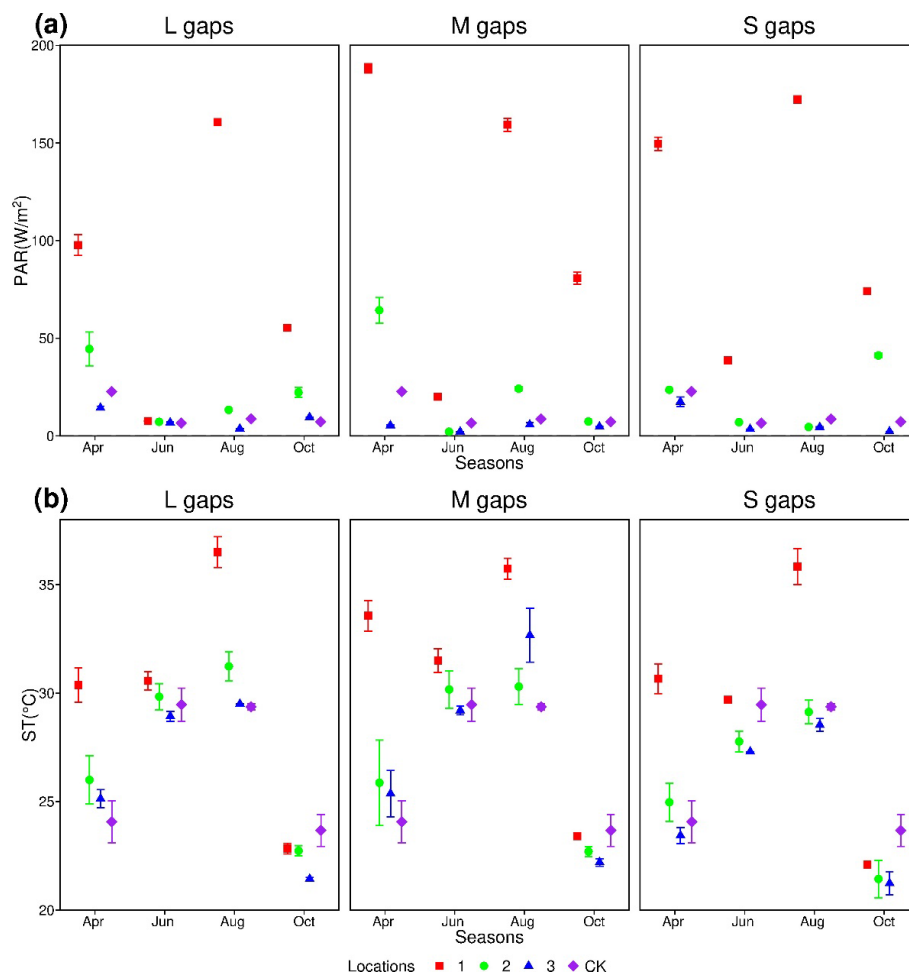


Figure 2. (a) Photosynthetically active radiation in different locations and gap sizes across seasons (b) Soil temperature in different locations and gap sizes across seasons. PAR: photosynthetically active radiation. ST: soil temperature. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

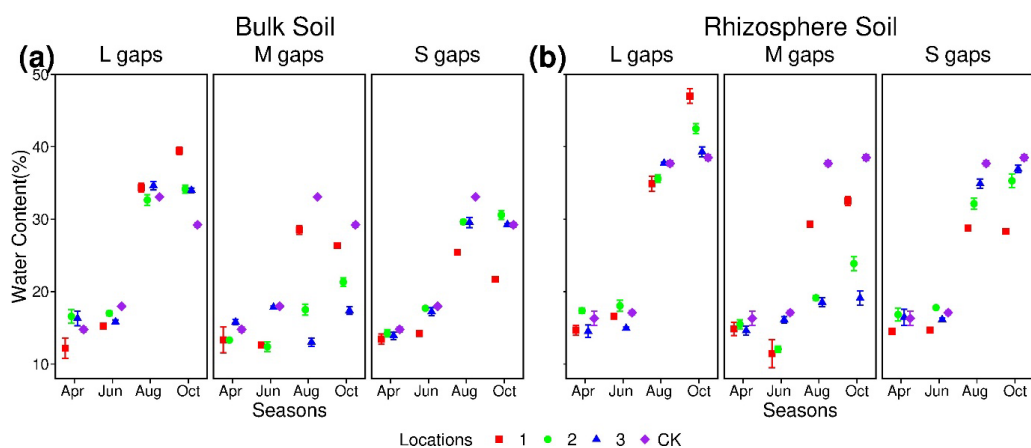


Figure 3. (a) Water content in different locations and gap sizes across seasons for: (a) bulk soil and (b) rhizosphere soil. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

