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# Effects of thinning intensity on nutrient concentration and enzyme activity in *Larix kaempferi* forest soils

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## Abstract

**Background:** As the decomposition of lignocellulosic compounds is a rate-limiting stage in the nutrient mineralization from organic matters, elucidation of the changes in soil enzyme activity can provide insight into the nutrient dynamics and ecosystem functioning. The current study aimed to assess the effect of thinning intensities on soil conditions. Un-thinned control, 20 % thinning, and 30 % thinning treatments were applied to a *Larix kaempferi* forest, and total carbon and nitrogen, total carbon to total nitrogen ratio, extractable nutrients (inorganic nitrogen, phosphorus, calcium, magnesium, potassium), and enzyme activities (acid phosphatase,  $\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -glucosaminidase) were investigated.

**Results:** Total carbon and nitrogen concentrations were significantly increased in the 30 % thinning treatment, whereas both the 20 and 30 % thinning treatments did not change total carbon to total nitrogen ratio. Inorganic nitrogen and extractable calcium and magnesium concentrations were significantly increased in the 20 % thinning treatment; however, no significant changes were found for extractable phosphorus and potassium concentrations either in the 20 or the 30 % thinning treatment. However, the applied thinning intensities had no significant influences on acid phosphatase,  $\beta$ -glucosidase,  $\beta$ -xylosidase, and  $\beta$ -glucosaminidase activities.

**Conclusions:** These results indicated that thinning can elevate soil organic matter quantity and nutrient availability, and different thinning intensities may affect extractable soil nutrients inconsistently. The results also demonstrated that such inconsistent patterns in extractable nutrient concentrations after thinning might not be fully explained by the shifts in the enzyme-mediated nutrient mineralization.

**Keywords:** Extracellular enzyme, Japanese larch, Nutrient availability, Soil organic matter, Thinning intensity

## Background

Forest soil is a key component in ecosystems as a major storage and source of plant-available nutrients. In addition, it can be affected by the changes in other living and non-living components of forests since they interact with each other (Attiwill and Adams 1993). Accordingly, anthropogenic activities on forests may directly or indirectly shift soil conditions, disturb nutrient cycle, and consequently hinder future forest productivity if they are inappropriately conducted (Grigal 2000). In this context,

the influences of common forest management practices on soil conditions have been extensively assessed to find optimal strategies for sustaining forest productivity in the long term (Boerner et al. 2008; Achat et al. 2015).

Thinning, the selective tree cutting to achieve various management purposes, is one of the most frequently applied forest management practices. It can affect various processes related to soil nutrient dynamics. For example, tree density reduction after thinning is known to alter microclimate by increasing soil temperature and moisture (Son et al. 2004; Masyagina et al. 2006). Thinning can also influence soil conditions such as the quantity and quality of soil organic matter (Son et al. 2004; Smolander et al. 2013) and extractable nutrient

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concentration (Hwang and Son 2006; Kim et al. 2015). These changes in microclimate and soil conditions are known to impact the activity of extracellular enzymes mediating the decomposition of lignocellulosic compounds (Giai and Boerner 2007; Adamczyk et al. 2015). As the decomposition of lignocellulosic compounds is a rate-limiting stage in the nutrient mineralization from organic matters, elucidation of the changes in soil enzyme activity can provide insight into the nutrient dynamics and ecosystem functioning (Sinsabaugh et al. 2008).

Japanese larch (*Larix kaempferi* Lamb.) is a major deciduous coniferous species in South Korea. This species accounts for approximately 16.5 % of the coniferous forest area in South Korea and has been widely used for reforestation and large log production (Korea Forest Service 2015). As large areas of *L. kaempferi* forests were established by national reforestation programs since the 1960s, thinning of *L. kaempferi* forests has become one of the principal challenges in the Korean forestry (Son et al. 2004; Hwang and Son 2006; Hwang et al. 2007; Lee et al. 2010; Ko et al. 2012).

The present study aimed to test the effects of thinning intensity on nutrient status and enzyme activity in *L. kaempferi* forest soils. To assess the effect on soil nutrient status, we measured concentrations of total carbon (TC) and nitrogen (TN), total carbon to total nitrogen ratio (CN ratio), inorganic N, and extractable phosphorous (P) and cations, including calcium (Ca), magnesium (Mg), and potassium (K). Moreover, we investigated activities of acid phosphatase (AP),  $\beta$ -glucosidase (BG),  $\beta$ -xylosidase (BX), and  $\beta$ -glucosaminidase (NAG) that are involved in decompositions of the phosphomonoesters, celluloses, hemicelluloses, and chitins, respectively (Baldrian and Štursová 2011). These enzymes are known to mediate nitrogen mineralization (NAG) and litter decomposition (BG, BX) and act as significant indices of nitrogen and phosphorus availability (AP and NAG) (Baldrian and Štursová 2011; Olander and Vitousek 2000; Tabatabai et al. 2010).

