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The growth of *Larix gmelinii* seedlings as affected by charcoal produced at two different temperatures

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Abstract

Fires burn forest with spatially heterogeneous intensity and charcoals generated at various temperatures during fires exhibit variable physical and chemical characteristics. These variable properties of charcoal may, in turn, influence germination and growth of tree seedlings. To examine the effects of different charcoal properties on the growth of Gmelin larch (*Larix gmelinii*) seedlings, we conducted an experiment with larch-branch derived charcoals produced at 400°C (LOW charcoal) and 800°C (HIGH charcoal), within charcoal combined with sand in three different rates (5%, 20% and 50%, v/v charcoal in sand). While the LOW charcoal application stimulated growth and the effect increased with the rate of mixture, the HIGH charcoal did not significantly influence at any contents in the pots. The LOW charcoal application resulted in the greater available P content, a lower N:P in needles and the greater growth of seedlings than HIGH charcoal application. In contrast, the growth of seedlings was not affected by the application of the HIGH charcoal at any content probably because the HIGH charcoal inhibited the seedling growth due to its high pH. These results indicate that charcoal produced at different temperatures during forest fires can affect the growth of Gmelin larch seedlings differently in the Russian Far East.

2

Introduction

61

62   Fire is the primary form of disturbance driving the vegetation succession and structure in the

63   boreal forests (Goldammer and Furyaev 1996; Makoto et al. 2007). Forest fires create

64   heterogeneous conditions that can directly affect the vegetation composition, succession, and

65   structure (Turner et al. 1994; Miller and Urban 1999). To understand how forest fires affect

66   vegetation structure, it is necessary to understand factors driving environmental changes in

67   post-fire forests. The highly variable surface soil conditions created by fire are known to

68   directly affect vegetation structure (e.g. Smithwick et al. 2005). Indeed, fires cause variable

69   rates of charcoal and ash deposition and heat transfer into surface soils resulting in variable

70   chemical, physical, and biological properties in the soil (Zackrisson et al. 1996, Wardle et al.

71   1998, Neary et al. 1999; Certini 2005; Smithwick et al. 2005, DeLuca and Sala 2006 Mackenzie

72   et al. 2008). The influence of charcoal on soil processes has been poorly studied but we know

73   that charcoal may regulate soil conditions, leading to the variable responses of seedling

74   following fires in the forest ecosystems (Wardle et al. 1998; Gundale and DeLuca 2007;

75   Mackenzie et al. 2008).

76   The primary mechanism though which charcoal influences plant growth is alteration of

77   nutrient and water availability in soil (Tryon 1948; Glaser et al. 2002; Lehmann et al. 2003).

78   The porous nature of charcoal can increase the water holding capacity of soil and coincidentally

79   retention of soluble nutrients in soil. Ash associated with charcoal and embedded in charcoal

80   pores, contains large contents of nutrients like NH_4^+ and PO_4^{3-} as well as oxides of alkaline

81   metals that can increase soil pH in the immediate vicinity of the charcoal (Tryon 1948; Urely et

82   al. 1993; Topoliantz et al. 2005; Steiner et al. 2007) with a better plant growth (Topoliantz et al.

83   2005). Charcoal can also increase the soil surface temperature by reducing surface albedo

84   (Glaser et al. 2002; Certini 2005).

85   Charcoal can have different characteristics (e.g., nutrient contents, pH, and capacity to adsorb

86   organic compounds) depending on the charring temperature (Glaser et al. 2002; Brown et al.

87   2006; Gundale and DeLuca 2006). Therefore, we hypothesize that fire creates variation in the

88   soil conditions by producing charcoals at different temperatures. However, currently there is

89   limited information of how different charcoal produced at different temperatures can affect tree

90   growth (Lehmann and Joseph 2009).

91   In this study, we examined the effects of various charcoals on the growth and nutrient

92   physiology of Gmelin larch seedlings. We studied the effects of two charcoals produced at

93   different temperatures and applied to sand at different rates on the nutrient status and the growth

94   of Gmelin larch seedlings. The Gmelin larch (*Larix gmelinii*) is the dominant species in Far East

95   Russia and its regeneration is deeply associated with forest fire (Goldammer and Xueying 1992;

96   Makoto et al. 2007). Based on these results, we discuss how charcoals produced at different

3

97 temperatures can influence the initial vegetation growth and nutrient uptake in a post-fire
98 Gmelin larch forest in Far East Russia. We used two types of charcoals produced at two
99 temperatures (400°C and 800°C), which are representative of the lower and upper thresholds of
100 charring temperatures during forest fires (Glaser et al. 2002; Gundale and DeLuca 2006;
101 Makoto 2010).