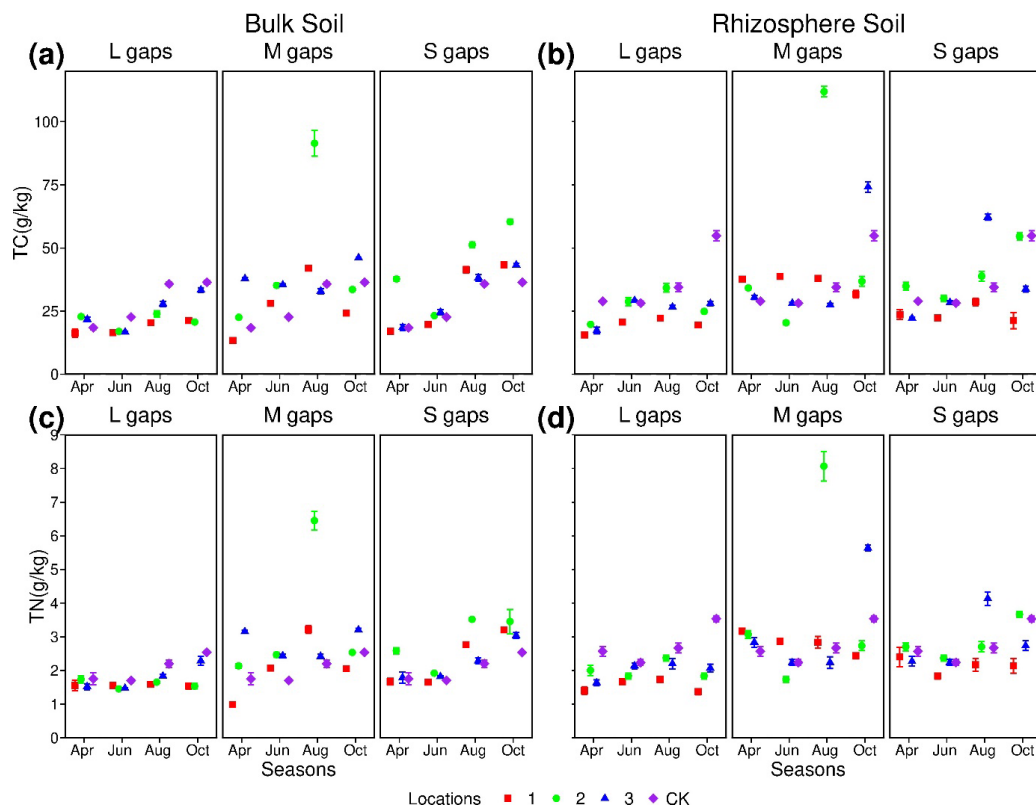


Figure 4. Total carbon content in different locations and gap sizes across seasons for: (a) bulk soil and (b) rhizosphere soil. Total nitrogen content in different locations and gap sizes across seasons for: (c) bulk soil and (d) rhizosphere soil. TC: total carbon; TN: total nitrogen. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

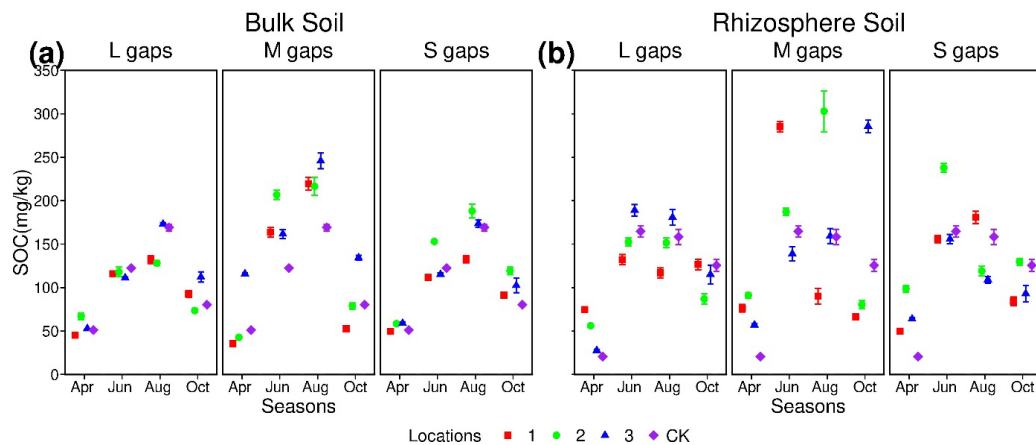


Figure 5. Soluble organic carbon content in different locations and gap sizes across seasons for: (a) bulk soil and (b) rhizosphere soil. SOC: soluble organic carbon. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

Figure 5b illustrates that in the rhizosphere soils, SOC levels at all locations except L3 were higher than CK in April, whereas in October, SOC levels at locations L2, M1, M2, S1, and S3 were lower than CK ($p < 0.05$).

As depicted in **Figure 6a**, in bulk soils, $\text{NH}_4^+\text{-N}$ levels were higher than CK at locations M2 and S2 in August, while they were lower than CK ($p<0.05$) at all locations in the L gaps in October. In the rhizosphere soil, as shown in **Figure 6b**, $\text{NH}_4^+\text{-N}$ levels were higher than CK at all locations in the M gaps in April and at the locations M2 and S2 in August, while they were lower than CK at all forest gap sizes in October ($p<0.05$). From **Figure 6c**, it is evident that in bulk soils, $\text{NO}_3^-\text{-N}$ levels at locations L1 and L2 were lower than CK in August, and higher than CK in August and October in the M and S gaps ($p<0.05$). Furthermore, as depicted in **Figure 6d**, in the rhizosphere soils, all locations in the L gaps had lower $\text{NO}_3^-\text{-N}$ levels than CK in October, while $\text{NO}_3^-\text{-N}$ levels in the M gaps and locations S2 and S3 were notably higher than CK in October ($p<0.05$).

Illustrated in **Figure 7a**, in bulk soils, MBC levels were lower than CK at all forest gap sizes in August ($p<0.05$). From **Figure 7b**, it is observed that in the rhizosphere soils, MBC levels were lower than CK at locations L1, M1, M2, and all locations of S forest gaps in October ($p<0.05$). Furthermore, as shown in **Figure 7c**, in bulk soils, MBN levels were lower in August at locations L2, L3, M1, and S3 compared to CK ($p<0.05$). Finally, from **Figure 7d**, it can be seen that in the rhizosphere soil, all locations except M3 had lower MBN levels than CK in October ($p<0.05$).

Rhizosphere effects of four soil enzyme activities and repeated-measures analysis of variance

Compared to CK, the RE on PER were elevated at locations M2 and S2 in June ($p<0.05$) and decreased at L3 and S2 locations in October ($p<0.05$) (**Figure 8**). The RE on DEH were lower than CK at locations M1, M2, and S3 in June ($p<0.05$) and lower than CK at all the locations except L2 in August ($p<0.05$) (**Figure 9**).