## Methods

### Study site

The study site is a *L. kaempferi* forest in Muju, South Korea (35° 52' N, 127° 50' E). The overstory is comprised of *L. kaempferi* trees planted in 1968, whereas the understory is dominated by broad-leaved species such as *Acer pseudosieboldianum*, *Cornus controversa*, *Lindera obtusiloba*, *Styrax obassia*, *Quercus mongolica*, and *Q. serrata*. The site is an experimental forest, designed to establish a sustainable forest management system as well as to produce large logs. The average altitude and slope are 910 m and 21° (Kim et al. 2016). The soil is an acidic soil with a loamy sand texture (79 % sand, 19 % silt, 2 % clay), and the gravimetric water content, pH, and cation

exchange capacity are 29.0 %, 4.8, and 16.2  $\text{cmol}_c \text{kg}^{-1}$ , respectively (Kim et al. 2016). Three thinning intensities, including un-thinned control, 20 % thinning (T20), and 30 % thinning (T30) according to the removed basal area, were conducted. Each thinning intensity included three circular plots (314  $\text{m}^2$ ) that were established on the same slope to minimize unexpected heterogeneity of initial conditions. The thinning treatments were applied in October, 2011, and the thinning residues were left in the site.

### Soil sampling and processing

The mineral soil samples were collected using a 10-cm-long cylindrical corer (7.2 cm in diameter) at 0–10-cm depth within three random points of each plot in May, 2014. This soil depth is known to have concentrated soil microbial biomass and nutrients (Hwang and Son 2006; Park et al. 2011). The collected soil samples were sealed in the plastic bags and brought to the laboratory. Some field-moist subsamples were separated from each soil sample and were stored in a refrigerator at 4 °C until measurements of the enzyme activities and inorganic N. Other soil samples were air dried and sieved through a 2-mm mesh screen before quantifications of TC, TN, CN ratio, and extractable P and cations including Ca, Mg, and K.

### Laboratory analysis

TC, TN, and CN ratio were determined by the dry combustion method using an elemental analyzer (vario Macro, Elementar Analysensysteme GmbH, Germany). Inorganic nitrogen (Inorganic N) including ammonium ion ( $\text{NH}_4^+$ ) and nitrate ion ( $\text{NO}_3^-$ ) were extracted with 6 g of the field-moist subsamples and 30 ml of 2 M KCl.  $\text{NH}_4^+$  was quantified by the salicylate method (Mulvaney 1996), and  $\text{NO}_3^-$  was determined using the griess and vanadium chloride ( $\text{VCl}_3$ ) reagents (Miranda et al. 2001). P was extracted by Bray No. 1 method (Bray and Kurtz 1945), and Ca, Mg, and K were extracted with 1 N ammonium acetate buffer (pH = 7). P, Ca, Mg, and K in the extracts were quantified by using an inductively coupled plasma optical emission spectrometer (ICP-OES) (730 series, Agilent, USA).

Activities of AP, BG, BX, and NAG were analyzed by the fluorogenic substrate method (DeForest 2009). Soil suspensions were made by mixing 2 g of the field-moist soil sample and 125 ml of sodium acetate buffer (pH = 5). The homogenized soil suspensions were incorporated into 96-well microplates with the 4-MUB or the 4-MUB-linked substrates, and were incubated at 29 °C for 2 h. The fluorescence were measured at 355-nm excitation and 460-nm emission levels using a Multilable Plate Reader (Victor 3, Perkin-Elmer, USA).

The enzyme activities were expressed as nmol 4-MUB  $\text{g}^{-1}$  dry soil  $\text{h}^{-1}$ .

### Statistical analysis

Differences in TC, TN, CN ratio, extractable nutrient concentrations, and enzyme activities among the three thinning intensities were assessed by the analysis of variance (ANOVA) with post hoc Tukey's test ( $P < 0.05$ ). In addition, a simple linear regression was carried out to describe relationships of the enzyme activities to TC, TN, CN ratio, and the extractable nutrients ( $P < 0.05$ ). If a simple linear regression model was not significant, a quadratic regression was applied ( $P < 0.05$ ). Mean values of three soil samples from each plot were treated as replicates ( $n = 3$  for ANOVA;  $n = 9$  for regression tests). Proc GLM procedure of the SAS 9.4 software was used for statistical analysis.

