Title: Root exudation of low molecular mass organic acids by six tree species alters the dynamics of calcium and magnesium in soil.

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Short title: Low molecular mass organic acids and dynamics of cations

Abbrevisions: LMMOAs, low molecular mass organic acids
ABSTRACT

Plantation of Cryptomeria japonica that planted in large areas throughout Japan have ~three-fold more exchangeable Ca compared to other types of forest vegetation even in a Ca poor environment. To explain mechanisms underlying this phenomenon, we determined the effect of root exudation rate of low molecular mass organic acids (LMMOAs) on exchangeable cations in soil. We conducted pot experiment using C. japonica and five dominant tree species in Japan, and measured the root exudation rates of LMMOAs and exchangeable nutrient concentrations in the soils. To estimate whether root exudation rate of LMMOAs is elevated response to Ca deficiency, we created variation in Ca availability by adding different amounts of crushed oyster shells. The root exudation rates of LMMOAs were two to five times higher for C. japonica than for other tree species, but not differ significantly among the different quantities of oyster shell. Exchangeable Ca and Mg were significantly higher in the soils with C. japonica and significantly correlated with the root exudation rate of LMMOAs ($R^2 > 0.24$) at high and moderate quantities of oyster shell. Therefore, variation among species, in terms of root exudation of organic acids, might be one important factor affecting the
cation dynamics in soil.

Introduction

Many studies have demonstrated that the dynamics of carbon (C), nitrogen (N), and phosphorus (P) can be altered by the physiological processes of organisms through the alteration of chemical properties, such as decreasing pH levels and increasing nutrient availability in soil (Riha et al. 1986; Vanni 2002). Supplies of low molecular mass organic acids (LMMOAs) from the root systems of trees can also alter soil nutrient dynamics (Clarholm et al. 2015). LMMOAs, such as citric acid and oxalic acid, are released by the roots of vascular plants (Tyler and Ström 1995) and fungi, forming ectomycorrhiza with tree roots (Wallander and Wickman 1999; Ahonen Jonnarth et al. 2000; van Hees et al. 2005). LMMOAs in soil can solubilize recalcitrant N and P, which are then absorbed by plant roots (Simpson et al. 2002; Clarholm et al. 2015). Polyvalent metal cations, such as calcium ions (Ca$^{2+}$), act as important pH buffers (Clarholm and Skyllberg 2013), and cations in soil particles and base rock are leached by LMMOAs (Dijkstra and Smits 2002; Simpson et al. 2002). As the supply of LMMOAs from roots
varies significantly among tree species (Aoki et al. 2012), differences in vegetation type
might affect the concentrations of exchangeable cations. This means that large-scale
changes in forest vegetation might modify the levels of exchangeable cations in soil. In
particular, monoculture tree plantations, which supply large quantities of LMMOAs,
might significantly increase soil exchangeable cation levels.

