Influence of soil properties on the heartwood colour of *Juglans mandshurica* var. *sachalinensis* in a cool temperate forest

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Abstract

Heartwood colour is often an important factor in determining timber prices. However, the determinants of intraspecific variation in heartwood colour, which is useful information for sustainable wood marketing, are little understood, especially at the local scale in cool temperate forests. Because heartwood is produced as a secondary compound and photosynthesis is regulated by nitrogen (N) in cool temperate forests, we hypothesized that (1) soil conditions determine heartwood colour even at a local scale within a tree species and (2) N, specifically, can be an important driver of the intraspecific variation in heartwood colour in the trees of cool temperate forests. To test these hypotheses, we investigated the relationship between the colour values (luminescence, redness, and yellowness) of heartwood from *Juglans mandshurica* var. *sachalinensis* and the soil parameters in a cool temperate forest. Among the soil properties, not soil N but soil magnesium (Mg) contents alone had a significant influence on the redness and yellowness of the heartwood. Higher soil Mg contents resulted in increased greenness and yellowness of the heartwood in our study, probably due to the increase in phenolics and the colouring of the tannins in the heartwood with Mg. Our results indicate that even at a local scale, soil condition can determine the intraspecific variation in heartwood colour and that forest managers can utilize edaphic information to predict heartwood colour for timber marketing.

Keywords: Soil nutrients, Heartwood colour, Ecosystem service, Local timber production

Introduction

Wood colour is often an important factor in determining timber prices. Thus, understanding the determinants of wood colour is useful for effectively using ecosystem services and the sustainable marketing of wood [1, 2]. Species differences are well known to be determinants of wood colour. On the other hand, even within the same species, wood colour can differ [2], making the economic value of the wood markedly different (e.g., the heartwood colour of birch in Japan, [3]). Previous studies have revealed that environmental factors such as soil properties are important in determining wood colour [2, 4]. For instance, in Costa Rican plantations across the country, the wood colour of *Tectona grandis* varies with soil type and coincidental difference of chemical properties; pH; and calcium (Ca), magnesium (Mg), and iron (Fe) contents [2]. For five tree species in Mali, soil type is an important factor, driving the intraspecific variation in wood colour (especially redness and yellowness) across the nation [4]. For *Juglans nigra* in the USA, the luminance of wood differs between two states due to differences...
in soil properties [5]. The heartwood colour of Cryptomeria japonica differs depending on the soil moisture contents across a topographic region and the coincidental soil conditions [6]. However, there is a large knowledge gap about the determinants of heartwood colour in cool biomes, although forests in cool biomes are important for timber production [7]. In summary, little is known about the drivers of intraspecific variation in heartwood colour at a local scale in cool biomes, which makes it difficult for local forest managers to predict wood colour, information that is needed for marketing.

Heartwood substances are heartwood-specific secondary metabolites produced during tree growth [8]. Furthermore, tree growth in cool biomes, such as cool temperate forests, is known to be regulated by nitrogen (N) availability [9]. Based on these facts, we hypothesize that (1) soil condition drives the intraspecific variation in heartwood colour at a local scale (within a few kilometres) and (2) N, specifically, can be the determining factor of heartwood colour within a tree species in cool temperate forests. To test these hypotheses, we investigated the relationship between the colour index of the heartwood of Juglans mandshurica var. sachaalinensis and the soil parameters in a cool temperate forest of northern Japan, which is the northern edge of the distribution of this species. J. mandshurica var. sachaalinensis is one of the most economically valuable tree species in northern Japan; thus, understanding the determinants of the heartwood colour of this species would be helpful in the utilization and marketing of timber resources by local forest managers.

**Methods/experimental**

**Study site**

Wood samples from mature individuals of *J. mandshurica* var. *sachaalinensis* were obtained in a natural mixed forest in Nakagawa town, northern Hokkaido, Japan. The understory was mainly covered by dwarf bamboo (*Sasa sananensis* (Fr. et Sav.) Rehder), which is the dominant species in northern Japan, and the thickness of the litter layer was about 2–3 cm. Between 1981 and 2010, the average mean annual air temperature of this area was 5.5 °C and the annual precipitation was 1225 mm (Japan Meteorological Agency 2020). To assess the importance of environmental gradients at a local scale (which is the important unite for the local forest manager), all the individuals were separated from each other by less than 3 km horizontally and 50 m vertically; therefore, the climate variables can be regarded as relatively homogeneous. The bedrock of the study site was terrace deposits from the late Holocene.