## Results and discussion

### TC, TN, and CN ratio

The mean TC ( $\text{g kg}^{-1}$ ) and TN ( $\text{g kg}^{-1}$ ) were 61.67 and 2.65 in the control, 78.48 and 4.08 in T20, and 97.70 and 5.14 in T30, respectively (Fig. 1a, b). TC and TN of T30 were 58.4 and 94.2 % higher compared to those of the control, and these differences were statistically significant. In contrast, no significant difference in TC and TN was found between T20 and the control. Meanwhile, CN ratio was 24.11 in the control, 19.46 in T20, and 19.29 in T30 (Fig. 1c). Even though CN ratio tended to be higher in the control than in T20 and T30, there was no significant difference in CN ratio among the three thinning intensities.

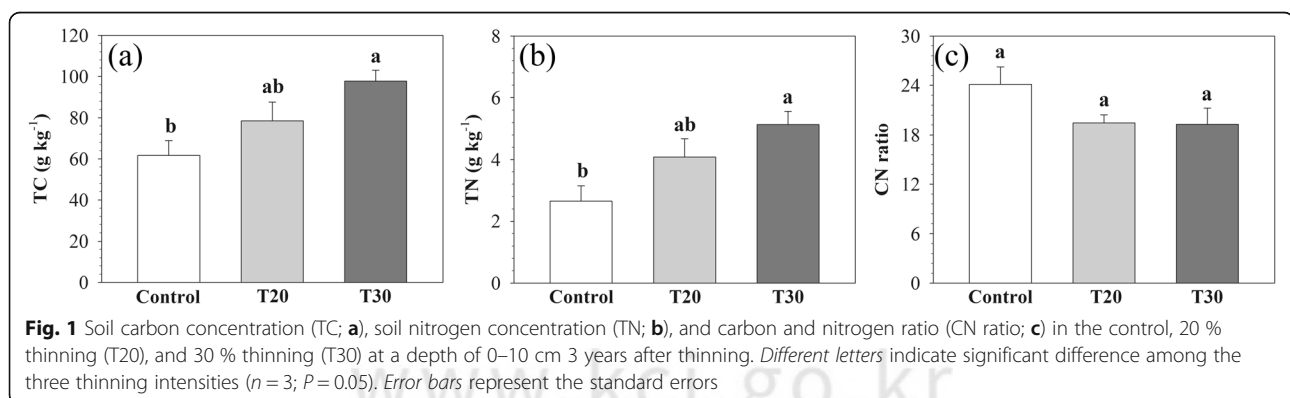
Our results demonstrate that TC and TN might be increased by thinning after 3 years. The increasing patterns in TC and TN are in agreement with the results from other studies on *L. kaempferi* forests (Masyagina et al. 2006; Hwang and Son 2006; Lee et al. 2010; Ko et al. 2012), which reported marginal or remarkable increase in TC and TN due to thinning. These patterns might result from the incorporation of thinning residues

and root mortality following thinning (Hwang and Son 2006). As the C and N in forest soils are known to mainly originate from soil organic matters (Attiwill and Adams 1993), TC and TN can be relevant measures for the soil organic matter quantity. Accordingly, the obtained results imply that T30 might substantially increase the quantity of soil organic matter whereas T20 might have only marginal effect on it.

CN ratio is generally considered an important index for soil organic matter quality because it controls microbial potentials for the nutrient mineralization and immobilization (Hodge et al. 2000). In the present study, the applied thinning intensities showed no significant influences on soil CN ratio. The result is consistent with the other study on a *L. kaempferi* forest, showing that the effect of thinning on soil CN ratio was not significant (Masyagina et al. 2006). These findings are unexpected since thinning is known to accelerate the mixing of low quality plant materials (i.e., thinning residues) into soils and could consequently increase soil CN ratio (Giai and Boerner 2007). We speculate that T20 and T30 might have not substantially shifted soil CN ratio in the short term because of the heterogenic distribution of thinning residues (Boerner et al. 2008).

