Carbon and Nitrogen Resorption of Needles As Affected by Compound Fertilizer in a Red Pine Stand

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\textbf{ABSTRACT}

This study aimed to examine carbon (C) and nitrogen (N) resorption efficiency between green needles and needle litter in response to compound fertilizer types in a red pine stand. Green needles and needle litter were collected during the growing season (July, September) and during the heavy litterfall season (November) from two compound fertilizer (N\textsubscript{3}P\textsubscript{4}K\textsubscript{1}, P\textsubscript{4}K\textsubscript{1}) and control treatments. The C concentration of green needles and needle litter was significantly higher in the N\textsubscript{3}P\textsubscript{4}K\textsubscript{1} than in the P\textsubscript{4}K\textsubscript{1} and control treatments. C and N resorption efficiency was not significantly affected by compound fertilizer, whereas the N concentration in green needles was significantly higher in the N\textsubscript{3}P\textsubscript{4}K\textsubscript{1} than in the P\textsubscript{4}K\textsubscript{1} and control treatments. N resorption efficiency was significantly affected by sampling month ($P < 0.05$). N resorption efficiency was significantly higher in November, followed by September and July. In addition, N resorption efficiency was lower in the current-year needles than in the 1- or 2-year-old needles. These results demonstrate a lack of clear relation between C or N resorption efficiency and N availability in response to the compound fertilizer.

\textbf{INTRODUCTION}

Foliar nutrient resorption (retranslocation) in forest trees is an important mechanism for the conservation of tree nutrients (Hagon-Thorn et al. 2006; Fife et al. 2008; Yuan and Chen 2015) and has been accepted as a good indicator of soil fertility (Weetman and Wells 1990). Many studies have reported that species from nutrient-poor sites have a higher nutrient resorption efficiency compared with species from nutrient-rich environments (Aerts 1996; Yuan and Chen 2015). However, foliar nutrient resorption efficiency is affected by many environmental factors, such as soil properties, season length, water supply, needle age (Lü and Han 2010; Chen et al. 2015) and fertilization (Yuan and Chen 2015).

Foliar nutrient analysis following fertilizer application has received considerable attention because the nutrient concentrations of foliage are an important parameter for assessing nutrient requirements and deficiencies in forest stands (Weetman and Wells 1990). However, there is no consistent trend regarding the effects of fertilizer application on nutrient resorption efficiency (Yuan and Chen 2015). For example, nutrient resorption efficiency was generally higher in the fertilized than unfertilized plots in sawtooth oak stands (Kim et al. 2013b), whereas nitrogen (N) resorption efficiency was reduced following N addition (Lü and Han 2010). Despite many studies on the nutrient responses of forest stands following fertilizer applications (Son et al. 2007; Kim et al. 2013b), there is still a lack of knowledge on the nutrient resorption efficiency in the response to foliage following compound fertilizer application. In addition, the nutrient resorption efficiency following compound fertilizer application should be evaluated due to multi-nutrient deficiency problems in Korean forest stands (Son et al. 2007; Kim et al. 2013a; Kim et al. 2013b).

The objective of this study was to determine the carbon (C) and N resorption of needle in response to compound fertilizer types in red pine (\textit{Pinus densiflora} S. et Z.), which is the most important conifer tree species throughout the country.

