Title	The growth of <i>Larix gmelinii</i> 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.

Keywords

Charcoal, charring temperature, Gmelin larch (Larix gmelinii), P availability, forest fire

Introduction

Fire is the primary form of disturbance driving the vegetation succession and structure in the boreal forests (Goldammer and Furyaev 1996; Makoto et al. 2007). Forest fires create heterogeneous conditions that can directly affect the vegetation composition, succession, and structure (Turner et al. 1994; Miller and Urban 1999). To understand how forest fires affect vegetation structure, it is necessary to understand factors driving environmental changes in post-fire forests. The highly variable surface soil conditions created by fire are known to directly affect vegetation structure (e.g. Smithwick et al. 2005). Indeed, fires cause variable rates of charcoal and ash deposition and heat transfer into surface soils resulting in variable chemical, physical, and biological properties in the soil (Zackrisson et al. 1996, Wardle et al. 1998, Neary et al. 1999; Certini 2005; Smithwick et al. 2005, DeLuca and Sala 2006 Mackenzie et al. 2008). The influence of charcoal on soil processes has been poorly studied but we know that charcoal may regulate soil conditions, leading to the variable responses of seedling following fires in the forest ecosystems (Wardle et al. 1998; Gundale and DeLuca 2007; Mackenzie et al. 2008).

The primary mechanism through which charcoal influences plant growth is alteration of nutrient and water availability in soil (Tryon 1948; Glaser et al. 2002; Lehmann et al. 2003). The porous nature of charcoal can increase the water holding capacity of soil and coincidentally retention of soluble nutrients in soil. Ash associated with charcoal and embedded in charcoal pores, contains large contents of nutrients like NH_4^+ and PO_4^{3-} as well as oxides of alkaline metals that can increase soil pH in the immediate vicinity of the charcoal (Tryon 1948; Urely et al. 1993; Topoliantz et al. 2005; Steiner et al. 2007) with a better plant growth (Topoliantz et al. 2005). Charcoal can also increase the soil surface temperature by reducing surface albedo (Glaser et al. 2002; Certini 2005).

Charcoal can have different characteristics (e.g., nutrient contents, pH, and capacity to adsorb organic compounds) depending on the charring temperature (Glaser et al. 2002; Brown et al. 2006; Gundale and DeLuca 2006). Therefore, we hypothesize that fire creates variation in the soil conditions by producing charcoals at different temperatures. However, currently there is limited information of how different charcoal produced at different temperatures can affect tree growth (Lehmann and Joseph 2009).

In this study, we examined the effects of various charcoals on the growth and nutrient physiology of Gmelin larch seedlings. We studied the effects of two charcoals produced at different temperatures and applied to sand at different rates on the nutrient status and the growth of Gmelin larch seedlings. The Gmelin larch (*Larix gmelinii*) is the dominant species in Far East Russia and its regeneration is deeply associated with forest fire (Goldammer and Xueying 1992; Makoto et al. 2007). Based on these results, we discuss how charcoals produced at different

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

Materials and Methods

Seeds, sand and charcoal

Seeds of the Gmelin larch (*Larix gmelinii* (Rupr.) Kuzen.) were imported from Amur in Far East Russia, sterilized with 20% H₂O₂, put into Petri dishes, and germinated in a growth chamber (EYELA MTI-202, Tokyo Rika, Japan) at 30°C with a day length of 16 h. Each germinated seed was planted in a 500 ml pot in a greenhouse of the Faculty of Agriculture, Hokkaido University, Sapporo, Northern Japan. The day length of 16 h was obtained with a supplementary light system with halogen lamps (60 W, Toshiba, Tokyo) and was to simulate the conditions of the growing season of Gmelin larch in Amur State, Russia (from May to August). The air temperature was maintained between 24°C and 32°C (night / day), which is the air temperature range during the summer in Amur. Water was applied every 1 or 2 days. The seedlings were grown for 4 months.

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

Experimental flow and analysis

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

we prepared 12 seedlings per treatment (72 seedlings in 72 independent pots for 6 treatments). Unfortunately, some seedlings died during the growing period leaving 9 to 12 seedlings in each treatment at the end of the experiment.