### Extractable nutrients

The mean concentrations of inorganic N and extractable P tended to be highest in T20, followed by T30 and the control (Table 1). The average inorganic N concentration in T20 was 102.5 % higher than that in the control and the difference was statistically significant. Although extractable P concentration was also higher in T20, there was no significant difference in extractable P between T20 and the control. Concentrations of inorganic N and extractable P in T30 were not significantly different compared to those in the control. Meanwhile, the mean concentrations of extractable cations were highest in T20 (Table 1). Extractable Ca and Mg concentrations were significantly higher in T20 than in the control, but no significant difference was found between T30 and the



**Table 1** Concentrations of the inorganic nitrogen (N) and the extractable phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K) in the control, 20 % thinning (T20), and 30 % thinning (T30) at a depth of 0–10 cm 3 years following thinning

Thinning intensity	Inorganic N (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )	Extractable Ca (cmol <sub>c</sub> kg <sup>-1</sup> )	Extractable Mg (cmol <sub>c</sub> kg <sup>-1</sup> )	Extractable K (cmol <sub>c</sub> kg <sup>-1</sup> )
Control	8.63 (0.26)b	17.50 (0.51)a	0.83 (0.08)b	0.27 (0.02)b	0.24 (0.04)a
T20	17.48 (1.91)a	20.03 (1.40)a	2.57 (0.56)a	0.57 (0.07)a	0.29 (0.02)a
T30	10.64 (1.37)b	18.14 (1.05)a	1.50 (0.17)ab	0.44 (0.05)ab	0.22 (0.01)a

Different letters indicate significant difference among the three thinning intensities ( $n = 3$ ;  $P = 0.05$ ). Values in parenthesis denote the standard errors

control. Extractable K concentration did not significantly differ among the three thinning intensities.

The obtained results indicate that T20 might increase extractable nutrient concentrations in soils. Significant increases in concentrations of several extractable nutrients might be attributed to the increase in total quantity of soil nutrient substrates following the initial addition of dead roots, leaves, and twigs of thinned trees (Smolander et al. 2013; Hwang et al. 2007). Moreover, decreases in tree density and nutrient demand of remaining trees might also result in the observed increases in the nutrient concentrations. In contrast, T30 had no significant effects on soil extractable nutrient concentrations. This pattern implies that different thinning intensities might affect extractable soil nutrient concentrations inconsistently. Several processes might be involved in this inconsistent pattern. Particularly, increase in thinning intensity could elevate the hydrological nutrient loss because lower canopy cover following thinning may decelerate direct rainfall interception (Kim et al. 2015; Siemion et al. 2011). The observed inconsistent effects of thinning might also be results of differences in the microbial immobilization since soil microbes may accumulate more extractable nutrients as microbial biomass when heavy intensity of thinning is applied (Chen et al. 2015). Thus, it is likely that these decreasing effects of thinning intensity might cancel out the increases in soil extractable nutrients following thinning.

#### Enzyme activities

The average activities of AP, BG, BX, and NAG were highest in T30, and the enzyme activities of T20 and T30 were 2.7–22.9 % and 16.9–118.5 % higher than

