



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

3

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12 **Key words:** *Cryptomeria japonica*, organic acid, cations, plantation, pot experiment.

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

14 **Abbreviations:** LMMOAs, low molecular mass organic acids

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16

17 ABSTRACT

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

33 cation dynamics in soil.

34

35 **Introduction**

36 Many studies have demonstrated that the dynamics of carbon (C), nitrogen (N), and

37 phosphorus (P) can be altered by the physiological processes of organisms through the

38 alteration of chemical properties, such as decreasing pH levels and increasing nutrient

39 availability in soil (Riha et al. 1986; Vanni 2002). Supplies of low molecular mass

40 organic acids (LMMOAs) from the root systems of trees can also alter soil nutrient

41 dynamics (Clarholm et al. 2015). LMMOAs, such as citric acid and oxalic acid, are

42 released by the roots of vascular plants (Tyler and Ström 1995) and fungi, forming

43 ectomycorrhiza with tree roots (Wallander and Wickman 1999; Ahonen Jonnarth et al.

44 2000; van Hees et al. 2005). LMMOAs in soil can solubilize recalcitrant N and P, which

45 are then absorbed by plant roots (Simpson et al. 2002; Clarholm et al. 2015). Polyvalent

46 metal cations, such as calcium ions (Ca^{2+}), act as important pH buffers (Clarholm and

47 Skyllberg 2013), and cations in soil particles and base rock are leached by LMMOAs

48 (Dijkstra and Smits 2002; Simpson et al. 2002). As the supply of LMMOAs from roots

49 varies significantly among tree species (Aoki et al. 2012), differences in vegetation type
50 might affect the concentrations of exchangeable cations. This means that large-scale
51 changes in forest vegetation might modify the levels of exchangeable cations in soil. In
52 particular, monoculture tree plantations, which supply large quantities of LMMOAs,
53 might significantly increase soil exchangeable cation levels.

54 Japanese cedar (*Cryptomeria japonica* D. Don, Cupressaceae) has been
55 planted in large areas throughout Japan, accounting for approximately 12% of the total
56 land area (Forestry Agency 2011), and stocked large biomass (90 kg C ha⁻¹ in average)
57 in Japan (Fukuda et al. 2003). Ohta et al. (2014a; 2014b) reported that vegetation in
58 catchments might alter the exchangeable Ca concentrations in soil in Ca poor
59 environment. Ohta et al. (2014a; 2014b) observed that the concentration of
60 exchangeable Ca in *C. japonica* plantations was approximately three-fold higher than
61 that in evergreen broad-leaved forests. Furthermore, a similar phenomenon, in which
62 the soil Ca concentration in *C. japonica* plantations was higher than in broad-leaved
63 forests, has been reported for many areas of Japan (Tsutsumi 1987; Baba et al. 2004).
64 These patterns suggest that *C. japonica* has the potential to alter the Ca dynamics in soil.

65 However, the mechanisms underlying this phenomenon are not understood. Therefore,
66 *C. japonica* must be assessed by a comparison of physiological processes that affect soil
67 cation dynamics, including the supply of LMMOAs from roots. In fact, a Cupressaceae
68 species (*Calocedrus decurrens*) cause higher contents of LMMOAs in soils as
69 compared to other broad-leaved tree species (Strobel, 2001). Therefore, the exudation
70 rates of LMMOAs from roots of Cupressaceae are higher than for other tree species.

71 In addition, some plant species could increase the release of LMMOAs from
72 roots in response to a nutrient deficiency (Ström et al. 1994; Van Schöll et al. 2006).
73 Ohta et al. (2014b) showed that the exchangeable Ca concentration in soil was higher in
74 *C. japonica* plantations in Ca-poor environments. Cations such as Ca and magnesium
75 (Mg) are essential elements for tree species. For instance, Ca is involved in some
76 manner in nitrogen metabolism and Mg is a constituent of chlorophyll molecule
77 (Pallardy 2007). Therefore, cations are leached by LMMOAs might be important
78 nutrients for tree species in poor cations soil. We predicted that *C. japonica* would
79 increase the release of LMMOAs from roots in response to a Ca deficiency.

