# Rhizosphere Priming Effects On Soil Extracellular Enzymatic Activity And Microbial Abundance During The Low-Temperature Dormant Season In A Northern Hardwood Forest

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# Abstract

### Purpose

Plant roots alter nutrient cycling within the soil surrounding them (rhizosphere). Recent studies have focused on nutrient uptake by plants in low-temperature seasons. This study aimed to reveal the nutrient dynamics in rhizosphere during low-temperature seasons in a northern hardwood forest in Japan.

### Methods

The potential extracellular enzymatic activity, bacterial, fungal, and archaeal abundances, and soil chemical properties in the rhizosphere of canopy trees and understory vegetation and non-rhizosphere bulk soil were measured at the beginning of the dormant season (November), end of the dormant season (April and May), and middle of the growing season (August) in a northern hardwood forest in Japan.

### Results

The abundance of fungi and the activity of nitrogen- and phosphorus-degrading enzymes were higher in the rhizosphere than in non-rhizosphere bulk soil regardless of the season. The concentration of extractable organic and inorganic N was higher in the rhizosphere than in the non-rhizosphere bulk soil at the beginning and end of the dormant season, but this trend was not observed in the middle of the growing season.

### Conclusion

Since the concentration of nutrients in the rhizosphere is determined by the balance between nutrient uptake by fine roots and root-induced acceleration of decomposition, our results suggest that plant roots would accelerate N and P cycles during the dormant season, even though the amount of nutrient uptake by plants was lower during the season.

# Introduction

Nutrient availability regulates the primary production of forests and consequently ecosystem C sequestration. In temperate forests, N often limits primary production (LeBauer and Treseder 2008). Phosphorus is also a key element, especially in volcanic ash soil such as andosols (Shoji and Takahashi 2002). Most nutrients are stored in complex organic materials, which plants cannot directly utilize in forest soils (Borie and Rubio 2003; Bormann et al. 1977). Instead, nutrients are transformed by microbial decomposition and mineralization into plant-available forms. Microbes such as bacteria and fungi produce diverse extracellular enzymes involved in organic matter decomposition and mineralization (Nikitina et al. 2010; Rodríguez and Fraga 1999; Talbot et al. 2008) and are the main drivers of nutrient cycling in forest soils (Isobe and Ohte 2014).

In the soil surrounding plant fine roots (rhizosphere), the processes of organic matter decomposition and mineralization are accelerated by the fine roots (Bengtson et al. 2012; Cheng et al. 2003; Dijkstra et al. 2009; Phillips and Fahey 2006; Yin et al. 2014). Plants continuously release labile organic compounds called root exudates into the rhizosphere. Since root exudates are the sources of energy and substrates for microbial activity, the activity and populations of microbes are higher in the rhizosphere than in non-rhizosphere bulk soil (Kuzyakov and Razavi 2019; Wang et al. 2019). Therefore, the activity of extracellular enzymes produced by microbes is higher in the rhizosphere (Kuzyakov and Razavi 2019). This alteration of microbial communities, enzyme activity, and, consequently, nutrient cycling within the rhizosphere is called the rhizosphere priming effect (RPE). Although the rhizosphere comprises a limited area of soil, the C and N mineralization accelerated by the RPE is reported to account for one-third of the total surface soil C and N mineralization in temperate forest soils (Finzi et al. 2015). Root exudates, which constitute photosynthetically produced organic C, are among the most important factors for the RPE (Shahzad et al. 2015); therefore, most studies on RPEs have focused on the plant growing season (Huo et al. 2017), when photosynthetic activity is high. However, there is a lack of knowledge on the RPE in dormant seasons, especially in forest soils.

The long-lasting winter in northern hardwood forests is considered a dormant season for plants. However, studies have shown that tree species, including deciduous trees, take up nutrients from soils even in winter (Andresen and Michelsen 2005; Ueda et al. 2015; Zavišić and Polle 2018). Furthermore, although photosynthetic activity is relatively low in winter (Voříškova et al. 2014), root exudates are released in winter by evergreen tree species (Phillips et al. 2008). Deciduous trees also release a certain amount of root exudates after leaf fall, at least at the beginning of the dormant season (Nakayama and Tateno 2018). Studies have also reported that microbial communities are active at sub-zero temperatures (Clein and Schimel 1995; Isobe et al. 2018), and organic matter decomposition and mineralization have been observed even under snow cover (Clein and Schimel 1995; Hishi et al. 2014; Isobe et al. 2018). Therefore, during plant dormant seasons, plants could stimulate microbial growth and extracellular enzyme production so as to take up nutrients efficiently from their rhizosphere—the nutrients taken up in winter are used for spring growth (Ueda et al. 2011). Moreover, because of climate change, the importance of understanding nutrient cycling processes during winter has been emphasized (Blankinship and Hart 2012; Campbell et al. 2005). Therefore, revealing the rhizosphere processes in winter is needed to better understand soil nutrient cycles and plant growth in forests.

In the northern hardwood forests of Japan, the forest floor is covered by a dense understory vegetation of evergreen dwarf bamboo (*Sasa* spp.). Fukuzawa et al. (2006) reported that dwarf bamboo prevented N loss by taking up excess N after the clear-cutting of canopy trees. The aboveground biomass of dwarf bamboo is lower than that of canopy trees (Fukuzawa et al. 2015), while the fine root biomass of dwarf bamboo is comparable to that of canopy trees (Fukuzawa et al. 2007, 2021; Tateno et al. 2020). Even though a meta-analysis reported that the magnitude of the RPE was higher for tree species than for herbaceous species (Huo et al. 2017), the contribution of understory vegetation, as well as that of canopy trees, should be considered regarding the RPE on the nutrient cycle. Additionally, the culm and leaf longevities of *Sasa nipponica* (Makino) Makino & Shibata, the understory vegetation at this study site, are

approximately 1 year, and several culms and leaves survive winter and have higher photosynthetic activity in May (Kayama and Koike 2018). The leafless season of canopy trees provides more favorable light conditions for understory vegetation rather than summer (Kudo et al. 2008). Considering that a large part of root exudates is recently assimilated C (Epron et al. 2011; Kuzyakov and Cheng 2001; Sanaullah et al. 2012), the RPE of dwarf bamboo would be higher and more significant than that of canopy trees at the beginning and end of the canopy tree dormant season. However, as we described above, previous studies on the RPE have mainly focused on the growing season.