Japanese cedar (Cryptomeria japonica D. Don, Cupressaceae) has been
planted in large areas throughout Japan, accounting for approximately 12% of the total
land area (Forestry Agency 2011), and stocked large biomass (90 kg C ha\(^{-1}\) in average)
in Japan (Fukuda et al. 2003). Ohta et al. (2014a; 2014b) reported that vegetation in
catchments might alter the exchangeable Ca concentrations in soil in Ca poor
environment. Ohta et al. (2014a; 2014b) observed that the concentration of
exchangeable Ca in C. japonica plantations was approximately three-fold higher than
that in evergreen broad-leaved forests. Furthermore, a similar phenomenon, in which
the soil Ca concentration in C. japonica plantations was higher than in broad-leaved
forests, has been reported for many areas of Japan (Tsutsumi 1987; Baba et al. 2004).
These patterns suggest that C. japonica has the potential to alter the Ca dynamics in soil.
However, the mechanisms underlying this phenomenon are not understood. Therefore, *C. japonica* must be assessed by a comparison of physiological processes that affect soil cation dynamics, including the supply of LMMOAs from roots. In fact, a Cupressaceae species (*Calocedrus decurrens*) cause higher contents of LMMOAs in soils as compared to other broad-leaved tree species (Strobel, 2001). Therefore, the exudation rates of LMMOAs from roots of Cupressaceae are higher than for other tree species. In addition, some plant species could increase the release of LMMOAs from roots in response to a nutrient deficiency (Ström et al. 1994; Van Schöll et al. 2006). Ohta et al. (2014b) showed that the exchangeable Ca concentration in soil was higher in *C. japonica* plantations in Ca-poor environments. Cations such as Ca and magnesium (Mg) are essential elements for tree species. For instance, Ca is involved in some manner in nitrogen metabolism and Mg is a constituent of chlorophyll molecule (Pallardy 2007). Therefore, cations are leached by LMMOAs might be important nutrients for tree species in poor cations soil. We predicted that *C. japonica* would increase the release of LMMOAs from roots in response to a Ca deficiency.
This study involved six tree species that are predominant in Japan. We created variation in Ca availability by adding different amounts of crushed oyster shells to the potting soil. Oyster shells contain CaCO$_3$ and MgO, which are major components of common volcanic or sedimentary rock (Fukushima and Tatsumi 2007; GSJ-AIST 2010). We compared the exudation rate of LMMOAs from the roots and levels of exchangeable cations in the soils. We tested the following hypotheses: (1) the exudation rates of LMMOAs from roots are higher for C. japonica than for other tree species, (2) the exudation rates of LMMOAs from roots are higher under Ca poor conditions, and (3) the concentration of exchangeable cations in soil will increase with increasing exudation rates of LMMOAs from roots.

Materials and Methods

Study trees

We conducted an experiment from 10 June to 10 August 2014 in the Wakayama Experimental Forest of Hokkaido University (33°40'N, 135°40'E; 447 ha). We prepared 72 plastic pots (diameter, 25 cm; height, 30 cm) and 72 seedlings of six dominant tree
species (12 pots per species): *C. japonica, Chamaecyparis obtusa, Fagus crenata, Quercus myrsinifolia, Quercus crispula and Quercus serrata.*

**Experimental system**

A total of 3 kg of commercial soil (Kanuma soil), which contained very few nutrients (Yoshida et al. 1992; Fukushima and Tatsumi 2007), was placed in each of the 72 pots. Equal amounts of KH$_2$PO$_4$ and CO(NH$_2$)$_2$ were added as fertilizer to the Kanuma soil to create soils with similar concentrations of nutrients to the soil in the Wakayama Experimental Forest (N: 15 mg g$^{-1}$; P: 2 mg g$^{-1}$; Ohta et al. 2014b). Broken oyster shells were also mixed with the soil in three different amounts (0.5, 2, and 10 mg oyster shell g$^{-1}$ soil) to vary the amounts of raw ingredients for exchangeable Ca and Mg. The Ca concentration at low quantities of oyster shell (0.5 mg oyster shell g$^{-1}$ soil) was similar to the concentration in Wakayama Experimental Forest soil (Ohta et al. 2014a). Three subsamples of broken oyster shells were collected to measure the elemental components. And then, the 72 seedlings of tree planted in each plastic pot. The plastic pots were arranged randomly under a plastic roof to prevent nutrient deposition by rainfall; they
were maintained for 60 days. The seedlings were supplied with 200 mL of water daily, in the morning.

After 60 days, LMMOAs from the roots were collected according to Phillips et al. (2008) and Aoki et al. (2012). The roots were carefully removed from the soil in each pot, and one newly developed root (< 1 mm in diameter) was selected from the Kanuma soil. After the roots were carefully rinsed with deionized water to remove attached soil, the root systems were placed in 30-mL syringes containing sterile acid-washed glass beads. The syringes were covered with aluminum (Al) foil to minimize the photolytic degradation of organic acids from the roots. We put a Ca-free nutrient solution (0.5 mM NH₄NO₃, 0.1 mM KH₂PO₄, 0.2 mM K₂SO₄, and 0.2 mM MgSO₄) in the syringes. The roots and syringes rinsed with the solution three times to remove contaminants. Next, we filled the syringes with 15 mL of the Ca-free nutrient solution. After 24 h, we used another syringe to collect the solution containing the accumulated exudates. The collected solution was filtered through 0.45-µm glass filters (Whatman, GF/C; GE Healthcare, Little Chalfont, UK) and stored at -30°C until analysis. Before collecting LMMOAs from the roots, we collected Kanuma soil samples
Sample processing