80 This study involved six tree species that are predominant in Japan. We
81 created variation in Ca availability by adding different amounts of crushed oyster shells
82 to the potting soil. Oyster shells contain CaCO₃ and MgO, which are major components
83 of common volcanic or sedimentary rock (Fukushima and Tatsumi 2007; GSJ-AIST
84 2010). We compared the exudation rate of LMMOAs from the roots and levels of
85 exchangeable cations in the soils. We tested the following hypotheses: (1) the exudation
86 rates of LMMOAs from roots are higher for *C. japonica* than for other tree species, (2)
87 the exudation rates of LMMOAs from roots are higher under Ca poor conditions, and
88 (3) the concentration of exchangeable cations in soil will increase with increasing
89 exudation rates of LMMOAs from roots.

90

91 **Materials and Methods**

92 *Study trees*

93 We conducted an experiment from 10 June to 10 August 2014 in the Wakayama
94 Experimental Forest of Hokkaido University (33°40'N, 135°40'E; 447 ha). We prepared
95 72 plastic pots (diameter, 25 cm; height, 30 cm) and 72 seedlings of six dominant tree

96 species (12 pots per species): *C. japonica*, *Chamaecyparis obtusa*, *Fagus crenata*,

97 *Quercus myrsinifolia*, *Quercus crispula* and *Quercus serrata*.

98

99 *Experimental system*

100 A total of 3 kg of commercial soil (Kanuma soil), which contained very few nutrients

101 (Yoshida et al. 1992; Fukushima and Tatsumi 2007), was placed in each of the 72 pots.

102 Equal amounts of KH_2PO_4 and $\text{CO}(\text{NH}_2)_2$ were added as fertilizer to the Kanuma soil to

103 create soils with similar concentrations of nutrients to the soil in the Wakayama

104 Experimental Forest (N: 15 mg g^{-1} ; P: 2 mg g^{-1} ; Ohta et al. 2014b). Broken oyster shells

105 were also mixed with the soil in three different amounts (0.5, 2, and 10 mg oyster shell

106 g^{-1} soil) to vary the amounts of raw ingredients for exchangeable Ca and Mg. The Ca

107 concentration at low quantities of oyster shell ($0.5 \text{ mg oyster shell g}^{-1}$ soil) was similar

108 to the concentration in Wakayama Experimental Forest soil (Ohta et al. 2014a). Three

109 subsamples of broken oyster shells were collected to measure the elemental components.

110 And then, the 72 seedlings of tree planted in each plastic pot. The plastic pots were

111 arranged randomly under a plastic roof to prevent nutrient deposition by rainfall; they

112 were maintained for 60 days. The seedlings were supplied with 200 mL of water daily,
113 in the morning.

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

128 (approximately 100 g fresh weight) near the roots that will extract LMMOAs.

129

130 *Sample processing*

131 To estimate the root exudation rates of LMMOAs, the concentrations of LMMOAs in

132 the liquid containing the root exudates were analyzed by high-performance liquid

133 chromatography (Shimadzu, Kyoto, Japan) according to van Hees et al. (1999) and

134 Aoki et al. (2012). The LMMOAs were separated on a Supelcogel C610-H ion

135 exclusion column, using 0.1% H₃PO₄ as the mobile phase at operating temperatures of

136 60°C for citric acid and 30°C for oxalic acid and malic acid, with UV detection at 210

137 nm. After the analysis of organic acids, we calculated the root exudation rates of

138 LMMOAs (mg g⁻¹ root h⁻¹).

139 To measure exchangeable nutrients in soil collected near the root that

140 extracted LMMOAs, we shook (160 rev min⁻¹) a 0.5 g (air-dried mass) subsample of

141 each soil sample in 100 mL of 1 M KCl for 1 h, filtered the sample through filter paper

142 (No. 5C; Advantec, Tokyo, Japan), and then stored the suspension at -30°C until

143 analysis. The KCl extracts of soil were analysed for concentrations of exchangeable

144 ions (Mg, Al, P, Ca, iron [Fe] and nitrate [NO₃]) per unit air-dried mass using an
145 inductively coupled plasma atomic emission spectrometer (iCAP 6200; Thermo
146 Scientific, Cambridge, UK) and the absorptiometric method (Sakata 2000).
147 Exchangeable elements in the samples were quantified with an internal standard
148 (yttrium), which was added to the extract before analysis. Al and Fe are major
149 components of Kanuma soil.