Here, we aim to reveal how plants in a northern hardwood forest affect rhizosphere processes at the beginning and end of the dormant season, which is the leafless season for canopy trees. For this purpose, we measured microbial abundance and extracellular enzyme activity involved in the nutrient cycle in the rhizospheres of canopy trees and understory vegetation as well as in the non-rhizosphere bulk soils. For the canopy tree *Quercus crispula* Blume, leaf-fall usually occurs in late October, and leaf flush occurs in late May to early June (Nakayama and Tateno 2018; Tateno et al. 2019). The soil is covered by snow from mid-November to April, and the soil is frozen from December to May (Hosokawa et al. 2017); therefore, we did not collect samples from the period when the soil was frozen during the mid-dormant season. Thus, we collected samples in November (beginning of the dormant season: after leaf-fall and before snow cover), May (end of the dormant season: after snowmelt and before leaf flush), and August (mid-growing season). We hypothesized that (1) both the canopy trees and understory vegetation have positive RPE on enzymatic activity and microbial abundance at the beginning and end of the dormant season as well as during the mid-growing season, enabling nutrients to be taken up effectively from their rhizosphere. (2) The magnitude of the RPE is higher for the understory vegetation than for canopy trees at the beginning and end of the dormant season but lower during the mid-growing season.

# Materials And Methods

2.1 Study site

This study was conducted in a cool-temperate deciduous forest in eastern Hokkaido, Japan (43°24.2' N, 144°38.5' E), managed by the Shibecha Branch of the Hokkaido Forest Research Station, Field Science Education and Research Center, Kyoto University. The soils at this site are andosols (IUSS Working Group WRB 2015). The mean annual air temperature and precipitation (1986–2015) at a meteorological station located approximately 9 km south of the study site were 6.3 ℃ and 1,189 mm, respectively. The daily average air temperatures measured at the meteorological station during the experiment are shown in Fig. 1.

Sampling was conducted in the four plots (15 m × 15 m) established in our previous study (Nakayama and Tateno 2018). In the plots, the canopy tree was *Q. crispula*, and the forest floor was densely covered with dwarf bamboo (*S. nipponica*). The plots were separated by at least 30 m from each other. The fine root density of *Q. crispula* at a depth of 0–10 cm in the plots was 132.9 ± 47.8 g $m^{-2}$ (Nakayama and

Tateno 2018), and that of *S. nipponica* was 88.0 ± 55.9 g $m^{-2}$. More detailed information on the study plots can be found in previous reports (Nakayama and Tateno 2018, 2021).

The soil temperature at 5 cm soil depth in the plots was measured once every 30 min during the experiment using a temperature sensor with a data logger (TR-52i; T & D Corporation, Nagano, Japan). The sensor was removed on November 26, 2019 and April 28 and May 22, 2020, for data collection and a battery change and reburied on the same day. The daily average soil temperatures in the study plots are shown in Fig. 1.

2.2 Soil sampling and treatment

Mineral soils at a 0–10 cm-depth (A horizon) were collected using a shovel from each plot after removing the litter layer by hand. Soil sampling was conducted for 4 days during each sampling season, November 21, 23, 27, and 29, 2019 (beginning of the dormant season; hereafter, early winter); April 29, May 1, 3, and 5 (end of the dormant season; hereafter, early spring); August 23, 25, 28, and 30, 2020 (mid-growing season; hereafter, mid-summer). Soil samples were placed on ice in the field and refrigerated at 4 ℃ until processing.

In the laboratory, soil samples were sieved using a 4 mm mesh sieve. Soil that passed the sieve was considered as non-rhizosphere bulk soil (BS). The fine roots left on the sieve were carefully picked up by hand and forceps and separated into three types, *Q. crispula* and *S. nipponica* roots and others, including dead roots and roots of other species, based on their morphological traits (e.g., mycorrhizal type, color, and branching pattern). The ‘other’ roots constituted a small proportion and were discarded. Next, the fine roots of *Q. crispula* and *S. nipponica* were gently shaken, after which the soil adhering to their roots was collected as the rhizosphere (Phillips and Fahey 2006) and considered as the canopy tree rhizosphere (TR) and understory rhizosphere (UR), respectively.

After sieving and separation, the samples were divided into wet, oven-dry, and frozen subsamples. Oven-dried subsamples were dried at 60 ℃ for more than 72 h. Wet and frozen subsamples were stored at 4 and − 20 ℃, respectively, until further processing.

2.3 Soil chemical analysis

To measure the gravimetric water content of the samples, the oven-dried subsamples were weighed before and after oven-drying. Total N and C contents of oven-dried samples were then measured using an elemental analyzer (Sumigraph NC-900; Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Next, 2 g of dried soil was extracted in 5 mL of deionized water, and then the pH of the extracts was measured using a pH meter (HORIBA D-51; Horiba, Ltd., Kyoto, Japan).

A portion of the frozen subsample was extracted in 2 *M* KCl (extracted from 1 g of the wet weight of the frozen subsample into 5 mL of 2 *M* KCl) to measure the concentration of total extractable N (TEN), $NO_3^-$-N, $NH_4^+$-N, and extractable organic N (EON). The concentration of $NO_3^-$-N and $NH_4^+$-N were

colorimetrically measured using the Griess assay and indophenol blue method (Miranda et al. 2001), using a microplate reader (Synergy HXT; BioTek, Winooski, VT, USA), at wavelengths of 540 and 636 nm, respectively. To measure the concentration of TEN, 1 mL of 2 *M* KCl extracts was mixed with 1 mL of deionized water and 200 µL of alkaline potassium persulfate solution and then autoclaved (121 ℃, 20 min). Thereafter, TEN concentration was measured as $NO_3^-$-N concentration using the aforementioned method. The concentration of EON was calculated by subtracting the sum of inorganic N ($NO_3^-$-N and $NH_4^+$-N) from TEN.