102

103 **Materials and Methods**

104 *Seeds, sand and charcoal*

105 Seeds of the Gmelin larch (*Larix gmelinii* (Rupr.) Kuzen.) were imported from Amur in Far
106 East Russia, sterilized with 20% H_2O_2, put into Petri dishes, and germinated in a growth
107 chamber (EYELA MTI-202, Tokyo Rika, Japan) at 30°C with a day length of 16 h. Each
108 germinated seed was planted in a 500 ml pot in a greenhouse of the Faculty of Agriculture,
109 Hokkaido University, Sapporo, Northern Japan. The day length of 16 h was obtained with a
110 supplementary light system with halogen lamps (60 W, Toshiba, Tokyo) and was to simulate
111 the conditions of the growing season of Gmelin larch in Amur State, Russia (from May to
112 August). The air temperature was maintained between 24°C and 32°C (night / day), which is the
113 air temperature range during the summer in Amur. Water was applied every 1 or 2 days. The
114 seedlings were grown for 4 months.

115 We used river sand so as to simulate the edaphic condition of the Amur River basin (Table 1;
116 Makoto et al. 2007). Charcoal was produced from the fresh branches of standing Japanese larch
117 (30 y old), planted at Hokkaido University, in an electric furnace (ADVANTEC, MFS, Inc.
118 Japan, KT-1053P) within a N atmosphere following Brown et al. (2006). Fresh branches were
119 set into the tube furnace and the temperature in the tube was increased from room temperature
120 to either 400°C (LOW charcoal) or 800°C (HIGH charcoal). These two temperatures are
121 representative of the lower and upper thresholds of charring temperatures during forest fires
122 (Glaser et al. 2002; Gundale and DeLuca 2006; Makoto 2010). We left the branches for 3 hours
123 as the target temperature to complete the charring process. The samples were left in the tube
124 overnight in order to allow them to cool. Both the LOW and HIGH temperature charcoals were
125 ground with a hammer, sieved 1 < mm and < 2-mm and used for the subsequent experiments.

126

127 *Experimental flow and analysis*

128 The LOW and HIGH charcoals were added at three rates respectively to the river sand.The
129 weights of LOW charcoal added to sand were 3.15g, 12.6g and 31.5g, and those of HIGH
130 charcoal were 3.6g, 14.4g and 36.0g respectively. The total volume of each pot including
131 charcoal and river sand was 300ml across all treatments. The mass of charcoal added to each pot
132 accounted for 5%, 20% and 50% of total media by volume. At the beginning of the experiment,

4

133   we prepared 12 seedlings per treatment (72 seedlings in 72 independent pots for 6 treatments).

134   Unfortunately, some seedlings died during the growing period leaving 9 to 12 seedlings in each

135   treatment at the end of the experiment.

136   Four months after the planting, we harvested the seedlings. The shoot (S) and the root (R) of

137   the seedlings were separated, and their dry mass was determined after drying for 4 d at 60°C.

138   The dried needles were ground for chemical analyses. The needle nitrogen (N) content was

139   analyzed with a CHNO analyzer (PE-2400 series, Perkin-Elmer, Norwalk, CT). The remaining

140   needles were digested according to the modified wet digestion method (Kayama et al. 2009),

141   and the P content was analyzed using inductively coupled plasma spectroscopy (IRIS, Jarrel

142   Ash, Franklin, MA).

143   The pH and electrical conductivity (EC) of both the LOW and the HIGH charcoals were

144   measured in slurries (1:4 and 1:2 charcoal / deionized water respectively) (Gundale and DeLuca

145   2006). A sand or charcoal (1g) was shaken with 2 M KCl (25 ml) for 1 h and the NH_4^+ and the

146   NO_3^- contents of the extract were analyzed with an auto-analyzer (AACS-4, BL-TEC, Inc,

147   Japan). The NH_4^+ was determined with indophenol blue absorption spectrometry, and the NO_3^-

148   was by the naphthyl ethylenediamine dihydrochloride spectrophotometric method. The

149   plant-available P was determined following the Bray II method (Bray and Kurtz 1945). The

150   contents of all of the nutrients and the average of the three replicates were expressed on a dry

151   mass basis by calculating the water contents of the fresh and air-dried samples.

