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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.

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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 **Abbrevistions**: **LMMOAs,** low molecular mass organic acids

15

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 $CaCO_3$ 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 $CO(NH_2)_2$ 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 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 NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, and 0.2 mM

122    $MgSO_4$) 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% H_3PO_4 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 [NO_3]) 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% HNO_3 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 (± 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 NO_3) 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 LMMMOAs*

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   NO_3 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 Skyllberg 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 Skyllberg 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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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, $P < 0.05$).

Tree species	Ca amendment	Mg (mg g⁻¹)	Al (mg g⁻¹)	P (mg g⁻¹)	Ca (mg g⁻¹)	Fe (mg g⁻¹)	NO₃ (mg g⁻¹)
Cryptomeria japonica	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
Chamaecyparis obtusa		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
Fagus crenata		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
Quercus serrata		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
Quercus crispula		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
Quercus myrsinaefolia		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
Cryptomeria japonica	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
Chamaecyparis obtusa		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
Fagus crenata		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
Quercus serrata		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
Quercus crispula		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
Quercus myrsinaefolia		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
Cryptomeria japonica	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
Chamaecyparis obtusa		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
Fagus crenata		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
Quercus serrata		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
Quercus crispula		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
Quercus myrsinaefolia		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).

		F value	d.f.	*P* 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).

		F value	d.f.	*P* 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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