2.4 Extracellular enzymatic activity measurements

The enzyme assays were conducted within 24 h of sampling using wet subsamples. The activities of six extracellular enzymes (Online Resource 1) involved in C, N, and P cycles were measured following the method of Saiya-Cork et al. (2002) with a few modifications. For the hydrolytic enzyme assays, i.e., *β*-xylosidase (XY), *β*-glucosidase (GL), acid phosphatase (AP), and *β*-1,4-N-acetylglucosaminidase (NAG), 1.0 g of wet soil sample was suspended in 100 mL of 50 mM sodium acetate (pH 5.0). The suspensions were mixed well using a magnetic stirrer for 1 min. Afterward, 800 µL of aliquots was dispensed into sample wells (four replicate wells per sample per enzyme) and quenching standard wells of 96-well deep-well plates. For the quenching standard wells, 200 µL of 4-methylumbelliferone (MUB) was added (concentrations: 0, 1, 2, 4, 10, 20, 40, and 100 µM). In the negative control well, 800 µL of acetate buffer was added. Subsequently, 200 µL of a 200 µM-substrate was added to the sample wells, after which the deep-well plates were incubated at 20 ℃ in the dark for 1 h. After incubation, the deep-well plates were centrifuged for 3 min at max speed (600 × g) (Bell et al. 2013). Thereafter, 250 µL supernatant was transferred into a 96-well black microplate; then the fluorescence was measured using a microplate reader (Synergy HXT, BioTek). The wavelengths of excitation and emission were 360 and 460 nm, respectively.

For the oxidative enzyme assays, phenol oxidase (PO) and peroxidase (PE), 800 µL of soil suspensions and 200 µL of 5 mM L-3,4-dihydroxyphenylalanine (DOPA) as the substrate were added to sample wells. Negative control wells contained 800 µL of acetate buffer and 200 µL of DOPA, and blank control wells contained 800 µL of soil suspensions and 200 µL of acetate buffer. For the PE assay, 40 µL of 0.3% $H_2O_2$ was further added to each well. Next, the deep-well plates were incubated in the dark at 20 ℃ for 10 h. The deep-well plates were then centrifuged for 3 min at max speed, and 250 µL of supernatants were transferred into a flat-bottom clear microplate. Enzyme activity was quantified by measuring absorbance at 450 nm using the same microplate reader.

2.5 DNA extraction and quantification of microbial gene abundance

Soil DNA was extracted from 0.25 g wet weight of frozen soil subsamples using a DNA extraction kit (DNeasy PowerSoil Pro Kit; QIAGEN, Hilden, Germany), following the manufacturer's protocol. The extracted DNA solution was stored at -20 ℃ until further analysis.

Microbial gene abundance quantification by real-time quantitative polymerase chain reaction (qPCR) was performed using a LightCycler 96 System (Roche Diagnostics K.K., Mannheim, Germany), with the intercalating dye SYBR Green I (FastStart Essential DNA Green Master; Roche Diagnostics K.K.). The bacterial and archaeal 16S rRNA genes and fungal ITS regions of the rRNA genes were quantified to estimate total abundances of bacteria, archaea, and fungi, respectively. In addition, we estimated the abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) by quantifying the bacterial and archaeal ammonia monooxygenase gene (*amoA*), respectively. The subsequent steps and primer set used for qPCR have been described in Nakayama et al. (2021).

2.6 Statistical analyses

To test the difference in soil chemical properties, enzymatic activity, and microbial gene abundance among sampling seasons (early winter, early spring, and mid-summer) and sample positions (BS, TR, and UR), a two-way analysis of variance (ANOVA) with random effects (random effects: sampling date and plots) was used. We conducted multiple comparisons using the emmeans function in R with Tukey's adjusted *P*-value to test the difference in soil chemical properties, enzymatic activity, and microbial gene abundance. The magnitude of the RPE was calculated as the percentage difference between the paired rhizosphere and bulk soil sample for each variable. To determine whether the magnitude of the RPE significantly differed from zero, a *t*-test was used. The differences in the RPE magnitude among sampling seasons and between sampling positions (TR vs. UR) were tested using a two-way ANOVA followed by multiple comparisons as described above. All statistical analyses were conducted in R ver. 4.0.5 (R Core Team 2021).

# Results

3.1 Soil chemical properties

Regardless of the sampling season, the pH of rhizospheres was significantly lower than that of BS, while the C/N ratio was stable among different seasons and sampling positions (Table 1). The EON concentrations were significantly higher in the rhizosphere, especially in TR, than in BS during the dormant season; however, this trend was not observed in mid-summer (Table 1). The concentration of $NH_4^+$-N was significantly higher in rhizospheres than in BS regardless of the season, and that in TR tended to be higher than that in UR during early winter and mid-summer (Table 1). In contrast to EON and $NH_4^+$-N concentration, the concentration of $NO_3^-$-N in UR was significantly higher than that in TR and BS during early winter and mid-summer, and there was a non-significant difference in the concentrations between TR and BS (Table 1). Thus, there was a non-significant effect of sampling season on soil chemical properties except for EON.

Table 1 Chemical properties of soils.