150 To measure the elemental components of oyster shells, subsamples of broken
151 oyster shells were extracted with 70% HNO₃ at 80°C for 24 h. The extracts were
152 analyzed for their Mg, Al, P, Ca, and Fe contents using an atomic emission
153 spectrometer (iCAP 6200; Thermo Scientific, Cambridge, UK). The weight percentage
154 of the nitrogen in the oyster shell was obtained by an elemental analyzer (Flash EA
155 1112, Thermo Finnigan, Milan, Italy). Mean concentrations (\pm SE) of each element in
156 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
157 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.

158

159 Statistical analysis

160 To test hypotheses 1 and 2, we analyzed the exudation rates of LMMOAs from root
161 using a two-way ANOVA with tree species and oyster shell quantity, followed by post
162 hoc comparisons using Tukey's honestly significant difference (HSD) test. If the result
163 of two-way ANOVA indicated the exudation rates of LMMOAs from root differed
164 significantly among the tree species, but not among the different quantities of oyster
165 shell, we conducted the post-Tukey comparisons between tree species regardless of the
166 differences in quantities of oyster shell. To test hypothesis 3, we analyzed the
167 concentrations of exchangeable nutrients (Mg, Al, P, Ca, Fe and NO₃) in the soils using
168 a two-way ANOVA with tree species and oyster shell quantity, followed by post hoc
169 comparisons using Tukey's HSD test. The concentrations of exchangeable nutrients for
170 each quantity of oyster shell were then analyzed according to a linear model, with the
171 root exudation rates of the total of all three LMMOAs. All statistical analyses were
172 performed using R version 3.0.1 (R Core Team 2013).

173

174 **Results**

175 *The effects of tree species and oyster shells on the exudation of LMMOAs*

176 We detected three types of LMMOAs (citric, malic, and fumaric acids), but we could
177 not detect other LMMOAs, such as acetic or oxalic acid. The exudation rates of citric,
178 malic, and fumaric acids from roots differed significantly among the tree species, but
179 not among the different quantities of oyster shell (Table 1; Fig. 1). Therefore, we
180 conducted the post-Tukey comparisons between tree species regardless of the
181 differences in quantities of oyster shell. The exudation rates of fumaric and malic acid
182 from the *C. japonica* roots were significantly higher than the rates from the other five
183 species (Fig. 1; Tukey's HSD test, $P < 0.001$). The exudation rates of citric acid from
184 the *C. japonica* roots were not significantly higher than the rates from the other five
185 species (Fig. 1; Tukey's HSD test, $P > 0.05$). The exudation rates of total three
186 LMMOAs from the *C. japonica* roots were significantly higher than the rates from the
187 other five species (Fig. 1; Tukey's HSD test, $P < 0.05$).

188

189 *Effects of LMMMOAs from different trees species and oyster shell on the exchangeable*

190 *Mg and Ca in the soil*

191 Table 1 show concentration of exchangeable nutrients in the soil for each tree
192 species and quantity of oyster shell. Exchangeable Mg and Ca in the soils differed
193 significantly among the tree species, and Ca differed significantly among the quantities
194 of oyster shell (Tables 1, 3). Exchangeable Ca in the soils with *C. japonica* was
195 significantly higher at high quantities of oyster shell than others (Table 1; Tukey's HSD
196 test). The amount of exchangeable Mg in the soils with *C. japonica* was significantly
197 higher at moderate and high quantities of oyster shell than at low quantity of oyster
198 shell (Table 1; Tukey's HSD test). However, the amount of exchangeable P, Al, Fe and
199 NO₃ in the soils did not differ significantly among tree species and quantities of oyster
200 shell (Tables 1, 3). In addition, we observed significant positive correlation between the
201 exudation rates of LMMOAs from roots and the amounts of exchangeable Ca and Mg in
202 soil at moderate and high quantities of oyster shell (Fig. 2). However, these significant
203 correlations were not observed at low quantity of oyster shell (Fig. 2).