To estimate the root exudation rates of LMMOAs, the concentrations of LMMOAs in the liquid containing the root exudates were analyzed by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) according to van Hees et al. (1999) and Aoki et al. (2012). The LMMOAs were separated on a Supelcogel C610-H ion exclusion column, using 0.1% H₃PO₄ as the mobile phase at operating temperatures of 60°C for citric acid and 30°C for oxalic acid and malic acid, with UV detection at 210 nm. After the analysis of organic acids, we calculated the root exudation rates of LMMOAs (mg g⁻¹ root h⁻¹).

To measure exchangeable nutrients in soil collected near the root that extracted LMMOAs, we shook (160 rev min⁻¹) a 0.5 g (air-dried mass) subsample of each soil sample in 100 mL of 1 M KCl for 1 h, filtered the sample through filter paper (No. 5C; Advantec, Tokyo, Japan), and then stored the suspension at -30°C until analysis. The KCl extracts of soil were analysed for concentrations of exchangeable
ions (Mg, Al, P, Ca, iron [Fe] and nitrate [NO$_3$]) per unit air-dried mass using an inductively coupled plasma atomic emission spectrometer (iCAP 6200; Thermo Scientific, Cambridge, UK) and the absorptiometric method (Sakata 2000). Exchangeable elements in the samples were quantified with an internal standard (yttrium), which was added to the extract before analysis. Al and Fe are major components of Kanuma soil.

To measure the elemental components of oyster shells, subsamples of broken oyster shells were extracted with 70% HNO$_3$ at 80°C for 24 h. The extracts were analyzed for their Mg, Al, P, Ca, and Fe contents using an atomic emission spectrometer (iCAP 6200; Thermo Scientific, Cambridge, UK). The weight percentage of the nitrogen in the oyster shell was obtained by an elemental analyzer (Flash EA 1112, Thermo Finnigan, Milan, Italy). Mean concentrations (± SE) of each element in the oyster shells were: N, 0.18 ± 0.01 mg g$^{-1}$; Mg, 8.2 ± 0.20 mg g$^{-1}$; Al, 0.2 ± 0.00 mg g$^{-1}$; P, 0.08 ± 0.003 mg g$^{-1}$; Ca, 480.1 ± 21.41 mg g$^{-1}$; Fe, 2 ± 0.01 mg g$^{-1}$.

Statistical analysis
To test hypotheses 1 and 2, we analyzed the exudation rates of LMMOAs from root using a two-way ANOVA with tree species and oyster shell quantity, followed by post hoc comparisons using Tukey’s honestly significant difference (HSD) test. If the result of two-way ANOVA indicated the exudation rates of LMMOAs from root differed significantly among the tree species, but not among the different quantities of oyster shell, we conducted the post-Tukey comparisons between tree species regardless of the differences in quantities of oyster shell. To test hypothesis 3, we analyzed the concentrations of exchangeable nutrients (Mg, Al, P, Ca, Fe and NO$_3$) in the soils using a two-way ANOVA with tree species and oyster shell quantity, followed by post hoc comparisons using Tukey’s HSD test. The concentrations of exchangeable nutrients for each quantity of oyster shell were then analyzed according to a linear model, with the root exudation rates of the total of all three LMMOAs. All statistical analyses were performed using R version 3.0.1 (R Core Team 2013).

Results

The effects of tree species and oyster shells on the exudation of LMMMOAs
We detected three types of LMMOAs (citric, malic, and fumaric acids), but we could not detect other LMMOAs, such as acetic or oxalic acid. The exudation rates of citric, malic, and fumaric acids from roots differed significantly among the tree species, but not among the different quantities of oyster shell (Table 1; Fig. 1). Therefore, we conducted the post-Tukey comparisons between tree species regardless of the differences in quantities of oyster shell. The exudation rates of fumaric and malic acid from the *C. japonica* roots were significantly higher than the rates from the other five species (Fig. 1; Tukey’s HSD test, *P* < 0.001). The exudation rates of citric acid from the *C. japonica* roots were not significantly higher than the rates from the other five species (Fig. 1; Tukey’s HSD test, *P* > 0.05). The exudation rates of total three LMMOAs from the *C. japonica* roots were significantly higher than the rates from the other five species (Fig. 1; Tukey’s HSD test, *P* < 0.05).