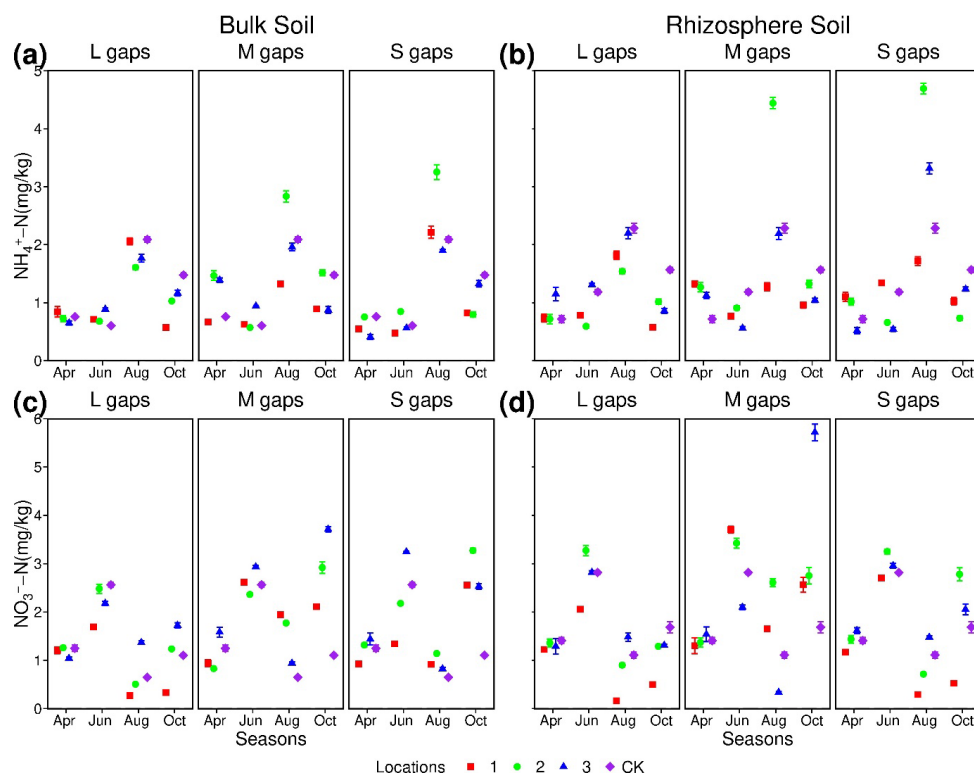


Figure 6. $\text{NH}_4^+\text{-N}$ content in different locations and gap sizes across seasons for: (a) bulk soil and (b) rhizosphere soil. $\text{NO}_3^-\text{-N}$ content in different locations and gap sizes across seasons for: (c) bulk soil and (d) rhizosphere soil. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

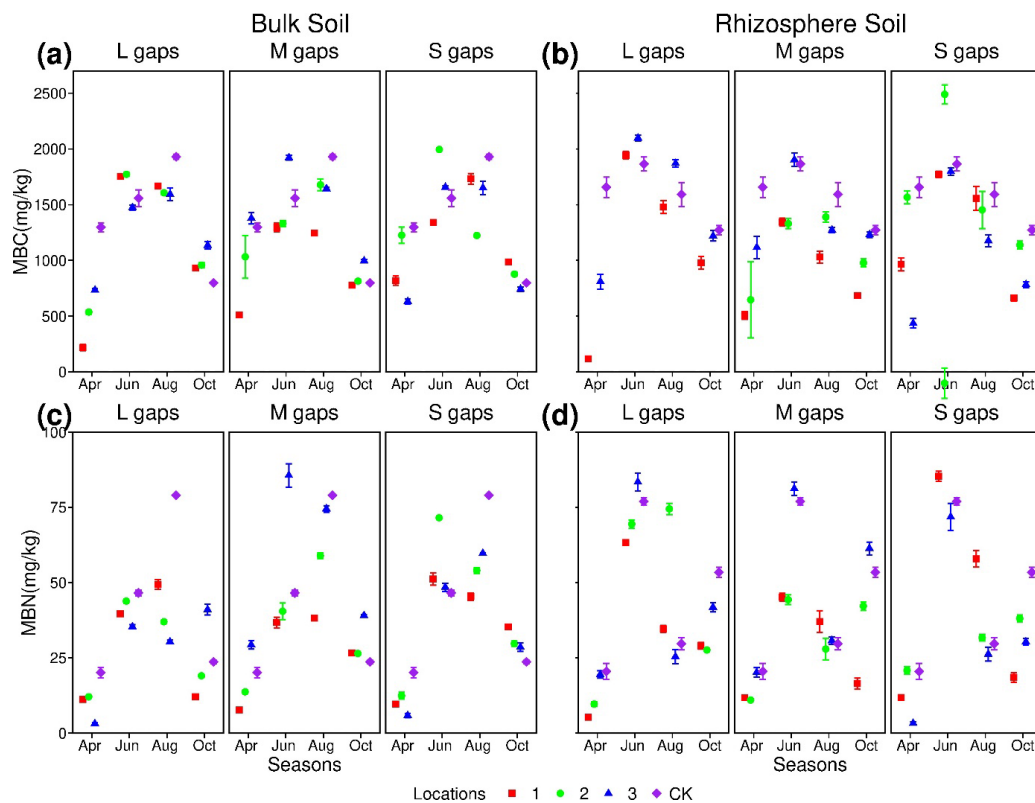


Figure 7. Microbial biomass carbon content in different locations and gap sizes across seasons for: (a) bulk soil and (b) rhizosphere soil. Microbial biomass nitrogen different locations and gap sizes across seasons for: (c) bulk soil and (d) rhizosphere soil. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