Four months after the planting, we harvested the seedlings. The shoot (S) and the root (R) of the seedlings were separated, and their dry mass was determined after drying for 4 d at 60°C. The dried needles were ground for chemical analyses. The needle nitrogen (N) content was analyzed with a CHNO analyzer (PE-2400 series, Perkin-Elmer, Norwalk, CT). The remaining needles were digested according to the modified wet digestion method (Kayama et al. 2009), and the P content was analyzed using inductively coupled plasma spectroscopy (IRIS, Jarrel Ash, Franklin, MA).

The pH and electrical conductivity (EC) of both the LOW and the HIGH charcoals were measured in slurries (1:4 and 1:2 charcoal / deionized water respectively) (Gundale and DeLuca 2006). A sand or charcoal (1g) was shaken with 2 M KCl (25 ml) for 1 h and the NH_4^+ and the NO_3^- contents of the extract were analyzed with an auto-analyzer (AACS-4, BL-TEC, Inc, Japan). The NH_4^+ was determined with indophenol blue absorption spectrometry, and the NO_3^- was by the naphthyl ethylenediamine dihydrochloride spectrophotometric method. The plant-available P was determined following the Bray II method (Bray and Kurtz 1945). The contents of all of the nutrients and the average of the three replicates were expressed on a dry mass basis by calculating the water contents of the fresh and air-dried samples.

The water holding capacity of river sand and charcoal was determined as following. The pots including river sand and charcoal at three rates (5%, 20% and 50%, v/v charcoal in sand) was put in a metal bath and soaked in distilled water for 24 h at room temperature to allow the sand or charcoal in the pots to absorb water by capillary action. The water depth was 0.5 cm, while that of the sand or charcoal in the pots was 2.0 cm. After 24 h of water absorption, we determined the weight of the pots. Then the pot was dried at 85°C for 3 days, weighed and the weight of the water absorbed by river sand or charcoal was calculated to determine the water holding capacity.

Charcoal effects on sand temperature

Before the experiment, the pots with sand and/or charcoal were placed in the refrigerating chamber at 4°C overnight to standardize the temperature of all of the pots. The experimental area was an outdoor area where no shade prevents the sunlight. The pots were taken from the refrigerating chamber and put into a white paper box in the experimental area in the field at about 11:30 am on the day of measurement. The sand temperature in the pots was then monitored with a thermal camera (Thermo-Tracer TH9100MV/WV, NEC, Japan).

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

degree of reflection. The thermal photographs were taken at 0, 1, 2, 5, 10, 30, 60, and 120 minutes after setting the pots in the box. A preliminary experiment confirmed that 120 minutes at midday is a sufficient time to saturate the pot temperatures. The experiment was repeated three times in different days and regarded as three replicates. Then, we evaluated the charcoal effects on sand temperature from the photographs taken with the thermal camera.

Statistical analysis

We analyzed the effects of type and amount of charcoal on sand properties and plant using generalized linear model (GLM) (Crawley 2005). The explanatory variables were the charring temperature, the charcoal content and their interaction, and the response variables were each parameter of plant growth and the needle nutrient contents. Correlations between the dry mass of the seedling and the needle nutrients and their balances were explored with Pearson's correlation tests or Spearman's rank correlation tests for the LOW and the HIGH charcoal treatments. For all of the analyses, significance was considered to be at $p < 0.05$. All of the analyses were performed using the R software package, version 2.6.0 (R Development Core Team 2007). In the case of sand temperature data, we did not conduct a statistical analysis and we considered the visible clear differences from the photography using the thermal camera.

Results

Chemical and physical properties of charcoal

The HIGH charcoal had a higher pH, EC and density and NO_3^- and available P contents than the LOW charcoal (Table 1). On the contrary, the LOW charcoal had more NH_4^+ content and water holding capacity than the HIGH charcoal (Table 1). Visually the increase in the charcoal content mixed increased in the sand surface temperature. The LOW charcoal increased the sand surface temperature more than the HIGH charcoal.