204

205 **Discussion**

206 The exudation rates of fumaric and malic acid from roots are higher for *C. japonica*
207 than for other tree species (Fig. 1, supporting hypothesis 1). This study shows that the
208 exudation rates of LMMOAs from roots differed significantly among tree species but
209 not among varying quantities of oyster shell (Table 2, opposing hypothesis 2), and that
210 the variation in the exudation rates of LMMOAs from roots might alter the cations
211 dynamics in soil, supporting hypothesis 3. There was a significantly positive correlation
212 between the root exudation rates of LMMOAs and exchangeable Ca and Mg at
213 moderate and high quantities of oyster shell, but this relationship was not evident at low
214 quantities of oyster shell. At low quantities of oyster shell, there might be very few
215 solutes that dissolve easily in LMMOAs, such as oyster shells in the soil. This finding
216 suggests that the exchangeable Ca and Mg were supplied by the oyster shells. Oyster
217 shells contain high concentrations of CaCO_3 and MgO , which are major components of
218 common volcanic and sedimentary rock (Fukushima and Tatsumi 2007; GSJ-AIST
219 2010). Therefore, root exudation of LMMOAs might dissolve Ca and Mg from
220 limestone, basalt, sand stone, and weathered soil. Ohta et al. (2014b) showed that the
221 amount of exchangeable Ca in soil weathered from sandstone was ~three times higher in

222 *C. japonica* plantations than in evergreen broad-leaved forests. Furthermore, the supply
223 of LMMOAs increases nutrient mobilization. LMMOAs might enhance the weathering
224 of base rock (Drever 1994; Drever and Stillings 1997). Therefore, large-scale planting
225 of a tree species that exhibit high exudation rates of LMMOAs from their roots might
226 increase the mobilization of nutrients and base rock disintegration.

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

236 On the other hand, the exudation rates of LMMOAs from roots did not differ
237 significantly among the quantities of oyster shell (Table 1; Fig. 1). Therefore, a Ca

238 deficiency might not contribute to an increase in the exudation rates of LMMOAs from
239 *C. japonica* roots. Aoki et al. (2010) suggested that the exudation of LMMOAs
240 contributes to P solubilization in soil and its uptake by plants in P-poor environments.
241 *Cryptomeria japonica* might absorb essential nutrients, such as N or P, through
242 increases in the exudation rate of LMMOAs from roots. Further research is needed to
243 understand why the exudation rates of LMMOAs from *C. japonica* roots are higher than
244 for other species.

245 Furthermore, the biomass and activity of microbe can alter the root exudation
246 rates of LMMOAs (Clarholm and Skjellberg 2013; Clarholm et al. 2015), and are
247 decreased by soil acidification (Vance et al. 1987). Therefore, LMMOAs might cause
248 soil acidification and decrease the biomass and activity of microbe. Because Ca in soil
249 has pH buffering ability, high quantity of oyster shell in soil might prevent soil
250 acidification by LMMOAs and maintain the exudation rate of LMMOAs from roots.
251 However, our results indicate the exudation rates of LMMOAs from roots did not differ
252 significantly among the quantities of oyster shell (Table 1; Fig. 1). Although we did not

253 measured pH levels in the soils, significant acidification by LMMOAs might not be
254 happened in our pot-systems.

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

265 The growth rate of the *C. japonica* root system is higher than that for other
266 dominant tree species in Japan (Karizumi 1987). Root length and depth distribution
267 (Dijkstra and Smits 2002), and the root N concentration (Mueller et al. 2012), might
268 also alter the dynamics of cations in soil. Therefore, to consider the effects of tree

269 performance on the dynamics of cations in soil, future studies should focus on
270 differences in these additional factors, such as length, depth distribution and N
271 concentration of root and microbial biomass in rhizosphere soil. Furthermore, high
272 biomass of arbuscular and ectomycorrhiza and high density of bacteria near plant roots
273 has been attributed to rhizodeposit compounds including LMMOAs (van Hees et al.
274 2005; Bais et al. 2006). Further studies are needed to clarify the ecological significance
275 of mineral weathering and carbon dynamics in arbuscular and ectomycorrhizal fungal
276 partnerships with trees (Koele et al. 2014; Thorley et al. 2015) also in Japanese forests.