\textbf{MATERIALS AND METHODS}

This study was conducted in approximately 40-year-old red pine stands in the Wola National Experimental Forest, Jinju, Korea. The highest amount of precipitation during the study period falls in the rainy season (July) with high temperature (Figure 1), whereas the annual average precipitation and temperature in this area are 1,490 mm yr\textsuperscript{-1} and 13.1 °C, respectively. Soil type based on forest soil classification of Korea is slightly dry, dark-brown forest soils (Inceptisols, US soil taxonomy). The site index of 8-10 indicates low forest productivity with poor soil fertility. The experimental design consisted of a completely randomized design with 2 blocks (35°12' 32" N, 128° 10' 23" E,
180 m; 35° 12’ 26” N; 128° 10’ 25” E, 195 m) involving total 18 plots [(3 treatments (N\_P\_K, P\_K, control) × 2 blocks × 3 replicated plots)] in a mature red pine stand. The compound fertilizer types were based on the guideline (N\_P\_K \_1 = 113:150:37 kg ha\(^{-1}\)) of forest fertilization in Korea forests and without nitrogen fertilizer (P\_K \_1 = 150:37 kg ha\(^{-1}\)). Urea, fused superphosphate, and potassium chloride fertilizers were used as sources of N, phosphorus (P), and potassium (K), respectively. The fertilizers were applied on forest floor by hand in early spring (April) in 2011. More specific information related to stand and soil in the study site could be found elsewhere (Kim et al. 2013b; Kim et al. 2017).

Fresh green needle samples following fertilizer application were collected at three times (1st of July, 8th of September, and 7th of November, 2011) using pole pruners from the mid-crown of two or three dominant trees per treatment plot. Senesced needle litter was collected from litter traps within each treatment plot at the same sampling times as fresh green needles. Litter traps in this study were used to determine the nutrient concentration of needle litter because nutrient leaching during a short period for litter collection does not appear to be significantly affecting nutrient resorption efficiency (Vergutz et al. 2012). In addition, throughfall inputs were similar between fertilizer and control treatments during the study period (Figure 2).

The green needle and needle litter samples were put into plastic zipper bags and then transported to the laboratory. Green needles were sorted by needle age classes (current-year, 1-year-old, 2-year-old needles) divided into the order of branching from twigs, whereas the age of needle litter was not determined because all-needle litter may involves all ages of needle (Griffin et al. 2011). However, the age of needle litter sampled in November (heavy litterfall season) could be over 3 years because average needle longevity of red pine was about 2.8 years (Kume et al. 2000). The green needles and needle litter samples were oven-dried at 65 °C for 48 h. The dried green needles and needle litter samples were ground in a Wiley mill and then passed through a 40-mesh stainless steel sieve. C and N concentrations from the ground materials were determined using an elemental analyzer (Thermo Scientific, Flash 2000, Italy).

The C or N resorption efficiency between the green needles and senesced needle litter was calculated using the following equation (Vergutz et al. 2012; Kim et al. 2013a; Yuan and Chen 2015):

\[
\text{C or N resorption efficiency} = \left( \frac{C \text{ or N concentration (green needle)} - i \cdot C \text{ or N concentration (senesced needle litter)}}{C \text{ or N concentration (green needle)}} \right) \times 100
\]

where \(i\) is the sampling time (July, September, November).

To measure the change of inorganic soil N concentrations following fertilizer applications, a 5-gram subsample of fresh mineral soil at 5-cm top soil was monthly sampled and extracted by a mechanical vacuum extractor (Model 24VE, SampleTek, Science Hill, KY, USA) with 50 ml of 2 M KCl solution immediately after sampling. The soil extract solutions were stored at 4 °C in a cooler. Ammonium (NH\(_4\) \_N) and nitrate (NO\(_3\) \_N) concentrations in the soil extract samples were determined using an Auto analyzer (AQ2 Discrete Analyzer, Southampton, UK). The inorganic N concentration was measured for 7 months (from May to November 2011) after fertilizer applications.
Green needle data were analyzed by three-way analysis of variance (ANOVA) to determine the significance of the main effects [compound fertilizer type (F), sampling month (M), needle age (A)] and their interactions (F × M, F × A, M × A, F × M × A), whereas needle litter data were analyzed by two-way ANOVA [compound fertilizer type (F), sampling month (M)] and their interactions (F × M). The main and interactive effects on the concentration and resorption of C and N in the foliage were tested at P < 0.05. The comparison of C and N concentration and C/N ratio between green needle and needle litter was examined using two sample T-test. Monthly inorganic soil N concentrations were analyzed by one-way ANOVA. All ANOVA and T-test were executed using the Proc GLM (general linear model) and Proc TTEST procedures in SAS (SAS Institute Inc. 2003). Tukey’s test was used for mean separations.