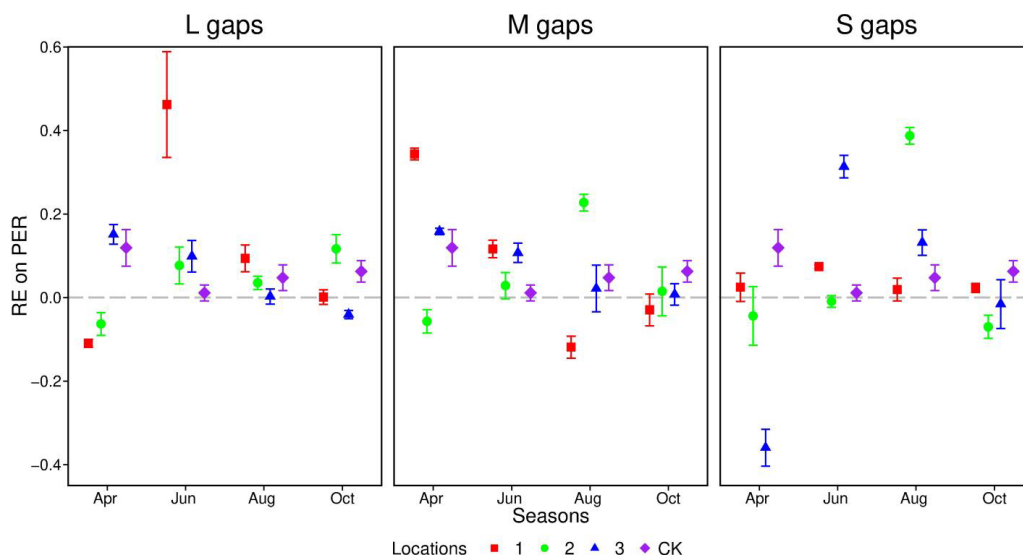


Figure 8. Rhizosphere effects on soil peroxidase activity content in different locations and gap sizes across seasons. RE: rhizosphere effect; PER: peroxidase. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

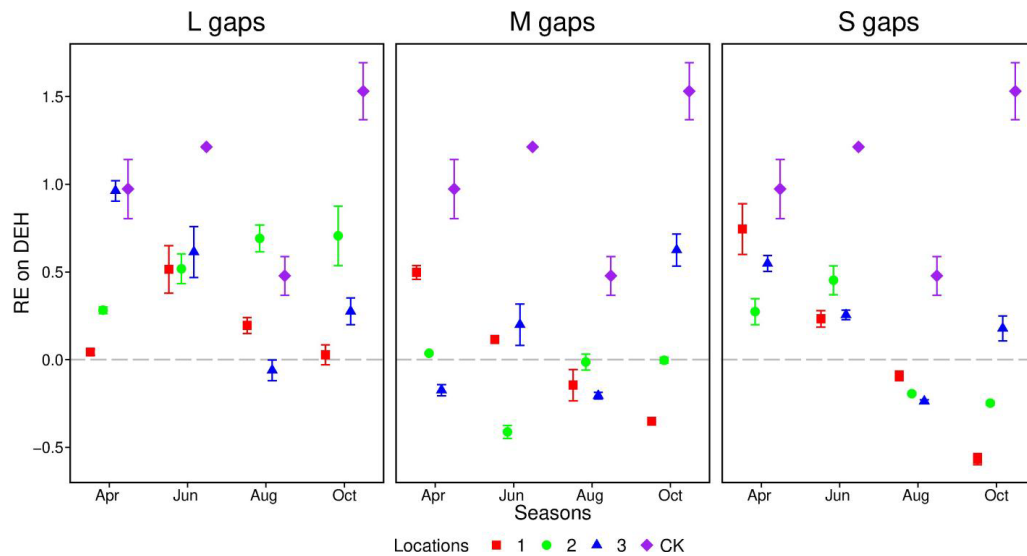


Figure 9. Rhizosphere effects on soil dehydrogenase activity content in different locations and gap sizes across seasons. RE: rhizosphere effect; DEH: dehydrogenase. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

In comparison to CK, the RE on URE were increased at locations L3, M1, and S1 in April ($p < 0.05$) and increased in L and S gaps but decreased in M gaps in June ($p < 0.05$) (Figure 10). In August, RE on URE were lower ($p < 0.05$) than CK at locations L1, L3, M2, M3, S1, and S2 but higher than CK at M1 and S3 ($p < 0.05$) (Figure 10). The RE on URE were lower than CK at L1, L2, L3, and S1 in October ($p < 0.05$) (Figure 10). The RE on INV were higher than CK at all the locations except locations L1 and M3 in April ($p < 0.05$) (Figure 11). Compared to CK, RE on INV were higher at locations L2 and S3 but lower at L3, M1, M2, M3, S1, and S2 locations in August ($p < 0.05$) (Figure 11). The RE on INV were lower than CK at all locations in October ($p < 0.05$) (Figure 11).

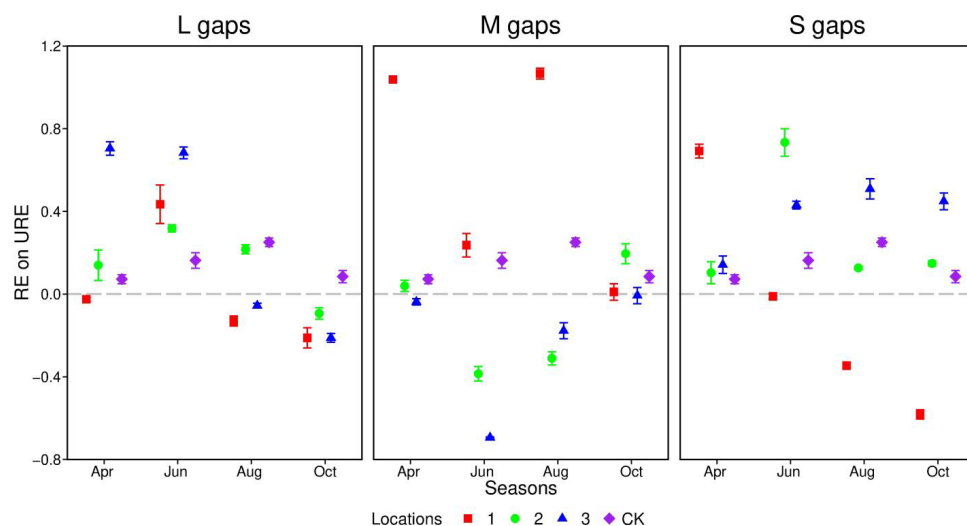


Figure 10. Rhizosphere effects on soil urease content in different locations and gap sizes across seasons. RE: rhizosphere effect; URE: urease. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