Effects of LMMOAs from different trees species and oyster shell on the exchangeable Mg and Ca in the soil
Table 1 shows concentration of exchangeable nutrients in the soil for each tree species and quantity of oyster shell. Exchangeable Mg and Ca in the soils differed significantly among the tree species, and Ca differed significantly among the quantities of oyster shell (Tables 1, 3). Exchangeable Ca in the soils with *C. japonica* was significantly higher at high quantities of oyster shell than others (Table 1; Tukey’s HSD test). The amount of exchangeable Mg in the soils with *C. japonica* was significantly higher at moderate and high quantities of oyster shell than at low quantity of oyster shell (Table 1; Tukey’s HSD test). However, the amount of exchangeable P, Al, Fe and NO₃ in the soils did not differ significantly among tree species and quantities of oyster shell (Tables 1, 3). In addition, we observed significant positive correlation between the exudation rates of LMMOAs from roots and the amounts of exchangeable Ca and Mg in soil at moderate and high quantities of oyster shell (Fig. 2). However, these significant correlations were not observed at low quantity of oyster shell (Fig. 2).

**Discussion**
The exudation rates of fumaric and malic acid from roots are higher for *C. japonica* than for other tree species (Fig. 1, supporting hypothesis 1). This study shows that the exudation rates of LMMOAs from roots differed significantly among tree species but not among varying quantities of oyster shell (Table 2, opposing hypothesis 2), and that the variation in the exudation rates of LMMOAs from roots might alter the cations dynamics in soil, supporting hypothesis 3. There was a significantly positive correlation between the root exudation rates of LMMOAs and exchangeable Ca and Mg at moderate and high quantities of oyster shell, but this relationship was not evident at low quantities of oyster shell. At low quantities of oyster shell, there might be very few solutes that dissolve easily in LMMOAs, such as oyster shells in the soil. This finding suggests that the exchangeable Ca and Mg were supplied by the oyster shells. Oyster shells contain high concentrations of CaCO$_3$ and MgO, which are major components of common volcanic and sedimentary rock (Fukushima and Tatsumi 2007; GSJ-AIST 2010). Therefore, root exudation of LMMOAs might dissolve Ca and Mg from limestone, basalt, sand stone, and weathered soil. Ohta et al. (2014b) showed that the amount of exchangeable Ca in soil weathered from sandstone was ~three times higher in
C. japonica plantations than in evergreen broad-leaved forests. Furthermore, the supply
of LMMOAs increases nutrient mobilization. LMMOAs might enhance the weathering
of base rock (Drever 1994; Drever and Stillings 1997). Therefore, large-scale planting
of a tree species that exhibit high exudation rates of LMMOAs from their roots might
increase the mobilization of nutrients and base rock disintegration.

In contrast, there was not a significant correlation between the root exudation
rates of LMMOAs and exchangeable Al and Fe (major components of Kanuma soil) at
any of the oyster shell quantities, perhaps because the ionization rates of Al and Fe are
lower than for Ca and Mg. Previous studies demonstrated that LMMOAs in soil can
solubilize recalcitrant Al and Fe in soil organic matter (Simpson et al. 2002; Clarholm
and Skyllberg 2013). In our experiment, because we used Kanuma soil, which contains
low amounts of organic matter, there were no organic substance layers in common in
forest soil. Therefore, the root exudation rates of LMMOAs did not increase the
amounts of exchangeable Al and Fe in our pots.

On the other hand, the exudation rates of LMMOAs from roots did not differ
significantly among the quantities of oyster shell (Table 1; Fig. 1). Therefore, a Ca
deficiency might not contribute to an increase in the exudation rates of LMMOAs from
*C. japonica* roots. Aoki et al. (2010) suggested that the exudation of LMMOAs
contributes to P solubilization in soil and its uptake by plants in P-poor environments.
*Cryptomeria japonica* might absorb essential nutrients, such as N or P, through
increases in the exudation rate of LMMOAs from roots. Further research is needed to
understand why the exudation rates of LMMOAs from *C. japonica* roots are higher than
for other species.