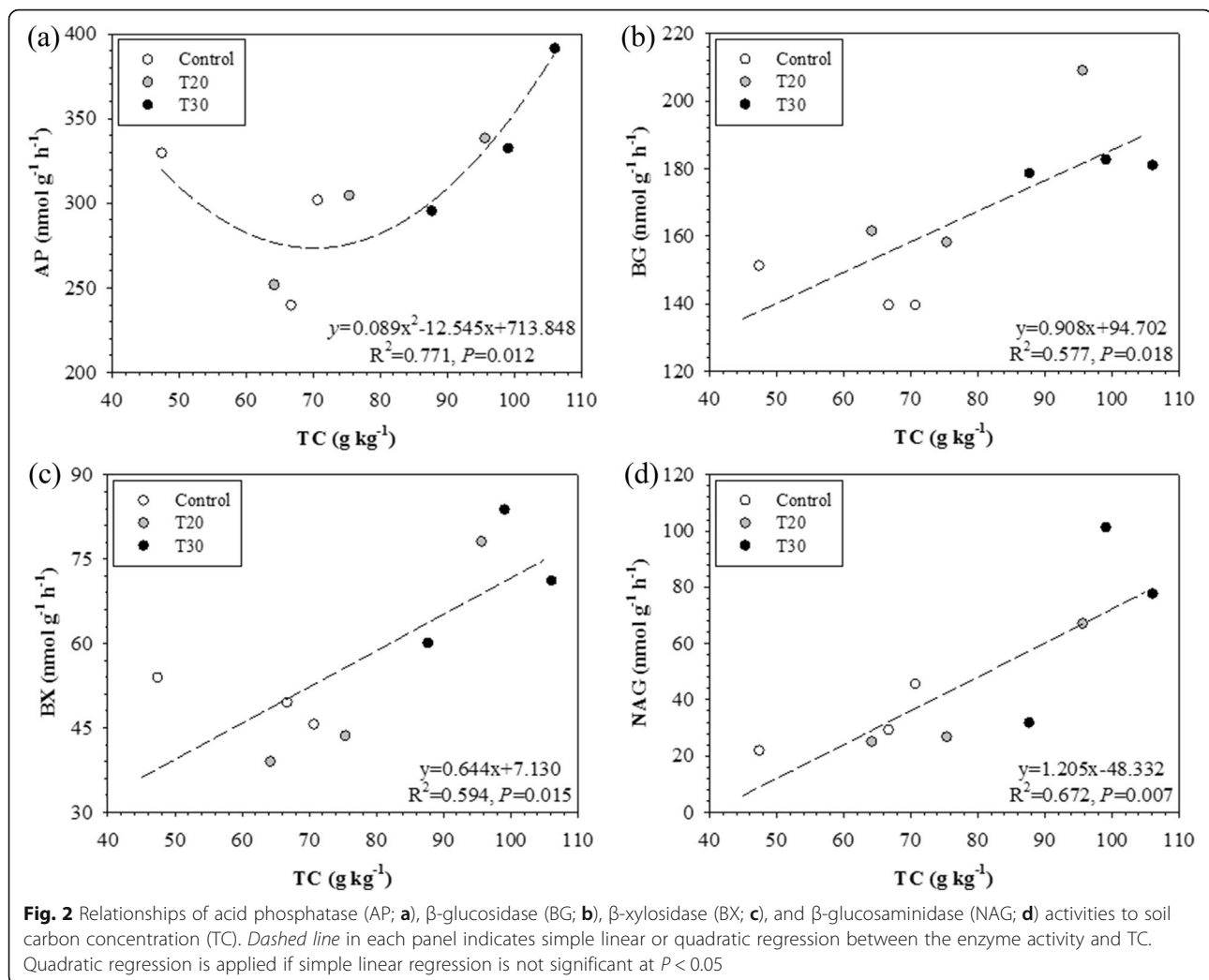
those in the control, respectively (Table 2). Nevertheless, the enzyme activities were not significantly different among the three thinning intensities. BG, BX, and NAG activities were positively correlated to TC (Fig. 2), while only BG activity showed a positive correlation with TN ( $P < 0.05$ ,  $R^2 = 0.54$ ). Though any simple linear regressions were not significant for AP activity, quadratic regression was significant between AP activity and TC or TN (Fig. 2a). Other soil properties including CN ratio and extractable nutrient concentrations exhibited no significant simple linear or quadratic regression with the enzyme activities ( $P > 0.05$ , data not shown).

As soil organic matter could stabilize the structure of soil enzymes and provide the substrates for soil microbes (Karaca et al. 2011; Brzostek et al. 2013), incorporation of dead plant materials following thinning could enhance the soil enzyme activities (Adamczyk et al. 2015). Although BG, BX, and NAG activities tended to be promoted with significant increases in TC due to thinning, these changes in enzyme activities were not significant in the present study. Similarly, previous studies on needle-leaved forests reported no significant influences of thinning on AP, BG, BX, and NAG activities in the short term (Boyle et al. 2005; Maassen et al. 2006; Geng et al. 2012). No significant differences in AP, BG, BX, and NAG activities might be due to the negative feedback between soil enzymes and nutrient availability. Generally, microbial decomposition for assimilating nutrients is accelerated when nutrient availability acts as a limiting factor for microbial growth and activity in soils; otherwise, it can be depressed (Olander and Vitousek 2000). In the present study, soil nutrient availability seemed to be, at least, not limited by thinning and T20

**Table 2** Activities of acid phosphatase (AP),  $\beta$ -glucosidase (BG),  $\beta$ -xylosidase (BX), and  $\beta$ -glucosaminidase (NAG) in the control, 20 % thinning (T20), and 30 % thinning (T30) at a depth of 0–10 cm 3 years following thinning