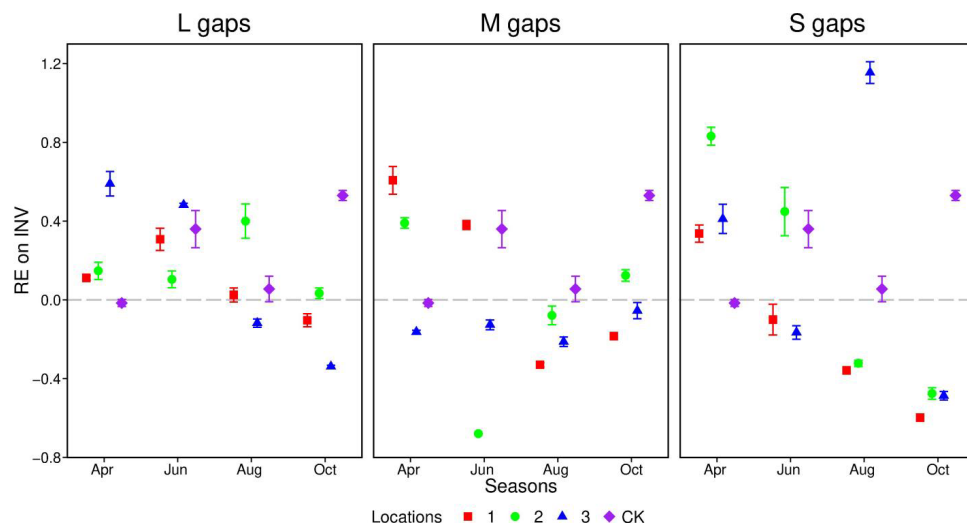


Figure 11. Rhizosphere effects on soil invertase activity content in different locations and gap sizes across seasons. RE: rhizosphere effect; INV: invertase. Locations 1, 2 and 3 represent 1 time the forest gap radius, 2 times the forest gap radius, and 3 times the forest gap radius, respectively.

From the RMANOVA (Table 3), gap size only significantly affected the RE on URE. Season significantly affected the RE on DEH (Table 3). The interaction of gap size and season strongly affected the RE on URE (Table 3). The interaction of gap size, location and season significantly affected the REs on DEH, URE and INV (Table 3).

Correlation analysis

We can observe the correlations between RE of soil enzyme activities and soil environmental factors in bulk and rhizosphere soil, respectively (Table 4). The correlation between RE on PER and ST was extremely significantly positive ($p < 0.01$), and the correlation between RE on DEH and PAR and ST was significantly positive ($p < 0.05$) (Table 4). In bulk soil, RE on PER was significantly positively correlated with $\text{NH}_4^+ \text{-N}$,

Table 3. *F* values in repeated-measures analysis of variance of the effect of gap size, location, season and their interaction on RE on the enzyme activities in the *Platycladus orientalis* plantation in Zhaotuan Forest Farm of Xuzhou City, China.

Effect	df	RE on PER	RE on DEH	RE on URE	RE on INV
Gap size	2	0.15	0.385	9.472**	1.37
Location	2	0.38	<0.001	0.802	0.032
Season	3	2.413	9.173**	0.01	2.376
Gap size × Location	4	0.193	0.723	0.69	2.925
Gap size × Season	6	0.190	3.728	12.184***	3.272
Location × Season	6	0.398	0.512	0.589	0.072
Gap size × Location × Season	12	3.435	15.601***	10.217**	5.446*

Note: *F* values are reported with *p* values indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. RE: rhizosphere effect; PER: peroxidase; URE: urease; DEH: dehydrogenase; INV: invertase.

Table 4. Pearson correlations between rhizosphere effect of enzymatic activity and environmental factors and soil physicochemical properties in rhizosphere and bulk soil in the *Platycladus orientalis* plantation in Zhaotuan Forest Farm of Xuzhou City, China.

Rhizosphere effect		PER	DEH	URE	INV
Environmental factors	PAR	ns	0.18*	ns	ns
	ST	0.25**	0.19*	ns	ns
Bulk Soil	WC	ns	ns	ns	-0.22*
	TC	ns	-0.3**	-0.38**	-0.36**
	TN	ns	-0.3**	-0.38**	-0.37**
	SOC	ns	ns	-0.36**	-0.32**
	MBC	0.3*	ns	ns	ns
	MBN	0.23*	-0.23*	-0.21*	-0.21*
	NO ₃ ⁻ -N	ns	ns	ns	-0.32**
	NH ₄ ⁺ -N	0.23*	-0.19*	-0.27**	ns
Rhizosphere Soil	WC	ns	ns	ns	0.22*
	TC	ns	ns	ns	ns
	TN	ns	ns	ns	ns
	SOC	ns	ns	ns	ns
	MBC	0.32**	ns	0.23*	ns
	MBN	0.19*	ns	0.25**	ns
	NO ₃ ⁻ -N	ns	ns	ns	ns
	NH ₄ ⁺ -N	0.34**	ns	0.22*	ns

Note: * Significant correlations ($p < 0.05$). ** Extremely significant correlations ($p < 0.01$). ns: non-significant. PAR: photosynthetically active radiation; ST: soil temperature; WC: water content; TC: total carbon; TN: total nitrogen; SOC: soluble organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; PER: peroxidase; URE: urease; DEH: dehydrogenase; INV: invertase.