Furthermore, the biomass and activity of microbe can alter the root exudation
rates of LMMOAs (Clarholm and Skyllberg 2013; Clarholm et al. 2015), and are
decreased by soil acidification (Vance et al. 1987). Therefore, LMMOAs might cause
soil acidification and decrease the biomass and activity of microbe. Because Ca in soil
has pH buffering ability, high quantity of oyster shell in soil might prevent soil
acidification by LMMOAs and maintain the exudation rate of LMMOAs from roots.
However, our results indicate the exudation rates of LMMOAs from roots did not differ
significantly among the quantities of oyster shell (Table 1; Fig. 1). Although we did not
measured pH levels in the soils, significant acidification by LMMOAs might not be
happened in our pot-systems.

Because physiological tolerance mechanism to Al stress involves the external
detoxification and exclusion of Al with LMMOAs (Barceló and Poschenrieder 2002;
Naik et al. 2009), woody plants release LMMOAs to rhizosphere at high Al condition
(Jones and Ryan 2003; Inostroza-Blancheteau et al. 2012). Some studies showed the
release of LMMOAs from root is enhanced by additive amount of Al (Qin et al. 2007;
Brunner and Sperisen 2013). Cronan and Grigal (1995) estimated that there is a 50%
risk of adverse impacts on tree growth or nutrition when the soil solution Ca/Al ratio is
as low as 1.0. In our experimental system, Ca/Al ratio in the soil solution were more
than 15. Therefore, it is consider that release of LMMOAs from root was not enhanced
by Al stress.

The growth rate of the C. japonica root system is higher than that for other
dominant tree species in Japan (Karizumi 1987). Root length and depth distribution
(Dijkstra and Smits 2002), and the root N concentration (Mueller et al. 2012), might
also alter the dynamics of cations in soil. Therefore, to consider the effects of tree
performance on the dynamics of cations in soil, future studies should focus on
differences in these additional factors, such as length, depth distribution and N
ccentration of root and microbial biomass in rhizosphere soil. Furthermore, high
biomass of arbuscular and ectomycorrhiza and high density of bacteria near plant roots
has been attributed to rhizodeposit compounds including LMMOAs (van Hees et al.
2005; Bais et al. 2006). Further studies are needed to clarify the ecological significance
of mineral weathering and carbon dynamics in arbuscular and ectomycorrhizal fungal
partnerships with trees (Koele et al. 2014; Thorley et al. 2015) also in Japanese forests.
ACKNOWLEDGMENTS

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poplar roots after exposure to Al, Cu and Zn. Tree Physiol. 27: 313-320.


Figure 1. Differences in the total root exudation rates of all three LMMOAs (A), fumaric acid, citric acid, and malic acid among tree species. Means and standard errors (+1 SE) are shown. L, M, and H indicate the added amounts of oyster shell. Significant differences ($P < 0.05$) between tree species are denoted by different letters. Cj, Cryptomeria japonica; Co, Chamaecyparis obtuse; Fc, Fagus crenata; Qs, Quercus serrata; Qc, Quercus crispula; Qm, Quercus myrsinifolia