Thinning intensity	AP (nmol g <sup>-1</sup> h <sup>-1</sup> )	BG (nmol g <sup>-1</sup> h <sup>-1</sup> )	BX (nmol g <sup>-1</sup> h <sup>-1</sup> )	NAG (nmol g <sup>-1</sup> h <sup>-1</sup> )
Control	290.16 (26.54)a	143.35 (3.87)a	49.62 (7.00)a	32.08 (7.00)a
T20	298.05 (25.23)a	176.14 (16.43)a	53.48 (12.34)a	39.44 (13.71)a
T30	339.45 (27.93)a	180.62 (1.17)a	71.60 (6.85)a	70.11 (20.38)a

Different letters indicate significant difference among the three thinning intensities ( $n = 3$ ;  $P = 0.05$ ). Values in parenthesis denote the standard errors



significantly increased some extractable nutrient concentrations (Table 1). Hence, we speculate that no nutrient shortage for soil microbes might have restricted increases in the enzyme activities following the organic matter incorporation due to thinning.

AP, BG, BX, and NAG are soil microbe-originated enzymes participating in the soil organic matter decomposition, and play a crucial role in the nutrient mineralization (Baldrian and Štursová 2011; Olander and Vitousek 2000). Their increase or decrease is generally expected to accompany stimulation of the mineralization process (Tabatabai et al. 2010). Thus, no significant shifts in AP, BG, BX, and NAG activities due to T20 and T30 indicate that application of these thinning intensities might result in only marginal changes in the enzyme-induced nutrient mineralization 3 years after the treatments. Our results are in line with a previous study finding that 10, 20, and 40 % thinning intensities had no significant influences on litter decomposition and N mineralization in

a *L. kaempferi* forest after 4 years (Son et al. 2004). In this context, increased extractable nutrient concentrations in T20 but no significant changes in T30 might not be fully explained by the consistently non-significant effects on the enzyme activities following thinning. From this, it is speculated that this inconsistent pattern might be closely related to other mechanisms such as reduced nutrient demand of trees, accelerated hydrological loss, and increased microbial immobilization (please refer to the “Extractable nutrient” section above). Elucidation of these processes remains further research priorities in order to clarify the nutrient status in thinned forests.

## Conclusions

TC, TN, CN ratio, extractable nutrients, and enzyme activities were investigated in a *L. kaempferi* forest where different thinning intensities were carried out. The obtained results showed that TC and TN were highest in T30 while no significant pattern was found for CN ratio.

Meanwhile, inorganic N, extractable Ca, and extractable Mg concentrations were significantly increased only in T20, and the effects of T20 and T30 appeared to be inconsistent for these extractable nutrients. As the activities of AP, BG, BX, and NAG were not significantly changed due to either T20 or T30, the enzyme-induced mineralization might not be enough to explain the inconsistent changes in extractable nutrient status in T20 and T30. Therefore, further studies on the inconsistent effects of thinning intensities on soil extractable nutrients are necessary for revealing the soil nutrient dynamics in thinned forests. We expect that the present study will contribute to the understanding of the nutrient status in *L. kaempferi* forests after thinning.

#### Abbreviations

ANOVA: Analysis of variance; AP: Acid phosphatase; BG:  $\beta$ -glucosidase; BX:  $\beta$ -xylosidase; Ca: Calcium; CN ratio: Total carbon to total nitrogen ratio; ICP-OES: Inductively coupled plasma optical emission spectrometer; Inorganic N: Inorganic nitrogen; K: Potassium; KCl: Potassium chloride; Mg: Magnesium; 4-MUB: 4-Methylumbelliferone; NAG:  $\beta$ -glucosaminidase;  $\text{NH}_4^+$ : Ammonium ion;  $\text{NO}_3^-$ : Nitrate ion; P: Phosphorus; T20: 20 % thinning treatment; T30: 30 % thinning treatment; TC: Total carbon; TN: Total nitrogen;  $\text{VCl}_3$ : Vanadium chloride

#### Acknowledgements

We would like to thank for the assistance of Soon Jin Yun, Jongyeol Lee, and Jiae An during the field and laboratory works.

#### Funding

This study was supported by the Forest Practice Research Center, National Institute of Forest Science (FM0101-2009-01) and the Korean Ministry of Environment (2016001300004).

#### Availability of data and materials

Please contact the author for data requests.

#### Authors' contributions

SK led the field and laboratory works, data analysis and discussion, and manuscript writing. SHH, GL, and TKY participated in the field and laboratory works, discussion of the data, and manuscript writing. STL and CK contributed to the experimental design and manuscript writing. YS supervised whole process including the experimental design, data analysis and discussion, and manuscript writing. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

Not applicable.

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Received: 1 September 2016 Accepted: 14 September 2016

Published online: 24 October 2016

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