MBC, and MBN ($p < 0.05$) (Table 4). Conversely, RE of DEH, URE, and INV were extremely significantly negatively correlated with TC and TN ($p < 0.01$), while they were significantly negatively correlated with MBN ($p < 0.05$) (Table 4). Rhizosphere effects on URE and INV were highly significantly negatively correlated with SOC ($p < 0.01$) (Table 4). Additionally, significantly negative correlations were found among various soil properties and RE of enzyme activities, such as between NO₃⁻-N and RE on INV and NH₄⁺-N and RE on DEH and URE ($p < 0.01$) (Table 4). In rhizosphere soil, RE on PER revealed extremely significantly positive correlations with NH₄⁺-N and MBC ($p < 0.01$) and was significantly positively correlated with MBN ($p < 0.05$) (Table 4). Furthermore, RE on URE demonstrated extremely significantly positive correlations with MBN ($p < 0.01$) and was significantly positively correlated with NH₄⁺-N and MBC ($p < 0.05$) (Table 4). Rhizosphere effect on INV was significantly positively correlated with WC ($p < 0.05$) (Table 4). However, correlation between RE on DEH and any factor in the rhizosphere soil is minor (Table 4).

Discussion

The RE on PER demonstrated a consistent positive trend across most seasons and sampling locations (Figure 8), showing a highly significant positive correlation with ST as indicated in Table 4. This finding suggests that the establishment of forest gaps had a marked stimulating effect on PER activity in the rhizosphere soil, with PER activity being greater sensitivity to fluctuations in soil temperature (Fei et al., 2023),

particularly in warmer months (August). Furthermore, MBC and MBN in both bulk and rhizosphere soils exhibited positive correlations with the RE on PER (Table 4), with notable increases at the M2 and S2 locations in August (Figure 8). This suggests that M and S gap sizes may create conditions that enhance microbial activity, and that this increase could be linked to the greater availability of organic substrates for microbial oxidation and decomposition. The strong positive correlation between RE on PER and $\text{NH}_4^+\text{-N}$ (Table 4) suggests that PER may play a critical role in nitrogen cycling in the rhizosphere, potentially facilitating the mineralization of organic matter into available forms. Furthermore, the significant correlation with MBC and MBN (Table 4) suggests that RE on PER may be involved in the breakdown of certain organic compounds, indirectly supporting microbial nutrient cycling in both rhizosphere and bulk soils. However, contrary to the hypothesis of our study, larger forest gaps and increased environmental heterogeneity did not result in enhanced RE of enzyme activity, nor did they extend range of influence on RE of enzyme activity. This result challenges our original expectations but aligns with findings from previous studies which suggested that smaller forest gaps are more conducive to higher soil enzyme activities (Muscolo et al., 2007; Yang et al. 2017). Additionally, Sinsabaugh (2010) emphasized a positive association between PER activity and litter quantity, proposing that smaller gaps tend to have higher litter accumulation, which can enhance enzymatic activity due to increased substrate availability.

Our results indicate that the RE on DEH were consistently lower than those of CK at most locations, with the exception of L2 in August (Figure 9). These findings suggest that the establishment of forest gaps significantly impacted DEH activity, particularly at M and S gap sizes, where environmental changes such as PAR and ST likely influenced microbial processes. Interestingly, the RE on DEH was more sensitive to fluctuations in PAR and ST than to soil factors in the rhizosphere (Table 4), pointing to bulk soil conditions as the primary drivers affecting DEH activity. Seasonal effects also played a key role in the RE on DEH (Table 3), with significant variations observed across different months. These seasonal variations could explain the observed increases in the RE on DEH during June and August (Figure 9), as higher temperatures and increased PAR likely stimulated microbial activity, leading to enhanced enzyme activity. This is in line with findings by Steinweg (2012), who highlighted the importance of temperature and moisture in regulating soil enzyme activities. Additionally, the negative correlations between RE on DEH and TC, TN, and MBN suggest that the availability of carbon and nitrogen might limit DEH activity under certain conditions (Table 4), possibly due to microbial competition for resources or imbalances in substrate availability.

Our results revealed a significant negative correlation between the RE on URE and $\text{NH}_4^+\text{-N}$ in bulk soil, while a positive correlation was observed in rhizosphere soil (Table 3). This differential response suggests that, under favorable hydrothermal conditions, substrate availability, particularly nitrogen, may limit URE activity in bulk soil following the forest gap establishment (Zuccarini et al., 2020, 2023). In comparison to the CK, the RE on URE showed an increasing trend in both L and S forest gaps in April and June, but declined in August and October (Figure 10). This temporal pattern highlights the greater sensitivity of URE activity to environmental changes in rhizosphere soils compared to bulk soils across different phases of the growing season (Xu et al., 2024). Notably, both in the rhizosphere and bulk soils, the RE of URE exhibited significant positive correlations with MBN, aligning with the findings of Fisher et al. (2017) and Corstanje et al. (2007), who emphasized the influence of microbial community composition on URE activity. These results further corroborate the observations by Wang et al. (2019b), who demonstrated the association between URE activity and soil nitrogen transformations, reinforcing the importance of microbial processes in mediating soil nitrogen cycling.

The changes in the RE on INV closely mirrored those of URE in our study, showing similar seasonal and spatial patterns (Figure 10 and 11). In April, the RE on INV increased across all forest gap sizes (Figure 11), indicating that forest gap creation positively influenced INV activity during the early growing season. However, by August and October, the RE on INV declined, following a distinct temporal pattern (Figure 11). This suggests that forest gaps initially enhanced INV activity by promoting favorable conditions for microbial activity, including increased substrate availability and rising temperatures in the rhizosphere soil.