| | | | Extractable nitrogen (mg-N $kg^{-1}$ dry soil) | | |
|---|---|---|---|---|---|
| | pH | C/N ratio | EON | $NH_4^+$-N | $NO_3^-$-N |
| Early winter | | | | | |
| Bulk soil | $5.2 \pm 0.3^a$ | 14.5 ± 0.6 | $67.8 \pm 17.9^c$ | $10.0 \pm 8.7^b$ | $1.0 \pm 2.3^b$ |
| Tree rhizosphere | $4.7 \pm 0.2^b$ | 14.8 ± 0.7 | $130.4 \pm 42.6^a$ | $18.7 \pm 7.7^a$ | $1.6 \pm 1.6^b$ |
| Understory rhizosphere | $4.8 \pm 0.3^b$ | 14.5 ± 0.7 | $105.3 \pm 24.9^b$ | $14.4 \pm 8.1^{ab}$ | $4.7 \pm 4.9^a$ |
| Early spring | | | | | |
| Bulk soil | $5.1 \pm 0.2^a$ | 14.7 ± 0.9 | $68.6 \pm 20.1^b$ | $13.8 \pm 11.8^b$ | 2.0 ± 2.8 |
| Tree rhizosphere | $4.7 \pm 0.2^b$ | 14.8 ± 0.9 | $114.8 \pm 19.4^a$ | $25.7 \pm 14.8^a$ | 2.8 ± 3.5 |
| Understory rhizosphere | $4.8 \pm 0.2^b$ | 14.8 ± 0.8 | $96.5 \pm 31.7^a$ | $27.9 \pm 17.0^a$ | 3.5 ± 4.3 |
| Mid-summer | | | | | |
| Bulk soil | $5.0 \pm 0.2^a$ | 14.6 ± 0.6 | 63.5 ± 12.8 | $5.8 \pm 4.7^c$ | $0.2 \pm 0.5^b$ |
| Tree rhizosphere | $4.5 \pm 0.2^b$ | 14.6 ± 0.7 | 77.9 ± 30.0 | $17.6 \pm 8.9^a$ | $0.4 \pm 0.5^b$ |
| Understory rhizosphere | $4.6 \pm 0.2^b$ | 14.5 ± 0.6 | 78.7 ± 15.6 | $12.5 \pm 6.6^b$ | $2.2 \pm 3.2^a$ |

Note: EON represents extractable organic nitrogen. The values are mean ± standard deviations. Different lowercase letters indicate a significant pairwise difference between soil positions at each sampling season.

3.2 Extracellular-enzyme activities

Although sampling season had a marginally significant effect on the activity of NAG and PE, there was no significant effect of season on enzyme activity (Online Resources 2 and 3). The activity of GL and NAG in TR was significantly higher than that in UR and BS, but there was no significant difference in the activities between UR and BS (Online Resource 2). The interaction effect of sampling season and position had a significant effect on the activity of AP and PO (Online Resource 3). Further analyses on the activity of AP and PO were conducted after considering sampling season and position separately. Sampling position significantly affected AP and PO activity regardless of sampling season except for PO during early winter (one-way ANOVA with random effects: AP, $F = 19.24$ and $P < 0.001$ for early winter; $F = 18.71$ and $P < 0.001$ for early spring; $F = 34.62$ and $P < 0.001$ for mid-summer and PO, $F = 1.22$ and $P = 0.307$ for early winter; $F = 8.12$ and $P < 0.01$ for early spring; $F = 18.92$ and $P < 0.001$ for mid-summer). The activity of AP in TR was higher than that in BS regardless of the sampling season and was higher than that in UR during early spring and mid-summer. Furthermore, the activity of AP in UR was higher than that in BS

during early winter and early spring. The activity of PO in TR was higher than that in BS and UR during early spring and mid-summer, but there was no significant difference in the activities between BS and UR regardless of the sampling season. Sampling season only significantly affected AP activity in BS (one-way ANOVA with random effects: $F = 6.10$, $P < 0.05$), and the activity of AP in TR during mid-summer was higher than that during early winter. The activity of XY and PE did not significantly vary among sampling seasons and positions (Online Resources 2 and 3).

The RPEs on extracellular enzyme activity varied among enzymes and between TR and UR (Fig. 2). The RPEs were significantly positive on AP and NAG activity regardless of the sampling season and TR and UR differences (Fig. 2). The magnitude of the RPE tended to be higher for TR than for UR regardless of the enzymes and seasons except for XY and PE during early spring (Fig. 2). However, the difference between TR and UR was significant only for NAG (two-way ANOVA with random effects: $F = 4.02$, $P < 0.05$). The difference in the RPE magnitude between TR and UR was marginally significant for PO (two-way ANOVA with random effects: $F = 2.48$, $P = 0.091$). Tukey's HSD test showed that the RPE on PO in TR was significantly higher during mid-summer than during early winter and early spring. The effect of sampling season on the RPE on AP was marginally significant (two-way ANOVA with random effects: $F = 3.50$, $P = 0.065$).

### 3.3 Microbial gene abundance

There was no clear difference in bacterial and archaeal 16S rRNA gene abundance among seasons and positions (Online Resources 4 and 5). In contrast, fungal ITS gene abundance was significantly higher during mid-summer than during early winter and early spring, and there was a non-significant difference in the fungal gene abundance between early winter and early spring (Online Resource 4). The fungal ITS gene was also significantly more abundant in TR and UR than in BS regardless of the season (Online Resource 4). There was a non-significant difference in fungal ITS gene abundance between TR and UR.

The magnitude of RPEs on the abundance of bacterial and archaeal 16S rRNA genes did not differ significantly from zero regardless of the season and position, except for the positive and marginally significant RPEs on the bacterial 16S rRNA gene observed during early spring in TR (Fig. 3). There were significant and positive RPEs on fungal ITS gene abundance regardless of the position and season except for UR during mid-summer (Fig. 3). There was no clear trend of the RPE magnitude on bacterial, archaeal, and fungal gene abundance among seasons and positions (Fig. 3). The magnitude of the RPE on fungal ITS in UR was significantly higher than that in TR only during early spring (one-way ANOVA with random effects: $F = 4.23$, $P < 0.05$), and the magnitude during early spring was significantly higher than that during mid-summer only in TR (one-way ANOVA with random effects: $F = 3.92$, $P < 0.05$).

Although the bacterial *amoA* gene abundance did not vary among seasons and positions (Online Resources 4 and 5), the archaeal *amoA* gene abundance was significantly lower in TR than in BS (Online Resources 4 and 5). There was a non-significant difference in the abundance of the archaeal *amoA* gene between TR and UR and between UR and BS (Online Resource 4).

The magnitude of the RPE on the bacterial *amoA* gene abundance did not differ from zero regardless of the season and position (Fig. 3). In contrast, significant and negative RPEs on the archaeal *amoA* gene abundance were observed regardless of the season and position except for UR during early winter (Fig. 3). Furthermore, the RPE on the archaeal *amoA* gene abundance tended to be more negative for TR than for UR regardless of the season; however, the difference in the gene abundance between TR and UR was not significant (two-way ANOVA with random effects: $F = 0.76$, $P = 0.385$).