152   The water holding capacity of river sand and charcoal was determined as following. The pots

153   including river sand and charcoal at three rates (5%, 20% and 50%, v/v charcoal in sand) was

154   put in a metal bath and soaked in distilled water for 24 h at room temperature to allow the sand

155   or charcoal in the pots to absorb water by capillary action. The water depth was 0.5 cm, while

156   that of the sand or charcoal in the pots was 2.0 cm. After 24 h of water absorption, we

157   determined the weight of the pots. Then the pot was dried at 85°C for 3 days, weighed and the

158   weight of the water absorbed by river sand or charcoal was calculated to determine the water

159   holding capacity.

160

Charcoal effects on sand temperature

162   Before the experiment, the pots with sand and/or charcoal were placed in the refrigerating

163   chamber at 4°C overnight to standardize the temperature of all of the pots. The experimental

164   area was an outdoor area where no shade prevents the sunlight. The pots were taken from the

165   refrigerating chamber and put into a white paper box in the experimental area in the field at

166   about 11:30 am on the day of measurement. The sand temperature in the pots was then

167   monitored with a thermal camera (Thermo-Tracer TH9100MV/WV, NEC, Japan).

168   Before the experiment, we determined the distance between the camera and pots and the

169 degree of reflection. The thermal photographs were taken at 0, 1, 2, 5, 10, 30, 60, and 120

170 minutes after setting the pots in the box. A preliminary experiment confirmed that 120 minutes

171 at midday is a sufficient time to saturate the pot temperatures. The experiment was repeated

172 three times in different days and regarded as three replicates. Then, we evaluated the charcoal

173 effects on sand temperature from the photographs taken with the thermal camera.

174

175 *Statistical analysis*

176 　　We analyzed the effects of type and amount of charcoal on sand properties and plant using

177 generalized linear model (GLM) (Crawley 2005). The explanatory variables were the charring

178 temperature, the charcoal content and their interaction, and the response variables were each

179 parameter of plant growth and the needle nutrient contents. Correlations between the dry mass

180 of the seedling and the needle nutrients and their balances were explored with Pearson's

181 correlation tests or Spearman's rank correlation tests for the LOW and the HIGH charcoal

182 treatments. For all of the analyses, significance was considered to be at $p<0.05$. All of the

183 analyses were performed using the R software package, version 2.6.0 (R Development Core

184 Team 2007). In the case of sand temperature data, we did not conduct a statistical analysis and

185 we considered the visible clear differences from the photography using the thermal camera.

186

Results

187

188 *Chemical and physical properties of charcoal*

189 　　The HIGH charcoal had a higher pH, EC and density and NO_3^- and available P contents than

190 the LOW charcoal (Table 1). On the contrary, the LOW charcoal had more NH_4^+ content and

191 water holding capacity than the HIGH charcoal (Table 1). Visually the increase in the charcoal

192 content mixed increased in the sand surface temperature. The LOW charcoal increased the sand

193 surface temperature more than the HIGH charcoal.

194

195 *Seedling growth*

196 　　Charring temperature and interaction of charring temperature with charcoal contents affected

197 the shoot dry mass of seedlings ($p<0.05$ and $p<0.001$; Fig. 1a). By increasing LOW charcoal

198 application increased shoot dry mass, whereas the HIGH charcoal application did not

199 significantly affect at any rates ($p<0.001$; Fig. 1a). Charcoal contents and charring temperature

200 interactively influenced the root dry mass of seedling ($p<0.01$; Fig. 1b). By increasing the LOW

201 charcoal application increased root dry mass, whereas the HIGH charcoal application did not

202 significantly affected at any rates ($p<0.001$; Fig. 1b). The HIGH charcoal application did not

203 affect S/R ratios at any rates, whereas the LOW charcoal significantly increased the S/R at 50%

204 contents ($p<0.05$; Fig. 1c).