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428 Figure 1. Differences in the total root exudation rates of all three LMMOAs (A),
429 fumaric acid, citric acid, and malic acid among tree species. Means and standard errors
430 (+1 SE) are shown. L, M, and H indicate the added amounts of oyster shell. Significant
431 differences ($P < 0.05$) between tree species are denoted by different letters. *Cj*,
432 *Cryptomeria japonica*; *Co*, *Chamaecyparis obtuse*; *Fc*, *Fagus crenata*; *Qs*, *Quercus*
433 *serrata*; *Qc*, *Quercus crispula*; *Qm*, *Quercus myrsinifolia*

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435 Figure 2. Relationships between the concentrations of exchangeable Ca and Mg and the
436 root exudation rates of LMMOAs at different quantities of oyster shell.

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444 Table 1. Concentration of exchangeable nutrients in the soil (mean \pm 1 SE) for each tree
 445 species and quantity of oyster shell. L, M, and H indicate the oyster shell quantities.
 446 Significant differences between the tree species for all oyster shell quantities are
 447 denoted by different letters (Tukey's HSD test, $P < 0.05$).

Tree species	Ca amendment	Mg (mg g ⁻¹)	Al (mg g ⁻¹)	P (mg g ⁻¹)	Ca (mg g ⁻¹)	Fe (mg g ⁻¹)	NO ₃ (mg g ⁻¹)
<i>Cryptomeria japonica</i>	L (0.5 mg oyster shell g soil ⁻¹)	0.0804 (0.005) ^a	0.0266 (0.019) ^a	0.0286 (0.009) ^a	1.1330 (0.306) ^a	0.0018 (0.0009) ^a	0.494 (0.097) ^a
<i>Chamaecyparis obtusa</i>		0.0691 (0.022) ^a	0.0221 (0.011) ^a	0.0294 (0.003) ^a	0.7323 (0.364) ^a	0.0011 (0.0002) ^a	0.462 (0.063) ^a
<i>Fagus crenata</i>		0.0631 (0.002) ^a	0.0123 (0.002) ^a	0.0135 (0.002) ^a	0.6575 (0.111) ^a	0.0006 (0.0003) ^a	0.454 (0.080) ^a
<i>Quercus serrata</i>		0.0636 (0.002) ^a	0.0330 (0.019) ^a	0.0261 (0.009) ^a	0.7067 (0.345) ^a	0.0007 (0.0004) ^a	0.512 (0.114) ^a
<i>Quercus crispula</i>		0.0725 (0.007) ^a	0.0010 (0.003) ^a	0.0232 (0.003) ^a	0.3679 (0.123) ^a	0.0019 (0.0012) ^a	0.422 (0.067) ^a
<i>Quercus myrsinaefolia</i>		0.0443 (0.004) ^a	0.0150 (0.008) ^a	0.0235 (0.003) ^a	0.2763 (0.077) ^a	0.0020 (0.0015) ^a	0.717 (0.204) ^a
<i>Cryptomeria japonica</i>	M (2 mg oyster shell g soil ⁻¹)	0.1155 (0.006) ^b	0.0154 (0.002) ^a	0.0193 (0.003) ^a	1.3684 (0.132) ^a	0.0027 (0.0011) ^a	0.617 (0.163) ^a
<i>Chamaecyparis obtusa</i>		0.0592 (0.008) ^a	0.0359 (0.016) ^a	0.0235 (0.004) ^a	0.7684 (0.406) ^a	0.0036 (0.0014) ^a	0.521 (0.199) ^a
<i>Fagus crenata</i>		0.0560 (0.021) ^a	0.0269 (0.012) ^a	0.0158 (0.002) ^a	0.8649 (0.328) ^a	0.0018 (0.0017) ^a	0.388 (0.092) ^a
<i>Quercus serrata</i>		0.0339 (0.013) ^a	0.0124 (0.005) ^a	0.0271 (0.002) ^a	0.5970 (0.229) ^a	0.0007 (0.0003) ^a	0.640 (0.163) ^a
<i>Quercus crispula</i>		0.0602 (0.011) ^a	0.0126 (0.003) ^a	0.0216 (0.008) ^a	0.5050 (0.108) ^a	0.0005 (0.0002) ^a	0.458 (0.098) ^a
<i>Quercus myrsinaefolia</i>		0.0394 (0.007) ^a	0.0049 (0.001) ^a	0.0267 (0.005) ^a	0.9793 (0.191) ^a	0.0003 (0.0002) ^a	0.451 (0.114) ^a
<i>Cryptomeria japonica</i>	H (10 mg oyster shell g soil ⁻¹)	0.1297 (0.018) ^b	0.0160 (0.003) ^a	0.0255 (0.002) ^a	3.0281 (0.452) ^b	0.0043 (0.0025) ^a	0.416 (0.059) ^a
<i>Chamaecyparis obtusa</i>		0.0546 (0.015) ^a	0.0095 (0.002) ^a	0.0235 (0.003) ^a	0.7648 (0.366) ^a	0.0044 (0.0040) ^a	0.486 (0.069) ^a
<i>Fagus crenata</i>		0.0634 (0.019) ^a	0.0141 (0.004) ^a	0.0283 (0.011) ^a	0.9492 (0.459) ^a	0.0007 (0.0004) ^a	0.572 (0.163) ^a
<i>Quercus serrata</i>		0.0587 (0.005) ^a	0.0382 (0.031) ^a	0.0297 (0.004) ^a	0.7771 (0.478) ^a	0.0008 (0.0002) ^a	0.368 (0.039) ^a
<i>Quercus crispula</i>		0.0441 (0.018) ^a	0.0073 (0.003) ^a	0.0235 (0.005) ^a	0.2095 (0.084) ^a	0.0014 (0.0002) ^a	0.649 (0.168) ^a
<i>Quercus myrsinaefolia</i>		0.0536 (0.012) ^a	0.0043 (0.001) ^a	0.0240 (0.006) ^a	0.8950 (0.099) ^a	0.0005 (0.0001) ^a	0.513 (0.128) ^a