(Zuccarini et al., 2023). In the later stage of the growing season, the decomposition of litter likely contributed substantial nutrients inputs to the bulk soil, potentially overshadowing the effects of forest gaps on INV activity in the rhizosphere. This shift could explain why INV activity in bulk soils was markedly higher than in the rhizosphere during the later months, and why the RE on INV was lower than CK in these periods. This finding aligns with previous research, which observed that INV activity was more responsive to changes in soil conditions during the early growing season, with a subsequent decline as the soil nutrient dynamics shifted, as seen in studies on organic mulching (Sun et al., 2021b) and straw returning practices (Wu et al., 2020). Additionally, in bulk soils, the RE on INV was negatively correlated with WC, TC, TN, SOC, MBN, and NO_3^- -N (Table 4). These results are consistent with the findings of Yang et al. (2017), who noted a decrease in INV activity in response to NH_4^+ -N additions during the early stages of plant growth, followed by an increase as the season progressed. Our study further emphasizes the complex interactions between soil nitrogen, carbon, and microbial activity, as these factors influence enzyme activity in response to varying forest gap sizes and the associated environmental changes.

In addition to the environmental factors discussed above, plant fine roots and microorganisms play a crucial role in shaping both bulk and rhizosphere soils (Sun et al., 2021b). Plants contribute organic substrates to the rhizosphere through litter deposition and root exudates, which regulate nutrient composition, enhance microbial habitat quality, and stimulate microbial abundance and metabolic activities (Sasse et al., 2018). The establishment of forest gaps significantly impacts these dynamics by altering soil properties and microbial communities. Given the pivotal role of plants in shaping the rhizosphere environment, the observed changes in enzyme activity following forest gap establishment can be attributed, in part, to the direct and indirect effects of plant root activity and microbial interactions on the soil environment.

Conclusions

During the growing season, forest gaps initially stimulated the RE of PER, URE, and INV, but these effects diminished as the season progressed, while the RE of DEH consistently decreased in both June and August. Additionally, forest gaps of L and S sizes influenced the RE of enzyme activity, with L gaps having the most pronounced impact. This study identified that the RE of soil enzyme activity did not increase with the enlargement of forest gap size. However, in L forest gaps, its impact range was more extensive, aligning with the assumptions made in Hypothesis 2 outlined earlier in this text.

Data availability: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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References

- Brookes PC, Landman A, Pruden G, Jenkinson DS, 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem* 17: 837-842. [https://doi.org/10.1016/0038-0717\(85\)90144-0](https://doi.org/10.1016/0038-0717(85)90144-0)
- Casida LE, Klein DA, Santoro T, 1964. Soil dehydrogenase activity. *Soil Sci* 98: 371-376. <https://doi.org/10.1097/00010694-196412000-00004>
- Chen X, Ding Z, Tang M, Zhu B, 2018. Greater variations of rhizosphere effects within mycorrhizal group than between mycorrhizal group in a temperate forest. *Soil Biol Biochem* 126: 237-246. <https://doi.org/10.1016/j.soilbio.2018.08.026>
- Corstanje R, Schulin R, Lark RM, 2007. Scale-dependent relationships between soil organic carbon and urease activity. *Eur J Soil Sci* 58: 1087-1095. <https://doi.org/10.1111/j.1365-2389.2007.00902.x>
- Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ, 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat Geosci* 8: 776-779. <https://doi.org/10.1038/ngeo2520>
- d'Oliveira MVN, Ribas LA, 2011. Forest regeneration in artificial gaps twelve years after canopy opening in Acre State Western Amazon. *For Ecol Manage* 261: 1722-1731. <https://doi.org/10.1016/j.foreco.2011.01.020>
- Fei F, Chen X, Guan Q, 2023. Short-term effects of forest gap size on soil enzyme activity in a *Platycladus orientalis* plantation. *Front Ecol Evol* 11: 1-9. <https://doi.org/10.3389/fevo.2023.1122796>
- Feng J, Wei K, Chen Z, Lü X, Tian J, Wang C, Chen L, 2019. Coupling and Decoupling of Soil Carbon and Nutrient Cycles Across an Aridity Gradient in the Drylands of Northern China: Evidence From Ecoenzymatic Stoichiometry. *Global Biogeochem Cycles* 33: 559-569. <https://doi.org/10.1029/2018GB006112>
- Filipović L, Romić M, Sikora S, Huić Babić K, Filipović V, Gerke HH, Romić D, 2020. Response of Soil Dehydrogenase Activity to Salinity and Cadmium Species. *J Soil Sci Plant Nutr* 20: 530-536. <https://doi.org/10.1007/s42729-019-00140-w>
- Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, Phillips RP, 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Glob Chang Biol* 21: 2082-2094. <https://doi.org/10.1111/gcb.12816>
- Fisher KA, Yarwood SA, James BR, 2017. Soil urease activity and bacterial ureC gene copy numbers: Effect of pH. *Geoderma* 285: 1-8. <https://doi.org/10.1016/j.geoderma.2016.09.012>
- Frankeberger WT, Johanson JB, 1983. Method of measuring invertase activity in soils. *Plant Soil* 74: 301-311. <https://doi.org/10.1007/BF02181348>
- Gray AN, Spies TA, Pabst RJ, 2012. Canopy gaps affect long-term patterns of tree growth and mortality in mature and old-growth forests in the Pacific Northwest. *For Ecol Manage* 281: 111-120. <https://doi.org/10.1016/j.foreco.2012.06.035>
- Joergensen RG, Mueller T, 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEN value. *Soil Biol Biochem* 28: 33-37. [https://doi.org/10.1016/0038-0717\(95\)00101-8](https://doi.org/10.1016/0038-0717(95)00101-8)
- Kandeler E, Gerber H, 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6: 68-72. <https://doi.org/10.1007/BF00257924>
- Kuzyakov Y, Razavi BS, 2019. Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biol Biochem* 135: 343-360. <https://doi.org/10.1016/j.soilbio.2019.05.011>
- Ma Y, Yue K, Heděnc P, Li C, Li Y, Wu Q, 2023. Global patterns of rhizosphere effects on soil carbon and nitrogen biogeochemical processes. *CATENA* 220: 106661. <https://doi.org/10.1016/j.catena.2022.106661>
- Makoi JHJR, Ndakidemi PA, 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. *African J Biotechnol* 7: 181-191. <https://doi.org/10.4314/ajb.v7i3.58355>
- Markkola AM, Ohtonen R, Tarvainen O, 1990. Peroxidase activity as an indicator of pollution stress in the fine roots of *Pinus sylvestris*. *Water Air Soil Pollut* 52: 149-156. <https://doi.org/10.1007/BF00283120>
- Miranda KM, Espey MG, Wink DA, 2001. A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. *Nitric Oxide* 5: 62-71. <https://doi.org/10.1006/niox.2000.0319>
- Muscolo A, Sidari M, Mercurio R, 2007. Influence of gap size on organic matter decomposition, microbial biomass and nutrient cycle in Calabrian pine (*Pinus laricio*, Poiret) stands. *For Ecol Manage* 242: 412-418. <https://doi.org/10.1016/j.foreco.2007.01.058>
- Muscolo A, Bagnato S, Sidari M, Mercurio R, 2014. A review of the roles of forest canopy gaps. *J For Res* 25: 725-736. <https://doi.org/10.1007/s11676-014-0521-7>