# Discussion

4.1 RPE on enzymatic activity and nutrient cycles

Recently, the importance of nutrient cycling processes during plant dormant seasons has been highlighted (Isobe et al. 2018). However, the RPE in the plant dormant season remains unclear. In the present study, we demonstrated that both the canopy tree and understory vegetation stimulated potential extracellular enzymatic activity at the beginning and end of the plant dormant season when the canopy trees are leafless (Fig. 2). The potential activity of extracellular enzymes reflects the amount of these enzymes (Baldrian et al. 2013). Thus, the differences in potential activity indicated higher concentrations of these enzymes in the rhizosphere compared to the non-rhizosphere bulk soil, implying that the rates of nutrient cycling processes, including decomposition and mineralization, were stimulated within the rhizosphere in the dormant season. We noted that the actual enzyme activity during early winter and early spring would be lower than the potential activity measured by the incubation at 25 ℃ because enzyme activity has been found to be affected by temperature (Baldrian et al. 2013).

In the plant dormant season, the concentration of EON within rhizospheres was higher than that in non-rhizosphere bulk soils, although this trend was not observed in the mid-growing season (Table 1). The amount of plant-available N in the soil is determined by the balance between uptake and root-primed decomposition and mineralization (Nakayama and Tateno 2021). Plants and mycorrhiza can take up organic and inorganic N (Liese et al. 2018; Schimel and Bennett 2004; Zhang et al. 2019). Ueda et al. (2015) demonstrated that plants, including *Q. crispula*, took up certain amount of inorganic N during mid-winter in sub-zero temperatures in the northern hardwood forest. Although it is unknown whether deciduous plant species can take up low-molecular-weight organic N during the dormant season, Zhang et al. (2019) reported that both the roots of evergreen conifers and their symbiotic mycorrhiza took up organic N during the dormant season as well as during the growing season. In addition, the organic and inorganic N uptake by herbaceous species during the dormant season has also been reported in a temperate coastal heath (Andresen and Michelsen 2005). Thus, there is a possibility that plants could have utilized EON and inorganic N during the dormant season in the present study. However, the amount of N taken up by plants during the dormant season should be lower than that take up in the mid-growing season in the present study, as shown for evergreen tree species (Zhang et al. 2019). Therefore, at the beginning and end of the plant dormant season, the lower EON uptake and positive RPE on N decomposition could lead to the higher EON concentration within the rhizospheres. In contrast, during the mid-growing season, the higher nutrient uptake activity by plant roots might mask the positive RPE.

During the plant dormant season, especially at its beginning and climax, decomposed N is retained by the microbial communities (Isobe et al. 2018). In snowy northern hardwood forests, the freeze-thaw event during the snowmelt season causes microbial cell disruption, releasing EON and reducing microbial abundance (Isobe et al. 2018). At the same time, the released organic matter stimulates microbial growth (Watanabe et al. 2019). In the current study, the magnitude of the RPE on bacterial and archaeal abundances at the beginning of the dormant season was more positive than that during the mid-growing season (Fig. 3), although the magnitudes were not significantly different from zero. Thus, excess bioavailable N transformed by root-induced decomposition and mineralization may be retained by microbes but not by the plants, at least at the beginning and end of the plant dormant season. Further research using $^{15}$N-tracers should reveal the fate of the excess N transformed by root-induced decomposition and mineralization during the plant dormant season.

4.2 Rhizosphere priming effects on the microbial abundance

It is well known that microbial abundance within the rhizosphere is higher than that in bulk soil (Kuzyakov and Razavi 2019). Our results were partly consistent with previous findings, i.e., only fungal abundance in the rhizospheres was higher than that in bulk soil—abundances of bacteria and archaea did not differ between the rhizosphere and bulk soils (Fig. 3). In general, the fungal community in the rhizosphere is characterized by mycorrhizal fungi (Baldrian 2017), and their abundance decreases with distance from the rhizoplane (Kuzyakov and Razavi 2019). Thus, arbuscular mycorrhizal fungi (AM; symbiotic with *S. nipponica*, the understory vegetation in the present study) and ectomycorrhizal fungi (EcM; symbiotic with *Q. crispula*, the canopy tree in the present study) would be relatively abundant within the rhizosphere at our study site. Mycorrhizal fungi are still active and are affected by tree C supply in winter (Kaiser et al. 2010, 2011). Therefore, the observed higher fungal abundance might be explained by a higher abundance of mycorrhizal fungi within the rhizospheres during the plant dormant season. However, there is also another contrasting explanation for this result. At the present study site, the relative abundance of ectomycorrhiza in the non-rhizosphere bulk soil was highest during the mid-growing season and decreased during other seasons (Nakayama et al. 2021), possibly because of reduced C supply from the symbiotic canopy trees. Žifčáková et al. (2017) reported that EcM activity was much lower in the bulk soil in winter than in summer, and the relative contribution of non-mycorrhizal fungi to extracellular enzyme production was higher during winter. The presence of mycorrhizal fungi, both AM and EcM, is known to suppress the activity of free-living saprotrophs (Frey 2019; Gadgil and Gadgil 1971). Therefore, the abundance of mycorrhiza within the rhizosphere might be reduced during the plant dormant season, and at the same time, this could stimulate non-mycorrhizal fungal activity in the rhizosphere, in which the nutrient conditions were relatively favorable. To test these contrasting hypotheses, further studies focusing on the fungal communities within the rhizosphere during the plant dormant season are needed.