**Soil measurements**

From the areas surrounding each tree, three subsamples of soil (from a depth of 0–20 cm) were obtained; the three subsamples were merged to form a composite representative sample for each tree. The soil analysis was conducted following the methods presented by a previous study [10]. In short, by using the total wet weight of the samples and gravimetric water contents, the bulk density of the soil was calculated. To determine the pH, 10 g of soil was shaken with 25 mL of deionized water, and the solution was measured with a pH meter (B212, Horiba, Japan). The total N concentration was measured with a CHNS/O analyser (2400II, Perkin Elmer, USA). Following [5], the exchangeable nutrients (P, Ca, Mg, K, Fe, Al, Cu, Mn, Zn, and S) were extracted with 100 mL 1N ammonium acetate solution from 10 g fresh samples, and their contents in the extracts were determined by inductively coupled plasma spectroscopy (IRIS, Jarrel Ash, Franklin, MA). The concentrations of all the

**Sampling and wood colour measurements**

A disc sample was obtained at a height of 30 cm from the bottom of the stem in each individual. In total, 17 mature individuals were sampled. The number of intraspecific individual replications was comparable to Montes et al. [4]. The average diameter at breast height (DBH) was 45 cm. The obtained samples were dried at 70 °C for 2 weeks. Although the drying process at 70 °C can change the wood colour from the original one, we employed this drying temperature because it is common to dry the wood at this temperature range for wood industry in Japan and one of our major aims of this study is the contribution to the better wood marketing. After drying, the surface of the flat-grained tangential face was carefully polished with sandpaper to prepare it for heartwood colour analysis. For each sample, three points were selected, and the heartwood colour was measured using a colour meter (ZE6000, Nihon Denshouk-kogyo, Japan) under stable temperature and humidity conditions. Before the measurements, calibration was conducted using a white standard reference supplied by the same company. The measurement was set within the visible range of 400–700 nm at intervals of 10 nm. According to HunterLab (1995), we estimated the heartwood wood colour in three coordinates: the L value (luminescence) represents the position on the black–white axis (L00 represents black; L0100 represents white), a value defines the position on the red–green axis (+100 values indicate red; −100 values indicate green), and b value defines the position on the yellow–blue axis (+100 values indicate yellow; −100 values indicate blue).

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nutrients and cations were determined on the basis of the soil dry mass.

Statistical analysis
The correlations between each of the colour values and each of the soil properties were analysed with a generalized linear model (GLM). The error distribution and the link function were set as Gaussian and log, respectively. The relationship between each of the wood colour values and the soil properties was regarded as significant when the p value was less than 0.05. The fit of the GLM was evaluated with the proportion of explained deviance of the model (D^2) [11]. All the statistical analyses were conducted with R 3.5.1 (R Core Team 2018).

Results and discussion
The soil Mg contents had a significant relationship with only the a value (redness) (Table 1, p < 0.01, D^2 = -0.48). High soil Mg contents resulted in more greenish heartwood in J. mandshurica var. sachalinensis (Fig. 1a). Furthermore, the b value was significantly correlated with the soil Mg content (Table 1, p < 0.01, D^2 = 0.48). The heartwood colour was more yellowish when the soil Mg content was high (Fig. 1b). These results indicate, as we hypothesized, that the soil condition significantly influenced the heartwood colour of J. mandshurica var. sachalinensis, even at a local scale. On the other hand, the soil N contents were not correlated with the heartwood colour of J. mandshurica var. sachalinensis, which is contrary to our hypothesis. Tree photosynthesis and coincidental productivity in cool temperate forests are known to be generally strongly regulated by N [9]. Our results indicated that rather than altering heartwood colour via the regulation of tree photosynthetic activity, which is related to growth, soil conditions alter heartwood colour via other physiological processes. The wood colour of Juglans species is related to the extractive contents and wood density [12]. The causes of intraspecific variation in wood colour are suggested to be driven by differences in the physiological processes of trees [13], although the specific physiological mechanism was not pointed out in their study. Furthermore, the soil physical properties are also known to influence the wood properties [14], which should be investigated in detail in future study.

In a Costa Rican plantation, intraspecific variation in the heartwood colour of T. grandis was driven by soil Mg contents [2]. In that study, together with other base cations, a high Mg content was regarded as a proxy of soil fertility in Ultisol, and fertility was suggested to change the heartwood colour. Similarly, soil Mg had a significant influence on wood colour of J. nigra [5]. These results, which were observed across a wide spatial scale, were consistent with our results, showing the importance of Mg in determining heartwood colour. In J. nigra and J. regia, ellagic acid derivatives, which are the components of hydrolysable tannins, are one of the major heartwood substances [1]. Similarly, gallic acid, ellagic acid and hydrolysable tannins have been found in heartwood in J. mandshurica var. sachalinensis [15, 16]. Mihara [17] reported that magnesium oxide reacts with tannin creating a different colour. Therefore, it is possible that Mg affect the heartwood colour of J. mandshurica var. sachalinensis by reacting with tannins. On the other hand, Mg is important for the various metabolic processes of trees [18]. Soil Mg contents function not