Figure 2. Relationships between the concentrations of exchangeable Ca and Mg and the root exudation rates of LMMOAs at different quantities of oyster shell.
<table>
<thead>
<tr>
<th>Tree species</th>
<th>Ca amendment</th>
<th>Mg (mg g⁻¹)</th>
<th>Al (mg g⁻¹)</th>
<th>P (mg g⁻¹)</th>
<th>Ca (mg g⁻¹)</th>
<th>Fe (mg g⁻¹)</th>
<th>NO₃ (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptomeria japonica</td>
<td>L (0.5 mg oyster shell g⁻¹)</td>
<td>0.0018 (0.002)</td>
<td>0.0266 (0.019)</td>
<td>0.0286 (0.009)</td>
<td>1.1330 (0.306)</td>
<td>0.0018 (0.0009)</td>
<td>0.494 (0.097)</td>
</tr>
<tr>
<td>Chamaecyparis obtusa</td>
<td>0.0619 (0.022)</td>
<td>0.0221 (0.011)</td>
<td>0.0294 (0.003)</td>
<td>0.7323 (0.364)</td>
<td>0.0011 (0.0002)</td>
<td>0.462 (0.063)</td>
<td></td>
</tr>
<tr>
<td>Fagus crenata</td>
<td>0.0613 (0.002)</td>
<td>0.0123 (0.002)</td>
<td>0.0135 (0.002)</td>
<td>0.6575 (0.111)</td>
<td>0.0006 (0.0003)</td>
<td>0.454 (0.080)</td>
<td></td>
</tr>
<tr>
<td>Quercus serrata</td>
<td>0.0636 (0.002)</td>
<td>0.0330 (0.019)</td>
<td>0.0261 (0.009)</td>
<td>0.7067 (0.345)</td>
<td>0.0007 (0.0004)</td>
<td>0.512 (0.114)</td>
<td></td>
</tr>
<tr>
<td>Quercus crispula</td>
<td>0.0725 (0.007)</td>
<td>0.0010 (0.003)</td>
<td>0.0232 (0.003)</td>
<td>0.3679 (0.123)</td>
<td>0.0101 (0.0012)</td>
<td>0.422 (0.067)</td>
<td></td>
</tr>
<tr>
<td>Quercus myrsinifolia</td>
<td>0.0443 (0.004)</td>
<td>0.0150 (0.008)</td>
<td>0.0235 (0.003)</td>
<td>0.2763 (0.077)</td>
<td>0.0020 (0.0015)</td>
<td>0.717 (0.204)</td>
<td></td>
</tr>
<tr>
<td>Cryptomeria japonica</td>
<td>M (2 mg oyster shell g⁻¹)</td>
<td>0.1155 (0.006)</td>
<td>0.0154 (0.002)</td>
<td>0.0193 (0.003)</td>
<td>1.3684 (0.132)</td>
<td>0.0207 (0.0011)</td>
<td>0.617 (0.163)</td>
</tr>
<tr>
<td>Chamaecyparis obtusa</td>
<td>0.0592 (0.008)</td>
<td>0.0359 (0.016)</td>
<td>0.0235 (0.004)</td>
<td>0.7684 (0.406)</td>
<td>0.0036 (0.0014)</td>
<td>0.521 (0.199)</td>
<td></td>
</tr>
<tr>
<td>Fagus crenata</td>
<td>0.0560 (0.021)</td>
<td>0.0269 (0.012)</td>
<td>0.0158 (0.002)</td>
<td>0.8649 (0.328)</td>
<td>0.0018 (0.0017)</td>
<td>0.388 (0.092)</td>
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<td>Quercus serrata</td>
<td>0.0339 (0.013)</td>
<td>0.0124 (0.005)</td>
<td>0.0271 (0.002)</td>
<td>0.5970 (0.229)</td>
<td>0.0007 (0.0003)</td>
<td>0.640 (0.163)</td>
<td></td>
</tr>
<tr>
<td>Quercus crispula</td>
<td>0.0502 (0.011)</td>
<td>0.0126 (0.003)</td>
<td>0.0216 (0.008)</td>
<td>0.5050 (0.108)</td>
<td>0.0005 (0.0002)</td>
<td>0.458 (0.098)</td>
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<tr>
<td>Quercus myrsinifolia</td>
<td>0.0394 (0.007)</td>
<td>0.0049 (0.001)</td>
<td>0.0267 (0.005)</td>
<td>0.9793 (0.191)</td>
<td>0.0003 (0.0002)</td>
<td>0.451 (0.114)</td>
<td></td>
</tr>
<tr>
<td>Cryptomeria japonica</td>
<td>H (10 mg oyster shell g⁻¹)</td>
<td>0.1297 (0.018)</td>
<td>0.0160 (0.003)</td>
<td>0.0255 (0.002)</td>
<td>3.0281 (0.452)</td>
<td>0.0043 (0.0025)</td>
<td>0.416 (0.059)</td>
</tr>
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<td>Chamaecyparis obtusa</td>
<td>0.0546 (0.015)</td>
<td>0.0095 (0.002)</td>
<td>0.0235 (0.003)</td>
<td>0.7648 (0.366)</td>
<td>0.0044 (0.0040)</td>
<td>0.486 (0.069)</td>
<td></td>
</tr>
<tr>
<td>Fagus crenata</td>
<td>0.0634 (0.019)</td>
<td>0.0141 (0.004)</td>
<td>0.0283 (0.008)</td>
<td>0.9492 (0.459)</td>
<td>0.0007 (0.0004)</td>
<td>0.572 (0.163)</td>
<td></td>
</tr>
<tr>
<td>Quercus serrata</td>
<td>0.0587 (0.005)</td>
<td>0.0382 (0.031)</td>
<td>0.0297 (0.004)</td>
<td>0.7771 (0.478)</td>
<td>0.0008 (0.0002)</td>
<td>0.368 (0.039)</td>
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</tr>
<tr>
<td>Quercus crispula</td>
<td>0.0441 (0.018)</td>
<td>0.0073 (0.003)</td>
<td>0.0235 (0.005)</td>
<td>0.2095 (0.084)</td>
<td>0.0014 (0.0002)</td>
<td>0.649 (0.168)</td>
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<td>Quercus myrsinifolia</td>
<td>0.0536 (0.012)</td>
<td>0.0045 (0.001)</td>
<td>0.0240 (0.006)</td>
<td>0.8950 (0.099)</td>
<td>0.0005 (0.0001)</td>
<td>0.513 (0.126)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Concentration of exchangeable nutrients in the soil (mean ± 1 SE) for each tree species and quantity of oyster shell. L, M, and H indicate the oyster shell quantities.