In contrast to our results, bacteria and archaea in the rhizosphere are typically much more abundant than in bulk soil (Kuzyakov and Razavi 2019). However, in the rhizosphere, some bacteria, such as pathogenic bacteria, are suppressed by root exudates (Bais et al. 2006). The significantly lower abundance of AOB and AOA within the rhizosphere (Fig. 3) also suggested the suppression of a part of bacterial and

archaeal communities. One possible explanation for these results is that the stimulation and suppression of bacterial and archaeal growth within the rhizospheres were well-balanced. Alternatively, the limited photosynthetic activity, especially the lack of photosynthesis by canopy trees, would also explain the bacterial and archaeal population results within the rhizosphere at the beginning and end of the dormant season. Wang et al. (2019) reported that the amount of root-exuded C positively correlated with the RPE on microbial biomass. Although the release of root exudates from tree species in winter or at least at the beginning of the dormant season has been observed in some studies (Nakayama and Tateno 2018; Phillips et al. 2008), the amount of root-exuded C at the beginning and end of the dormant season should be lower because a majority of root exudates are recently assimilated organic C (Epron et al. 2011; Nakayama and Tateno 2018; Sanaullah et al. 2012). However, there is limited knowledge on root exudation in winter; therefore, further research on root exudates and microbial community structures within the rhizosphere in the winter dormant season is needed to reveal the relationships among plant roots, root exudates, and rhizosphere microbes. Unlike total bacterial and archaeal abundance, the population of AOA was suppressed within the rhizosphere seasonally (Fig. 3). Studies have shown that plants have some functions that inhibit nitrification steps and growth of microbes responsible for nitrification partly to prevent N loss from ecosystems (reviewed in Moreau et al. 2019). There is still limited knowledge on the inhibition of ammonia-oxidizing communities by forest plants; our results suggest that plants suppressed the nitrification processes driven by AOA at the beginning and end of the plant dormant season as well as during the mid-growing season.

### 4.3 Differences in the RPE between canopy trees and understory vegetation

The magnitude of the RPE was higher for the canopy tree than for understory vegetation during the growing season (Figs. 2 and 3). This may be explained by the advantage of photosynthetic activity in canopy trees relative to understory vegetation during the growing season (Kuzyakov and Cheng 2001, 2004). However, in contrast to our second hypothesis, the magnitude of the RPE was higher for the canopy tree than for understory vegetation during the dormant as well as the growing seasons (Figs. 2 and 3). At the beginning and end of the plant dormant season, *Q. crispula* had no leaves, whereas *S. nipponica* was considered to have photosynthetic activity (Kayama and Koike 2018; Tateno et al. 2019). Thus, the advantage of photosynthesis was not simply explained by the difference in the magnitude of the RPE.

One possible factor that may result in the RPE differences between the canopy tree and understory vegetation irrespective of seasons is mycorrhiza type. Huo et al. (2017) reported that trees tended to have higher RPEs than herbaceous species in a meta-analysis using RPE data obtained during the growing season. They surmised that the difference between trees and herbaceous species is a result of differences in mycorrhiza, i.e., EcM (trees) vs. AM (herbaceous species). In general, EcM fungi have a greater capacity for producing extracellular enzymes than AM fungi (Frey 2019), and the enzymatic production activity of symbiotic mycorrhizal fungi are also important factors for the RPE on enzymatic activity (Phillips and Fahey 2006). Furthermore, mycorrhizal fungi are active during winter (Kaiser et al. 2010), although their activity would be lower during the plant dormant season than during the growing

season (Žifčáková et al. 2017). Therefore, the difference in mycorrhizal type could explain the higher RPE in TR on the enzymatic activity during leafless seasons.

The negative rhizosphere effect on the abundance of AOB and AOA was stronger in TR than in UR (Fig. 3). EcM symbiotic species tended to suppress nitrification more than AM symbiotic plants (Phillips et al. 2013; Tatsumi et al. 2020). Most mineralized N at the present study site undergoes nitrification processes in laboratory incubation without living roots and mycorrhiza (Nakayama et al. 2021). Thus, our results indicating a higher $NH_4^+$-N concentration and not a higher $NO_3^-$-N concentration in the rhizosphere in TR but not in UR (Table 1) suggest that *Q. crispula* had a stronger nitrification suppression effect than of *S. nipponica*. Plants take up each of the low-molecular-weight organic N, $NH_4^+$-N, and $NO_3^-$-N (Jones et al. 2005; Liese et al. 2018); however, there is a source preference for plant N uptake (Nordin et al. 2001). Ectomycorrhizal symbiotic trees prefer organic N and $NH_4^+$-N to $NO_3^-$-N compared with AM symbiotic species (Liese et al. 2018; Phillips et al. 2013). Tateno et al. (2020) reported that *Q. crispula* preferred $NH_4^+$-N to $NO_3^-$-N and *S. nipponica* utilized both types of inorganic N at the present study site. Therefore, our results implied that canopy tree species, which have symbiotic relationships with EcM, suppressed the AOA population and consequently, nitrification processes within the rhizosphere were stronger than those in the understory vegetation, which has symbiotic relationships with AM, regardless of the season. In the present study, there is a limitation that only one of each AM and EcM symbiotic plants were investigated; thus, studies using diverse plant species are needed to clarify the difference of the RPE between AM and EcM symbiotic plants.

# Conclusion

In the present study, we demonstrated that plants in the northern hardwood forests accelerated nutrient cycling within the rhizosphere during the non-growing season. Although recent studies have shown that the nutrients taken up by plants in winter are important for subsequent spring growth, our results also suggest that nutrient uptake by plants and the abundance of their root symbiotic mycorrhiza was less during the non-growing season compared with the growing season. Therefore, our results implied that both the canopy tree and understory vegetation hasten nutrient cycling processes, including decomposition and mineralization at the beginning and end of the dormant season. However, a large part of the nutrients transformed by root-induced decomposition was retained in the microbial biomass rather than in the plants. These retained nutrients would be released later during the subsequent growing season and be utilized for plant growth.

# Declarations


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### Conflicts of interest

The authors declare that they have no conflict of interest.

### Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Code availability

Not applicable

### Authors' contributions

MN and RT designed the experiment. MN performed the experiment and data analysis and prepared figures and tables. MN and RT wrote the manuscript.