| Component | df | C | N | C/N ratio in green needle | C | N | C/N ratio in needle litter | C resorption efficiency | N resorption efficiency |
|-----------|----|---|---|--------------------------|---|---|--------------------------|------------------------|------------------------|
| Fertilizer (F) | 2  | 0.6158 | 0.002 | 0.011 | 0.371 | 0.705 | 0.410 | 0.063 | 0.427 |
| Sampling month (M) | 2  | <0.001 | 0.624 | 0.731 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Needle age (A) | 2  | <0.001 | 0.005 | 0.002 | 0.672 | 0.396 | 0.670 | 0.584 | 0.036 |
| M × F | 4  | 0.612 | 0.393 | 0.392 | 0.672 | 0.396 | 0.670 | 0.584 | 0.036 |
| M × A | 4  | 0.068 | 0.626 | 0.212 | – | – | – | 0.092 | 0.489 |
| F × A | 4  | 0.566 | 0.270 | 0.567 | – | – | – | 0.596 | 0.698 |
| M × F × A | 8  | 0.371 | 0.949 | 0.968 | – | – | – | 0.447 | 0.995 |

Bold values denote a significance at P < 0.05. –: not determined

**Figure 4.** Carbon and nitrogen concentration of green needle or needle litter by compound fertilizer types, sampling month, and needle age in a red pine stand. Carbon and nitrogen concentration of needle litter in needle age was the mean values of three sampling times. Vertical bars represent one standard error. Different letters on the bars represent significant difference among treatment at P < 0.05. Asterisks represent a significant difference between green needle and needle litter at P < 0.05.
Results

Inorganic soil N (NH$_4^+$-N and NO$_3^-$-N) concentration rapidly increased with the N$_3$P$_4$K$_1$ treatment following compound fertilizer application (Figure 3). Mean soil NH$_4^+$-N and NO$_3^-$-N concentrations over the study period were 23.55 mg kg$^{-1}$ and 0.53 mg kg$^{-1}$ for the N$_3$P$_4$K$_1$, 3.46 mg kg$^{-1}$ and 0.13 mg kg$^{-1}$ for the P$_4$K$_1$ and 2.22 mg kg$^{-1}$ and 0.12 mg kg$^{-1}$ for the control treatments, respectively.

The C concentration in green needles was not significantly (P > 0.05) affected by the compound fertilizer types (Table 1, Figure 4), whereas the N concentration was significantly higher in the N$_3$P$_4$K$_1$ treatment than in the P$_4$K$_1$ and control treatments (Table 1, Figure 4). However, the C and N concentrations in the needle litter were not affected by compound fertilizer types. The C concentration of green needles was significantly higher in July than in November, but the concentration in the needle litter was highest in November. The N concentration in green needles was not related to sampling season, while the N in the needle litter was lowest in November. The C concentration in green needles was significantly lower in the current-year or 1-year-old needles than in the 2-year-old needles (Figure 4). The C/N ratio of green needles was significantly higher in the N$_3$P$_4$K$_1$ treatment than in the P$_4$K$_1$ and control treatments, and the C/N ratio was significantly higher in the 2-year-old needles than in the current-year or 1-year-old needles. The C/N ratio in the needle litter was significantly higher in November than in July or September (Figure 5).

The N resorption efficiency between the green needles and needle litter demonstrated a significant two-factor interactive effect between the sampling month and the compound fertilizer types (Table 1), whereas C resorption efficiency was not significantly affected by compound fertilizer types (Table 1). There was a significant main effect of C resorption efficiency on the sampling month and needle age but no significant two-way interaction (Table 1). N resorption efficiency was not significantly affected by compound fertilizer types (Figure 6). The C and N resorption efficiency was significantly different between sampling month and needle age. N resorption efficiency was significantly higher in November, followed by September and July. In addition, N resorption efficiency was significantly lower in the current-year needles than in the 1-year-old and 2-year-old needles.