Significant differences between the tree species for all oyster shell quantities are denoted by different letters (Tukey's HSD test, \( P < 0.05 \)).
Table 2 Results of statistical analysis for differences in root exudation rates of LMMOAs each pot (Two way-ANOVA).

<table>
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<tr>
<th></th>
<th>F value</th>
<th>d.f.</th>
<th>P value</th>
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<td>0.527</td>
</tr>
<tr>
<td>Interaction between Tree species and amount of oyster shell</td>
<td>0.783</td>
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<td>Malic acid</td>
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<td>0.981</td>
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<td>0.945</td>
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<td>Citric acid</td>
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<tr>
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<tr>
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Table 3 Results of statistical analysis for differences in concentration of exchangeable cations each pot (Two way-ANOVA).

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<th>P value</th>
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<td>Al</td>
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<td>0.918</td>
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<td>0.524</td>
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</table>
Fig. 1

- **A**: Total of all three LMNOAs (mg/L, root·h⁻¹)
- **B**: Fumaric (mg/g root·h⁻¹)
- **C**: Citric (mg/g root·h⁻¹)
- **D**: Malic (mg/g root·h⁻¹)

Legend:
- a
- b
- c
- d
- ab
- bc
- cd
- abc
- abcd

Concentration (mg/g)
- 0.0
- 0.2
- 0.4
- 0.6
- 0.8
- 1.0

Concentration (mg/L, root·h⁻¹)
- 0.0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
- 3.0

Concentration (mg/L, root·h⁻¹)
- 0.0
- 0.8
- 1.6
- 2.4
- 3.2
- 4.0
- 4.8
- 5.6
- 6.4
- 7.2
- 8.0

Legend:
- L
- M
- H

Other labels:
- QsM
- QcL
- QcH
- QmM
- FcL
- FcH
- CoM
- CjL
- CjH
Exchangeable cations in the soil (mg g⁻¹)

**Calcium**
- Low quantity of oyster shell
  - \( R^2 = 0.0138 \)
  - \( P = 0.580 \)
- Moderate quantity of oyster shell
  - \( R^2 = 0.3703 \)
  - \( P = 0.002 \)
- High quantity of oyster shell
  - \( R^2 = 0.2487 \)
  - \( P = 0.013 \)

**Magnesium**
- Low quantity of oyster shell
  - \( R^2 = 0.0006 \)
  - \( P = 0.912 \)
- Moderate quantity of oyster shell
  - \( R^2 = 0.4043 \)
  - \( P < 0.001 \)
- High quantity of oyster shell
  - \( R^2 = 0.404 \)
  - \( P < 0.001 \)

Root exudation rate of LMMOAs
- (mg g⁻¹ root · h⁻¹)