### Table 1 Statistical results about the relationship between soil chemical properties and heartwood colour values

|                     | L   | a        | b        |
|---------------------|-----|----------|----------|
| Bulk density        | N.S | N.S      | N.S      |
| Water contents      | N.S | N.S      | N.S      |
| pH                  | N.S | N.S      | N.S      |
| Total C contents    | N.S | N.S      | N.S      |
| Total N contents    | N.S | N.S      | N.S      |
| Exchangeable P contents | N.S | N.S      | N.S      |
| Exchangeable Ca contents | N.S | N.S      | N.S      |
| Exchangeable Mg contents | N.S | -0.69**  | 0.64**   |
| Exchangeable K contents | N.S | N.S      | N.S      |
| Exchangeable Fe contents | N.S | N.S      | N.S      |
| Exchangeable Al contents | N.S | N.S      | N.S      |
| Exchangeable Cu contents | N.S | N.S      | N.S      |
| Exchangeable Mn contents | N.S | N.S      | N.S      |
| Exchangeable Zn contents | N.S | N.S      | N.S      |
| Exchangeable S contents | N.S | N.S      | N.S      |

L value is for luminescence that represents the position on the black–white axis, a value that defines the position on the red–green axis, and b value that defines the position of the yellow–blue axis (see the details in Material and Method section). The number in the table is D^2 values calculated based on D^2 value by [3]. Asterisk (**) show the statistically significant relationship (p < 0.01) determined by GLM.
only as photosynthetic pigments that are directly related to carbon fixation, but also as accelerators of phenolic accumulation. Li et al. [18] reported that Mg contents can change the activity of phenylalanine ammonia-lyase (PAL), which relates to the production of phenolic compounds, and that of polyphenol oxidase (PPO) and guaiacol peroxidase (GPX), which relates to the oxidation of phenolic compounds in roots. Phenolic compounds are one of the major components of heartwood substances [19] and are also known to influence the colour of heartwood of Juglans species [1]. In *Acacia mangium* and *Vochysia guatemalensis*, phenolic contents affect colour of wood [20]. Therefore, it is possible that Mg can change the phenol-related metabolism in the stems of trees (which is non-assimilatory organs such as roots), consequently changing the amount and composition of phenolic compounds in the heartwood of *J. mandshurica* var. *sachalinensis*. However, to the best of our knowledge, it is not known whether the increase of phenolic compounds directly result in then increase of colouring material or indirectly change the colour reaction in *Juglans* species, which should be clarified in the future study.

Because it takes time for Juglans trees to start producing heartwood, a long-term experiment in which Mg is added to soil may be necessary to understand the role of soil nutrients (in this case, Mg) in determining heartwood colour; in turn, this knowledge could be applied to control Juglans wood colour, supporting sustainable wood production. Notably, there was not a clear relationship between tree diameter and any of the wood colour parameters, which implicate that, among the matured trees, the edaphic condition influences the heartwood colour rather than the tree age (data not shown). In USA, the average rate of diameter growth and tree age of *Juglans nigra* did not correlate with any parameters of wood colour (luminance, dominant wavelength, and purity), while soil properties (specifically, soil pH, available P, exchangeable Mg, K, Ca, and total N) were correlated with the above-mentioned wood colour parameters [5]. In their study, the age of investigated individuals of *Juglans nigra* ranges from 48 to 122 years, which indicates that, together with our observation, the age of *Juglans* trees has little influence on wood colour among the matured individuals.

There were two points where the exchangeable Mg concentration was under detectable (Fig. 1). By considering that other soil macro-nutrients (Ca, K, Fe, Al) were detected in the same solutions from two points [21], the failure of the detection for exchangeable Mg in soil would be not due to the artefact during analysis but to the little solubility of Mg in the soils of these two points. Exchangeability of Mg in soil is strongly determined by the soil depth to the bedrock, slope position and acidity in temperate forests [22]. In the future study, we should also clarify the determinant of exchangeable Mg in soil across the landscape.

The GLM revealed that none of the soil properties had a significant effect on the *L* value. In [5], the *L* (luminance) value of *J. nigra* differed between two regions in the USA. In their study, the combination of exchangeable Mg and available P predicted 30% of the variation in heartwood luminance. The contrasting effect of Mg and P on the *L* value between our study and the previous study might be partly due to the difference in context between the studies (e.g., spatial scale, difference in absolute amount of nutrients, and climate), even within the same genus. It is better to investigate the determinants of heartwood colour for economically valuable species at the local scale. Our results indicate that the local forest managers can utilize edaphic conditions (specifically soil Mg) to predict the heartwood colour of harvested walnut for efficient marketing in northern Japan.

**Conclusion**

The heartwood colour of *Juglans mandshurica* var. *sachalinensis* showed large variation according to soil properties. Our study demonstrated that soil Mg content could be an important factor in determining the variation of heartwood colour of this species at a local scale. Specifically, high soil Mg contents resulted in more reddish and more yellowish heartwood.

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**Authors’ contributions**

MK, TI, ST, TN, YT designed the research. MK, TI, ST, TN and YT conducted the wood sampling. ST and TI conducted the wood colour measurements. MK and SE conducted the soil sampling and the statistical analysis. MK led the writing of the manuscript. All authors contributed significantly for the improvement of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The dataset used in this study is available at Dryad [https://doi.org/10.5061/dryad.3bk3j9kjr](https://doi.org/10.5061/dryad.3bk3j9kjr)

**Declarations**

**Competing interests**

The authors have no conflicts of interest directly relevant to the content of this article.

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