Discussion

The C concentration in green needles and needle litter was not affected by compound fertilizer types. Inter- and intraspecific variation in C concentration in tree species is determined by genetic and environmental factors (Bert and Danjon 2006; Zhang et al. 2009) rather than by a change in nutrient availability following fertilizer application (Jeong et al. 2010). For example, the C concentration in the green needles or needle litter in this red pine stand was significantly affected by genetic and environmental factors such as needle age and sampling month. The seasonal patterns of C concentration could be attributed to the translocation of carbohydrates to actively growing tissues (Mugasha et al. 1999). In addition, the increased C concentration in the needle litter in November and in 2-year-old needles could be due to mineral concentration reductions by nutrient resorption before leaf abscission (Poorter and De Jong 1999).

The increased N concentrations in the green needles of the N$_3$P$_4$K$_1$ treatment compared with the P$_4$K$_1$ and control treatments may have resulted from responses to the increased N availability following N addition in compound fertilizer, as tree species with high N availability tend to produce needles that have high N concentrations (Sariyildiz and Anderson 2005; Kim et al. 2013b). In contrast, the N concentration in the needle litter did not appear to be affected by N addition in compound fertilizer types, as the N concentration in the needle litter is controlled by such factors as the combined effects of soil available N status, tree growth, climatic factors and N resorption rates (Lu and Han 2010; Yuan and Chen 2015). The N concentration in the green needles was not related to sampling season, but the decrease in N concentration of the needle litter during the heavy needle fall season (November) or in 2-year-old green needles could be attributed to N resorption before leaf abscission.
The compound fertilizer types were associated with a change in the C/N ratio of green needles because of the high N concentration in the N3P4K1 treatment compared with the P4K1 and control treatments. However, the high C/N ratio of the 2-year-old needles or needle litter in November could be due to increased N resorption before the heavy litterfall season (Hagont hern et al. 2006).

C and N resorption efficiencies between the green needles and needle litter in this study did not respond to changes in nutrient availability such as fertilizer application. Most studies have reported that plants in nutrient-poor environments show more resorption than do plants in nutrient-rich environments (Fife et al. 2008; Lü and han 2010; Yuan and Chen 2015) whereas there was no clear effect of nutrient availability in soil on nutrient resorption because of the increase in both leaf mass and leaf nutrient concentrations in response to fertilization (Aerts 1996). In the present study, C resorption efficiency was not affected by compound fertilizer types because the C in the needles is generally not limiting tree growth. However, the N resorption efficiencies were also not responsive to compound fertilizer types. The lack of a clear relation between N availability and N resorption efficiency might be due to the increases in N concentrations in mature needle after fertilizer application or the negative effect on N resorption efficiency from combined N and P fertilization (Yuan and Chen 2015). Similarly, N resorption efficiency of Chinese fir leaves was not altered by N addition because of increased leaf N concentration (Chen et al. 2015).

The C and N resorption efficiency demonstrated different patterns during the sampling month and for the different needle age classes. For example, N showed resorption, while C accumulated over the three sampling months. In this study, the average N resorption efficiency (56%) in the heavy litterfall season (November) was slightly lower than the global average (62.7%) of conifers (Vergutz et al. 2012). However, the efficiency in the growing season (July: 6%; September: 21%) was much lower than that during the heavy litterfall season. The low N resorption efficiency in the growing season could be due to increased N concentrations by fresh green needle inputs during the growing season. The resorption efficiency of C increased with
increasing needle age, while the efficiency of N decreased with increasing needle age class. The low N resorption efficiency in 2-year-old needles could be due to low N concentrations with increased needle age class.