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# Figures

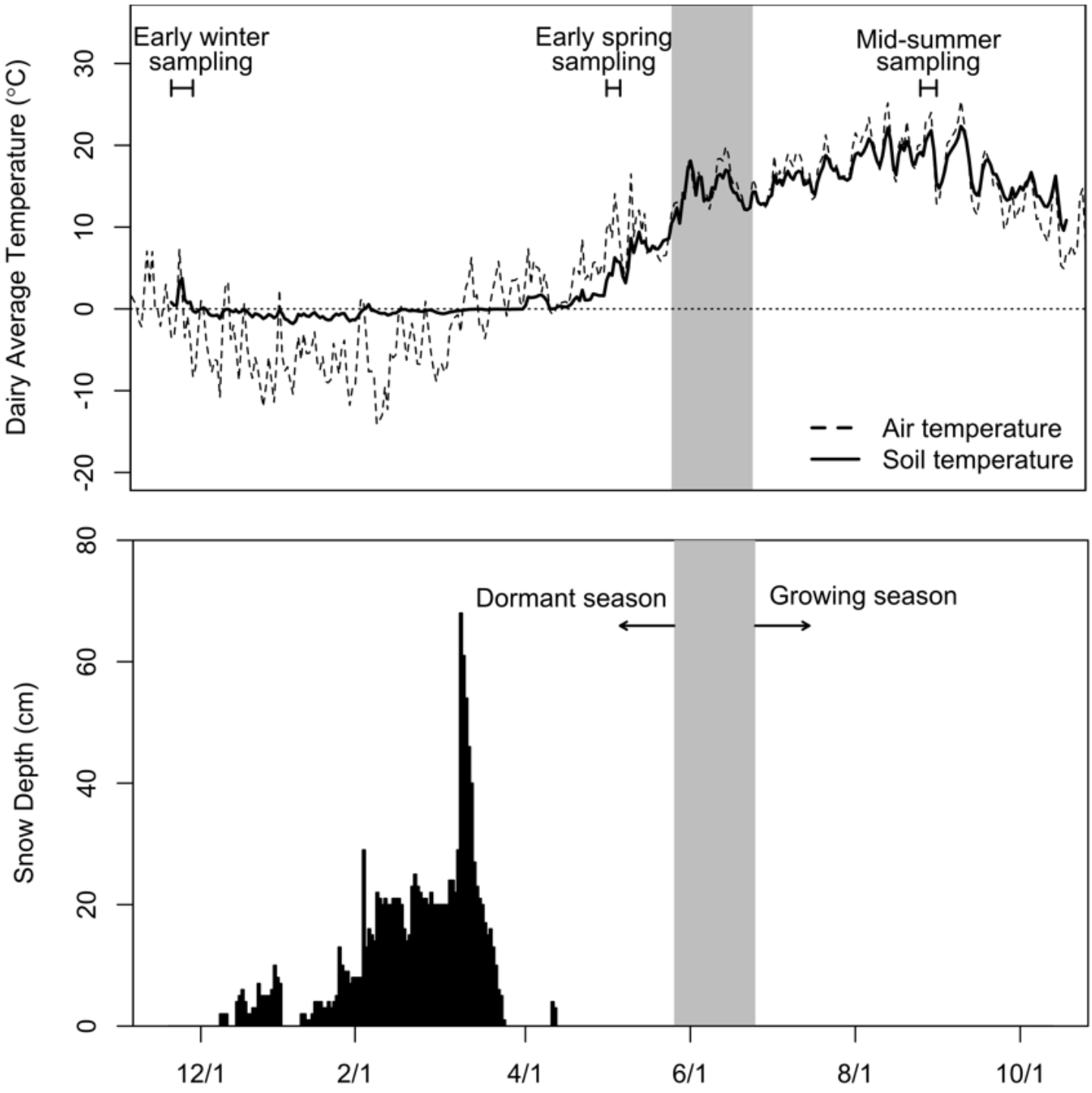
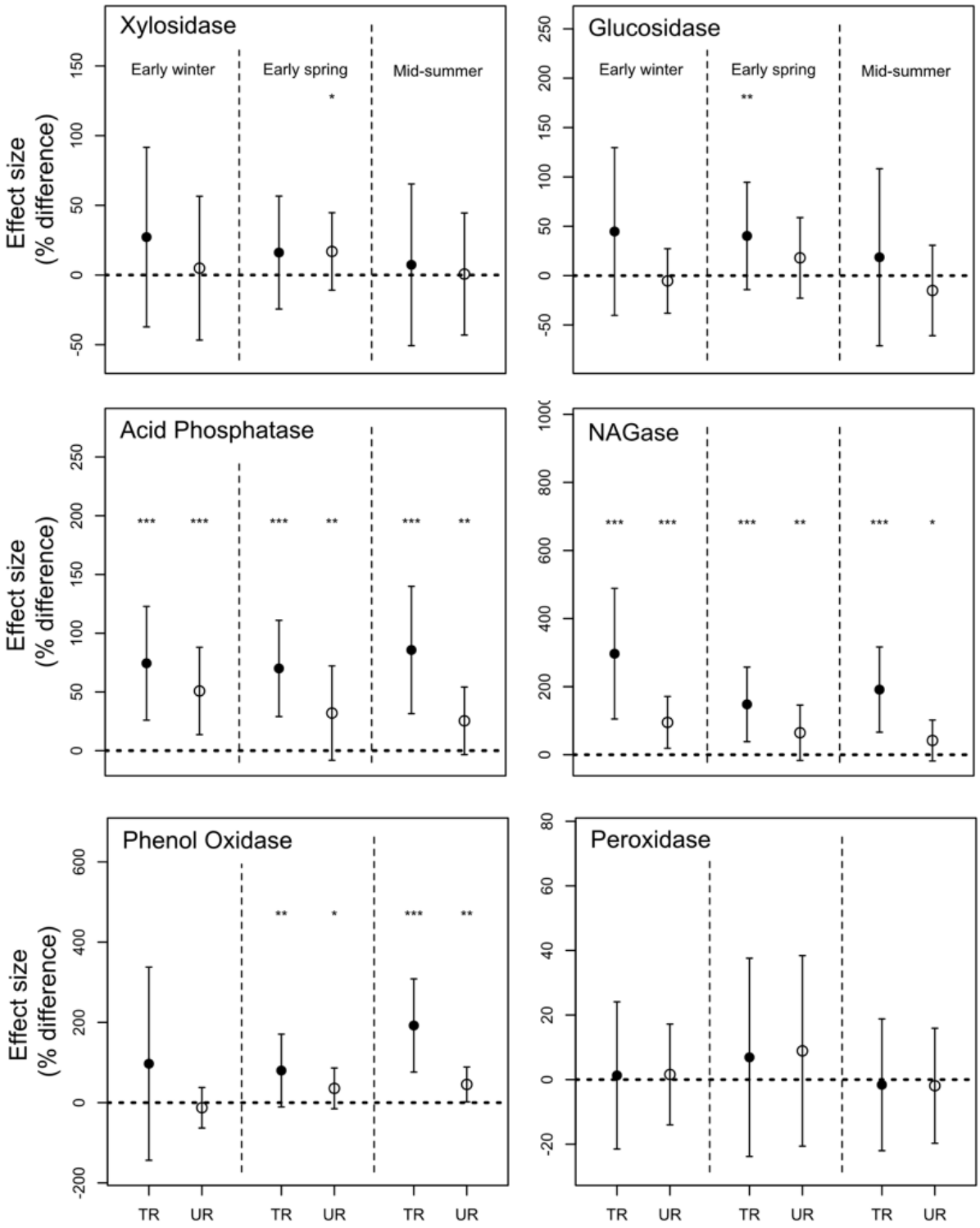