- Nakayama M, Tateno R, 2022. Rhizosphere effects on soil extracellular enzymatic activity and microbial abundance during the low-temperature dormant season in a northern hardwood forest. *Rhizosphere* 21: 100465. <https://doi.org/10.1016/j.rhisph.2021.100465>
- Pausch J, Kuzyakov Y, 2011. Photoassimilate allocation and dynamics of hotspots in roots visualized by ¹⁴C phosphor imaging. *J Plant Nutr Soil Sci* 174: 12-19. <https://doi.org/10.1002/jpln.200900271>
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH, 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11: 789-799. <https://doi.org/10.1038/nrmicro3109>
- Sasse J, Martinoia E, Northen T, 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends Plant Sci* 23: 25-41. <https://doi.org/10.1016/j.tplants.2017.09.003>
- Sinsabaugh RL, 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol Biochem* 42: 391-404. <https://doi.org/10.1016/j.soilbio.2009.10.014>
- Steinweg JM, Dukes JS, Wallenstein MD, 2012. Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. *Soil Biol Biochem* 55: 85-92. <https://doi.org/10.1016/j.soilbio.2012.06.015>
- Sun X, Ye Y, Guan Q, Jones DL, 2021a. Organic mulching masks rhizosphere effects on carbon and nitrogen fractions and enzyme activities in urban greening space. *J Soils Sediments* 21: 1621-1632. <https://doi.org/10.1007/s11368-021-02900-7>
- Sun X, Ye Y, Ma Q, Guan Q, Jones DL, 2021b. Variation in enzyme activities involved in carbon and nitrogen cycling in rhizosphere and bulk soil after organic mulching. *Rhizosphere* 19: 100376. <https://doi.org/10.1016/j.rhisph.2021.100376>
- Uto EB, Tewari L, 2015. Soil enzymes as bioindicators of soil ecosystem status. *Appl Ecol Environ Res* 13: 147-169. https://doi.org/10.15666/aecr/1301_147169
- Vajari KA, Jalilvand H, Pourmajidian MR, Espahbodi K, Moshki A, 2012. Effect of canopy gap size and ecological factors on species diversity and beech seedlings in managed beech stands in Hyrcanian forests. *J For Res* 23: 217-222. <https://doi.org/10.1007/s11676-012-0244-6>
- Vance ED, Brookes PC, Jenkinson DS, 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19: 703-707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6)
- Wang Q, Xiao J, Ding J, Zou T, Zhang Z, Liu Q, Yin H, 2019a. Differences in root exudate inputs and rhizosphere effects on soil N transformation between deciduous and evergreen trees. *Plant Soil* 458: 277-289. <https://doi.org/10.1007/s11104-019-04156-0>
- Wang Z, Yang H, Wang D, Zhao Z, 2019b. Spatial distribution and growth association of regeneration in gaps of Chinese pine (*Pinus tabulaeformis* Carr.) plantation in northern China. *For Ecol Manage* 432: 387-399. <https://doi.org/10.1016/j.foreco.2018.09.032>
- Wu L, Ma H, Zhao Q, Zhang S, Wei W, Ding X, 2020. Changes in soil bacterial community and enzyme activity under five years straw returning in paddy soil. *Eur J Soil Biol* 100: 103215. <https://doi.org/10.1016/j.ejsobi.2020.103215>
- Xu H, Gan Q, Huang L, Pan X, Liu T, Wang R, Wang Limengjie, Zhang L, Li H, Wang Lixia, Liu S, Li J, You C, Xu L, Tan B, Xu Z, 2024. Effects of forest thinning on soil microbial biomass and enzyme activity. *CATENA* 239: 107938. <https://doi.org/10.1016/j.catena.2024.107938>
- Yang Y, Geng Y, Zhou H, Zhao G, Wang L, 2017. Effects of gaps in the forest canopy on soil microbial communities and enzyme activity in a Chinese pine forest. *Pedobiologia (Jena)* 61: 51-60. <https://doi.org/10.1016/j.pedobi.2017.03.001>
- Zuccarini P, Sardans J, Asensio L, Peñuelas J, 2023. Altered activities of extracellular soil enzymes by the interacting global environmental changes. *Glob Chang Biol* 29: 2067-2091. <https://doi.org/10.1111/gcb.16604>
- Zuccarini P, Asensio D, Ogaya R, Sardans J, Peñuelas J, 2020. Effects of seasonal and decadal warming on soil enzymatic activity in a P-deficient Mediterranean shrubland. *Glob Chang Biol* 26: 3698-3714. <https://doi.org/10.1111/gcb.15077>