6

205

Needle nutrient content and balances

207   None of the charcoal treatments significantly affected the N content in the larch needles (Fig.

208   2a). The charring temperature solely and interaction of charring temperature with charcoal

209   contents affected the needle P contents ($p<0.001$ and $p<0.01$; Fig. 2b). The needle P contents

210   increased with the rates of LOW charcoal application, but it was not significantly affected by

211   the HIGH charcoal application ($p<0.01$; Fig. 2b). The rates of LOW charcoal at 5% and 20%

212   increased the needle N/P ratios more than the application of the LOW charcoal at 50%, whereas

213   HIGH charcoal application did not affect N/P ratios at any contents ($p<0.05$; Fig. 2c).

214

Relationship between nutrient conditions in the needles and the seedling growth

216   There was no significant relationship between the needle N contents and the total dry mass

217   (shoot plus root dry mass) of seedlings. The needle P contents had a significant positive

218   correlation with the total dry mass of seedlings in the application of LOW charcoal ($r=0.562$;

219    $p<0.001$), whereas there was no significant relations with the HIGH charcoal application. The

220   needle N/P ratios had a significant positive correlation with the total dry mass of seedlings with

221   the LOW charcoal ($r=0.48$; $p<0.001$), but not with the HIGH charcoal.

222

Discussion

224   The LOW charcoal application enhanced growth of Gmelin-larch seedlings (Fig. 1a and 1b).

225   The significant positive relationship between the dry mass of seedling with the LOW charcoal

226   and P content and N/P ratio (negative) suggests that P availability limits the seedling growth.

227   The LOW charcoal contained greater inorganic N (mainly NH_4^+) than the HIGH charcoal

228   probably due to lower volatilization of organic N of plant biomass during combustion at lower

229   temperature (Certini 2005). The presence of inorganic N would likely shift the limiting factor

230   from N to P availability (Chapin 1980, Aerts and Chapin 2000). As a consequence, the

231   alleviation of P limitation with the more application of the LOW charcoal would correspond

232   with the more growth of seedling.

233   The HIGH charcoal application did not significantly affect the growth of seedlings at any

234   rates ($p<0.001$; Fig. 1) but it increased needle P contents. Therefore there was another limiting

235   factor for seedling growth beyond P and N availability in the case of the HIGH charcoal

236   application. We believe that there are four plausible scenarios by which the HIGH charcoal did

237   not have significant effects on the seedling growth based on the results: (1) sand pH, (2) sand

238   moisture and (3) sand temperature. The pH of HIGH charcoal was 9.6 (Table 1). Ishii and

239   Kadoya (1994) suggested that higher amounts of charcoal caused a higher pH and inhibited

240   plant growth. The HIGH charcoal had a higher pH than LOW charcoal due to the higher

7

241 contents of ash and oxides of Ca and Mg as a result of higher mineralization during the
242 combustion, and by hydration, oxides can produce more OH^- than LOW charcoal. The high pH
243 (over pH 7.0) can cause deficiency in the macronutrients that are essential for the plant growth
244 (Brady and Weil 2002; Hiradate et al. 2007). Differences in the water holding capacity of
245 treated sand were not likely the influential factors limiting seedling growth when HIGH
246 charcoal was applied. Even though the HIGH charcoal had less water holding capacity than the
247 LOW charcoal, both charcoals had more water holding capacity than sand (Table 1). As a
248 consequence, application of both types of charcoal increased availability of water for seedlings.
249 However, only application of LOW charcoal increased seedling growth. The high sand
250 temperature was also not a plausible explanation of the obtained data in the present. Higher soil
251 temperatures than 15^oC can inhibit the root elongation of larch species (Qu et al. 2009).
252 Charcoal can generate heat through the adsorption of far infrared radiation (Tai et al 1999) and
253 charcoal produced at high temperatures generated more heat through the greater adsorption of
254 far infrared radiation than charcoal generated at low temperature (Reeves et al 2008). However,
255 we observed the higher sand temperature in the pots with the LOW than HIGH charcoal. These
256 contradictory results suggest the need for future research on the affect of charcoal produced at
257 different temperature on the sand temperature and its mechanism. At least in the present study,
258 we suggest that high temperature is not the cause of the inhibition of root and aboveground
259 growth. The possible mechanism of the negative effects of charcoal on plant growth have been
260 discussed (Gundale and DeLuca 2007; Warnock et al 2007). However, at present, we cannot
261 conclusively determine the factors that reset the positive effect of charcoal on plants. Charcoal
262 produced at high temperatures has a high capacity for adsorbing organic substances (Gundale
263 and DeLuca 2006) including those necessary for the functioning rhizosphere of plants (e.g.,
264 organic acids and enzyme, Reuban et al. 2008).   In addition to the basic parameters
265 investigated in this study, further information is needed regarding negative impacts of charcoal
266 on soil processes including enzyme activity, microbial activity, and availability of other
267 macronutrients as well as plant physiological functions including water relations, uptake of
268 other macronutrients.