Conclusions
The results of this study indicate that there was no clear effect on the C and N resorption efficiency from compound fertilizer. However, the C and N resorption efficiency in red pine stands was easily influenced by seasonal patterns and needle age class rather than by changes in nutrient availability following fertilizer application.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
Aerts R. 1996. Nutrient resorption from senescing leaves of perennials: Are there general patterns? J of Ecol. 84(4):597–608.
Bert D, Danjon F. 2006. Carbon concentration variations in the roots, stem and crown of mature Pinus pinaster (Ait.). For Ecol Manage. 222(1-3):279–295.
Chen FS, Niklas KJ, Liu Y, Fang XM, Wan SZ, Wang H. 2015. Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fir leaves and twigs differing in age. Tree Physiol 35:1106–1117.
Fife DN, Nambari EKS, Saur E. 2008. Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. Tree Physiol. 28:187–196.
Griffin JM, Turner MG, Simard M. 2011. Nitrogen cycling following mountain pine beetle disturbance in lodgepole pine forests of Greater Yellowstone. For Ecol Manage 261:1077–1089.
Hagen-Therm A, Varnagiye T, Nihlgard B, Armolaitis K. 2006. Autumn nutrient resorption and losses in four deciduous forest tree species. For Ecol Manage. 228(1-3):33–39.
Jeong J, Park JH, Kim JI, Lim JT, Lee SR, Kim C. 2010. Effects of container volumes and fertilization on red (Pinus densiflora) and black pine (Pinus thunbergii) seedlings growth. For Sci Tech. 6(2):80–86.
Kim C, Byun JK, Park JH, Ma HS. 2013a. Litter fall and nutrient status of green leaves and leaf litter at various compound ratios of fertilizer in sawtooth oak stands, Korea. Ann For Res 56(2):339–350.
Kim C, Ju NG, Lee HY, Lee KS. 2013b. Effects of fertilizer on growth, carbon and nitrogen responses of foliage in a red pine stand. Korean J Soil Sci Fert. 46(1):1–7.
Kim C, Baek G, Park SW, Kim S. 2017. Inorganic nitrogen dynamics of throughfall following fertilization in a red pine stand. For Sci Tech. 13(4):187–191.
Kume A, Tsuoi N, Satomura T, Suzuki M, Chirwa M, Nakane K, Sakurai N, Horikoshi T, Sakugawa H. 2000. Physiological characteristics of Japanese red pine, Pinus densiflora Sieb. Et Zucc., in declined forests at Mt. Gokurakuji in Hiroshima Prefecture, Japan. Tree 14:305–311.
Lü XT and Han XG. 2010. Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. Plant Soil 327:481–491.
Mugasha AG, Pluth DJ, Macdonald SE. 1999. Effects of fertilization on seasonal patterns of foliar mass and nutrients of tamarack and black spruce on undrained and drained minerotrophic peatland sites. For Ecol Manage. 116(1-3):13–31.
Poorter H, De Jong R. 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytol. 143(1):163–176.
Sariyildiz T, Anderson JM. 2005. Variation in the chemical composition of green leaves and leaf litters from three deciduous tree species growing on different soil types. For Ecol Manage. 210(1-3):303–319.
SAS Institute Inc. 2003. SAS/STAT Statistical Software. Version 9.1. Cary (NC): SAS Publishing.
Son Y, Lee MH, Noh NJ, Kang BO, Kim KO, Kim MJ. 2007. Fertilization effects on understory vegetation biomass and structure in four different plantations. J Korean For Soc. 49(3):520–527.
Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB. 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Mono. 82(2):205–220.
Weetman GF, Wells CG. 1990. Plant analyses as an aid in fertilizing forests. p. 659–690. R.L. Westerman (ed). 3rd ed. Soil testing and plant analysis. SSSA, Wisconsin, USA.
Zhang Q, Wang C, Wang X, Quan X. 2009. Carbon concentration variability of 10 Chinese temperate tree species. For Ecol Manage. 258(5):722–727.
Yuan ZY, Chen HYH. 2015. Negative effects of fertilization on plant resorption. Ecology 96(2):373–380.