460 Table 2 Results of statistical analysis for differences in root exudation rates of

461 LMMOAs each pot (Two way-ANOVA).

		<i>F</i> value	d.f.	<i>P</i> value
Total of all three LMMOAs	Tree species	12.563	5	< 0.001
	Amount of oyster shell	0.649	2	0.527
	Interaction between Tree species and amount of oyster shell	0.783	10	0.645
Malic acid	Tree species	22.702	5	< 0.001
	Amount of oyster shell	0.019	2	0.981
	Interaction between Tree species and amount of oyster shell	0.392	10	0.945
Citric acid	Tree species	2.985	5	0.019
	Amount of oyster shell	0.198	2	0.821
	Interaction between Tree species and amount of oyster shell	0.987	10	0.466
Fumaric acid	Tree species	21.074	5	< 0.001
	Amount of oyster shell	0.951	2	0.393
	Interaction between Tree species and amount of oyster shell	0.950	10	0.497

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471 Table 3 Results of statistical analysis for differences in concentration of exchangeable

472 cations each pot (Two way-ANOVA).

		<i>F</i> value	d.f.	<i>P</i> value
Mg	Tree species	9.403	5	< 0.001
	amount of oyster shell	0.446	2	0.643
	Interaction between Tree species and amount of oyster shell	1.447	10	0.185
Al	Tree species	1.297	5	0.279
	amount of oyster shell	0.287	2	0.754
	Interaction between Tree species and amount of oyster shell	0.718	10	0.704
P	Tree species	0.593	5	0.705
	amount of oyster shell	0.597	2	0.554
	Interaction between Tree species and amount of oyster shell	0.718	10	0.704
Ca	Tree species	8.808	5	< 0.001
	amount of oyster shell	3.643	2	0.033
	Interaction between Tree species and amount of oyster shell	2.187	10	0.033
Fe	Tree species	1.666	5	0.159
	amount of oyster shell	0.350	2	0.706
	Interaction between Tree species and amount of oyster shell	0.603	10	0.805
NO ₃	Tree species	0.170	5	0.973
	amount of oyster shell	0.015	2	0.985
	Interaction between Tree species and amount of oyster shell	0.918	10	0.524

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