269

270 *Implications for the role charcoal for tree regeneration in the Far Eastern Larch forests*

271   This study implies that the variation of burning intensities within a forest fire (i.e., the
272 charring of biomass at variable temperatures) may be an important driver for different
273 conditions preferred by some trees. It is possible that charcoal produced at low temperatures
274 promotes the seedling growth of Gmelin larch while high temperature char has no positive
275 effect on the seedling growth. In the larch forests of Far East Russia, relatively low severity
276 surface fires are the predominant fire (Wirth 2004; Makoto et al. 2007) generally resulting in

8

low temperature combustion of biomass. This suggests that most fires occurring in the Russian Far East should produce charcoal and thus conditions that are preferable for larch seedlings. Makoto et al. (2010) reported that the charcoal produced by a surface fire affected the seedling response, and that the effect was seen in the middle layer of the soil profile rather than at the surface. These results indicate that the charcoal produced by surface fire and later buried in the soil can provide a suitable habitat for larch seedlings in the Far Eastern larch forests.

Conclusion and further perspective

Charcoals produced at different temperature affect the growth of Gmelin larch seedlings differently. Such effects imply that the distribution of charcoal provides more complex habitat for the larch than can be imagined based on the spatial variation of charcoal in post-fire forest. For an appropriate understanding of the role of charcoal in the forest ecosystem and its mechanism, we should conduct experiments based on the manipulation of soil conditions such as the pH, the water content, and the temperature which can have potential to change the charcoal effect.

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Captions to Fig

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422

423 **Fig. 1** Shoot (a) and root (b) dry masses and their balances (S/R) (c) of Gmelin larch seedlings

424 grown under various contents of charcoal produced at different temperatures. White bar: dry

425 mass grown with charcoal produced at 400°C, black bar: grown with charcoal produced at

426 800°C. Vertical bars represent the standard error of the mean (SE). The legend shows the

427 statistical analysis of effects of treatments on each parameter by GLM. ***: $p<0.001$, **:

428 $p<0.01$, *: $p<0.05$. Contents: Effect of charcoal treatment at different contents; Temperature:

429 Effect of charcoal treatment produced at different temperatures; Contents×Temperature:

430 Interactive effect of both treatments; n.s.: Not significant.

431

432 **Fig. 2** Needle nitrogen (a), phosphorous (b) contents and their balances (N/P) (c) of Gmelin

433 larch seedlings grown under various contents of charcoal produced at different temperatures.