**Figure 1**

Seasonal fluctuations in the daily averages of soil (5 cm-depth) and air temperatures and snow depth during the experiment. Soil temperature was measured at the study plots, and air temperature and snow depth were measured at a meteorological station 9 km from the study site. The shaded area represents the approximate period from start to full expansion of leaf flushing of Q. crispula (data was shown in Tateno et al. 2019)




## Figure 2

Rhizosphere priming effects on the activity of xylosidase (XY), glucosidase (GL), acid phosphatase (AP), NAGase (NAG), phenol oxidase (PO), and peroxidase (PE). The filled and open circles represent the rhizosphere effect by the canopy tree and understory vegetation, respectively. The bars represent standard deviation. The symbols (* and †) indicate that the effect significantly differs from zero. The symbols are as follows: † P < 0.1, * P < 0.05, ** P < 0.01, and *** P < 0.001.

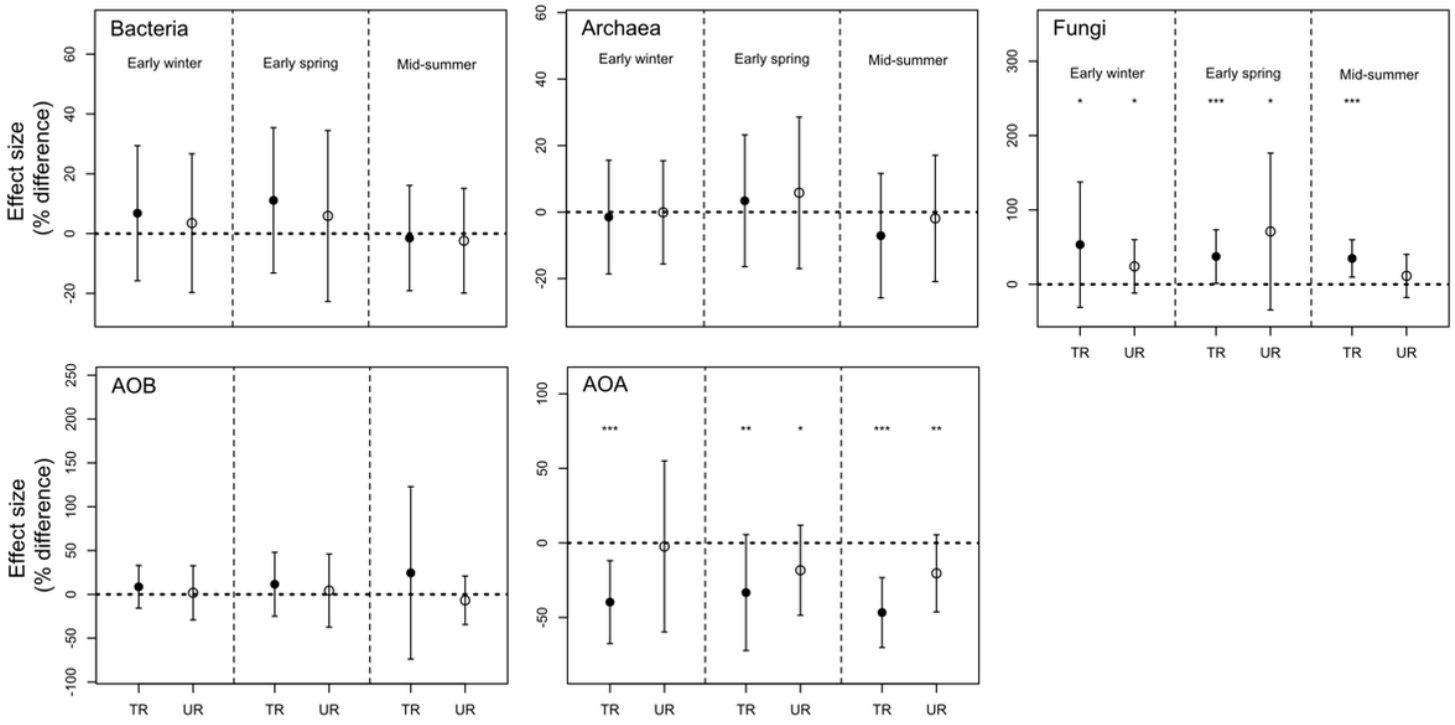



**Figure 3**

Rhizosphere priming effects on the abundance of bacterial 16S rRNA, archaeal 16S rRNA, fungal ITS region, bacterial amoA gene, and archaeal amoA gene. The filled and open circles represent the rhizosphere effect of the canopy tree and understory vegetation, respectively. The bars represent standard deviation. The symbols (* and †) indicate that the effect significantly differs from zero. The symbols are as follows: † P< 0.1, * P < 0.05, ** P < 0.01, and *** P < 0.001.

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