Seedling growth

Charring temperature and interaction of charring temperature with charcoal contents affected the shoot dry mass of seedlings ($p < 0.05$ and $p < 0.001$; Fig. 1a). By increasing LOW charcoal application increased shoot dry mass, whereas the HIGH charcoal application did not significantly affect at any rates ($p < 0.001$; Fig. 1a). Charcoal contents and charring temperature interactively influenced the root dry mass of seedling ($p < 0.01$; Fig. 1b). By increasing the LOW charcoal application increased root dry mass, whereas the HIGH charcoal application did not significantly affected at any rates ($p < 0.001$; Fig. 1b). The HIGH charcoal application did not affect S/R ratios at any rates, whereas the LOW charcoal significantly increased the S/R at 50% contents ($p < 0.05$; Fig. 1c).

Needle nutrient content and balances

None of the charcoal treatments significantly affected the N content in the larch needles (Fig. 2a). The charring temperature solely and interaction of charring temperature with charcoal contents affected the needle P contents ($p < 0.001$ and $p < 0.01$; Fig. 2b). The needle P contents increased with the rates of LOW charcoal application, but it was not significantly affected by the HIGH charcoal application ($p < 0.01$; Fig. 2b). The rates of LOW charcoal at 5% and 20% increased the needle N/P ratios more than the application of the LOW charcoal at 50%, whereas HIGH charcoal application did not affect N/P ratios at any contents ($p < 0.05$; Fig. 2c).

Relationship between nutrient conditions in the needles and the seedling growth

There was no significant relationship between the needle N contents and the total dry mass (shoot plus root dry mass) of seedlings. The needle P contents had a significant positive correlation with the total dry mass of seedlings in the application of LOW charcoal ($r = 0.562$; $p < 0.001$), whereas there was no significant relations with the HIGH charcoal application. The needle N/P ratios had a significant positive correlation with the total dry mass of seedlings with the LOW charcoal ($r = 0.48$; $p < 0.001$), but not with the HIGH charcoal.

Discussion

The LOW charcoal application enhanced growth of Gmelin-larch seedlings (Fig. 1a and 1b). The significant positive relationship between the dry mass of seedling with the LOW charcoal and P content and N/P ratio (negative) suggests that P availability limits the seedling growth. The LOW charcoal contained greater inorganic N (mainly NH_4^+) than the HIGH charcoal probably due to lower volatilization of organic N of plant biomass during combustion at lower temperature (Certini 2005). The presence of inorganic N would likely shift the limiting factor from N to P availability (Chapin 1980, Aerts and Chapin 2000). As a consequence, the alleviation of P limitation with the more application of the LOW charcoal would correspond with the more growth of seedling.

The HIGH charcoal application did not significantly affect the growth of seedlings at any rates ($p < 0.001$; Fig. 1) but it increased needle P contents. Therefore there was another limiting factor for seedling growth beyond P and N availability in the case of the HIGH charcoal application. We believe that there are four plausible scenarios by which the HIGH charcoal did not have significant effects on the seedling growth based on the results: (1) sand pH, (2) sand moisture and (3) sand temperature. The pH of HIGH charcoal was 9.6 (Table 1). Ishii and Kadoya (1994) suggested that higher amounts of charcoal caused a higher pH and inhibited plant growth. The HIGH charcoal had a higher pH than LOW charcoal due to the higher

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

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

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

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

Fig. 1 Shoot (a) and root (b) dry masses and their balances (S/R) (c) of Gmelin larch seedlings grown under various contents of charcoal produced at different temperatures. White bar: dry mass grown with charcoal produced at 400°C, black bar: grown with charcoal produced at 800°C. Vertical bars represent the standard error of the mean (SE). The legend shows the statistical analysis of effects of treatments on each parameter by GLM. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$. Contents: Effect of charcoal treatment at different contents; Temperature: Effect of charcoal treatment produced at different temperatures; Contents×Temperature: Interactive effect of both treatments; n.s.: Not significant.

Fig. 2 Needle nitrogen (a), phosphorous (b) contents and their balances (N/P) (c) of Gmelin larch seedlings grown under various contents of charcoal produced at different temperatures. Treatment codes and legend are as in Fig 1. Vertical bars represent the standard error of the mean (SE)

Table and footnote

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

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

Fig.1

Fig. 2