434 Treatment codes and legend are as in Fig 1. Vertical bars represent the standard error of the
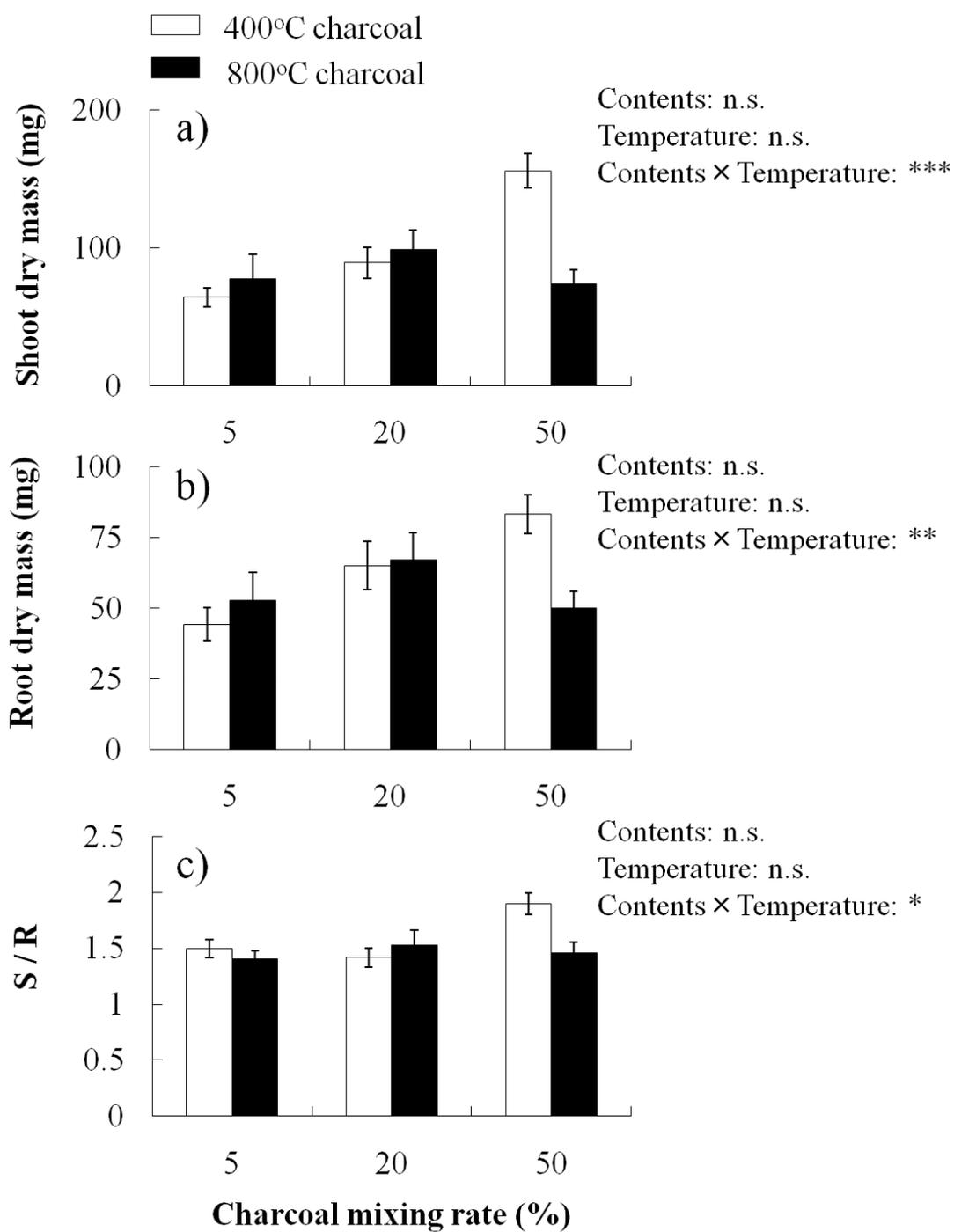
435 mean (SE)

436

Table and footnote

438 **Table 1**. Chemical properties of charcoal and sand used in this study.

	Sand			400°C			800°C		
pH	5.8	±	0.1	6	±	0.1	9.6	±	0.4
EC (ms cm^{-1})	28	±	2	43	±	8	84	±	9
Density (g ml^{-1})	1.9	±	0.16	0.21	±	0.02	0.24	±	0.02
NH$_4$ (mg kg^{-1})	5.6	±	0.4	14.4	±	1.2	7.6	±	1.7
NO$_3$ (mg kg^{-1})	0.5	±	0.02	0.68	±	0.19	1.02	±	0.23
Available P (mg kg^{-1})	27.9	±	1.2	42.7	±	0.9	44.2	±	1.3
Water holding capacity (g L^{-1})	125	±	7.2	272.2	±	30.9	188.9	±	11.1

439

440 Each value shows mean ±SE analyzed from three subsamples from the prepared materials
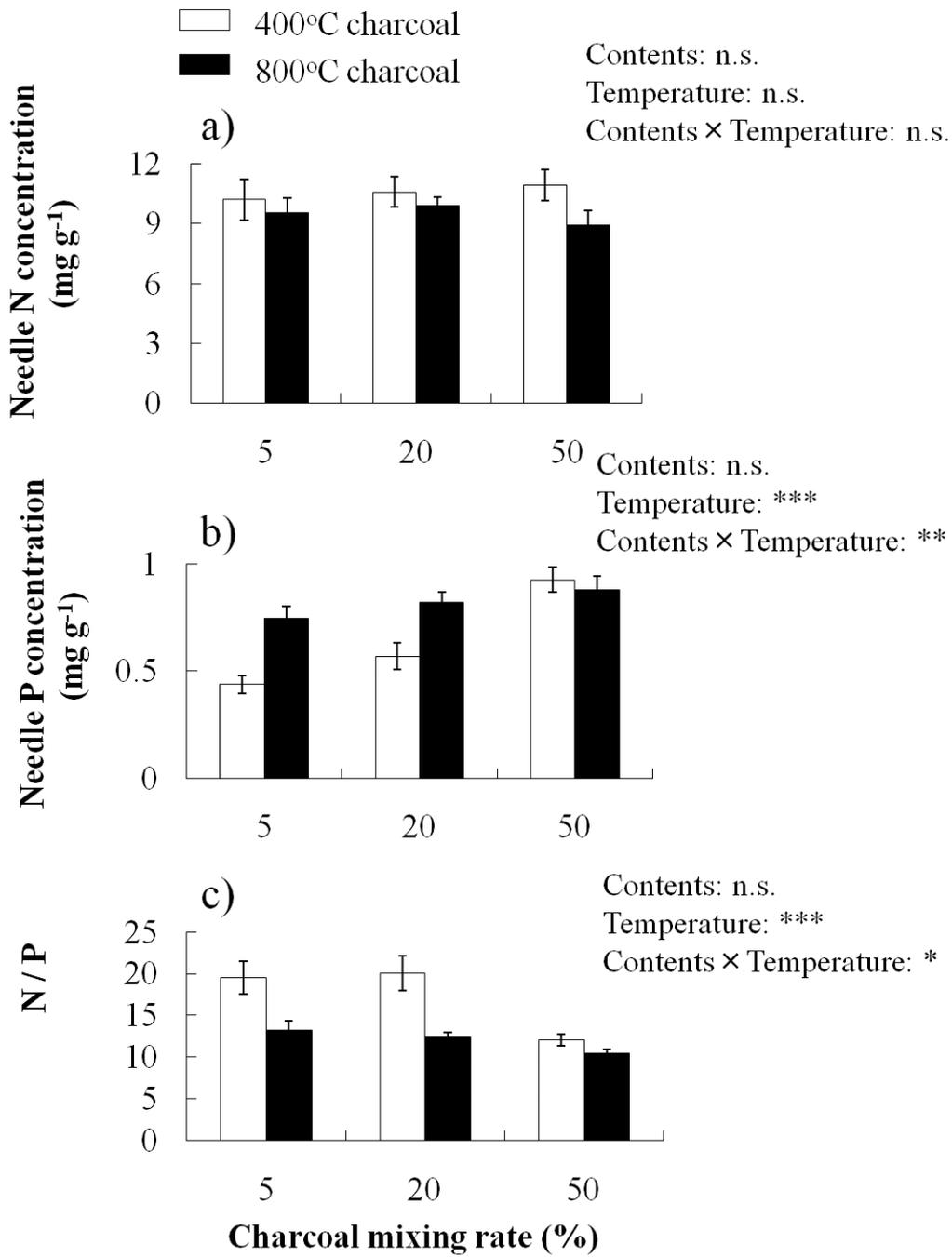
441 (n=3).

13

Fig.1

400°C charcoal
800°C charcoal

a)

Shoot dry mass (mg)

200

100

0

Contents: n.s.
Temperature: n.s.
Contents × Temperature: ***

5   20   50

b)

Root dry mass (mg)

100

75

50

25

0

Contents: n.s.
Temperature: n.s.
Contents × Temperature: **

5   20   50

c)

S / R

2.5

2

1.5

1

0.5

0

Contents: n.s.
Temperature: n.s.
Contents × Temperature: *

5   20   50

Charcoal mixing rate (%)

a)

b)

c)

Contents: n.s.
Temperature: n.s.
Contents × Temperature: n.s.

Contents: n.s.
Temperature: ***
Contents × Temperature: **

Contents: n.s.
Temperature: ***
Contents × Temperature: *

□ 400°C charcoal
■ 800°C charcoal

Needle N concentration (mg g^{-1})

Needle P concentration (mg g^{-1})

N / P

Charcoal mixing rate (%)