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Article

Effects of Fertilizer on Growth, Carbon and Nitrogen Responses of Foliage in a Red Pine Stand

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This study was to examine growth, carbon and nitrogen responses in foliage following forest fertilization in a red pine stand. Two types of fertilizer (N:P:K=113:150:37 kg ha^{-1}; P:K=150:37 kg ha^{-1}) were applied on late April 2011. Growth, carbon and nitrogen responses of foliage were monitored 3 times (July, September, November) after fertilization. Morphological growth responses (dry mass, leaf area, specific leaf area) with foliage age were not significantly ($P > 0.05$) affected by fertilizer application, while needle dry mass and leaf area of July were significantly lower in current-year-old than in one-year-old or two-year-old needles of September or November. Carbon concentration and content in foliage was little affected by fertilizer application compared with sampling month or needle age, while the NPK fertilizer produced high nitrogen concentration and content of foliage. The results indicate that nitrogen concentration and content in foliage may serve as an indicator of the nitrogen status by fertilization in a red pine stand.

Key words: Carbon, Fertilization, Foliage analysis, Nitrogen, Red pine

Introduction

Needle analysis following fertilizer application has received considerable research attention because the nutrient concentrations of foliage are an important parameter to assess the nutrient requirements and deficiencies in forest stands (Weetman and Wells, 1990; Barron-Gafford et al., 2003; Tausz et al., 2004). Generally, nutrient concentrations of foliage have been accepted as good indicators of growth and soil fertility on site (Bauer et al., 1997), although the nutrient status in foliage could be attributed to many environmental factors, such as soil properties, season length, water supply, and other environmental factors (Weetman and Wells, 1990).

Fertilizer application increased tree growth by the positive effects on leaf area and foliar photosynthetic rate (Garrison et al., 2000; Amponsah et al., 2005). Also, the nutrient concentrations in the living parts (needles, leaves, bark, and root) of trees increased following fertilizer application because the nutrient status of the tree is dependent on the nutrient status of the soil (Miller et al., 2006). However, the status of nutrient in foliage was depended on fertilizer types and dose, site characteristics, and tree species (Mugasha et al., 1999; Brockley, 2000; Miller et al., 2006).

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There have been a myriad of nutritional problems such as nitrogen and phosphorus deficiency on forest stands in Korea (Byun et al., 2006; Son et al., 2007; Park et al., 2008). Despite of many studies on the nutrient status of forest stands following fertilizer application, there still is a lack of knowledge about growth, carbon and nitrogen responses of foliage involved in the fertilizer reaction in Korea forest stands. In addition, an understanding of nutrient status is essential for designing and timing of fertilizer application in forest stands. The objective of this study was to determine the growth, carbon and nitrogen responses of the foliage by fertilizer application, sampling month, and needle age in red pine (*Pinus densiflora* S. et Z.) which is the most widespread conifer tree species throughout the country.

Materials and Methods

This study was conducted in about 40-year-old natural red pine stands in the Wola National Experimental Forest administered by the Southern Forest Research Center, Korea Forest Research Institute. The annual average precipitation and temperature in this area are 1,490 mm y^{-1} and 13.1℃, respectively. The soil is slightly dry, dark-brown forest soil (mostly Inceptisol, United States Soil Classification System) originating from sandstone or shale with a silt loam texture (Table 1 and 2). The site index indicates low forest productivity (site index, 8-10 at 20-year-old base age) suggesting poor soil fertility. The treatment plots were established on the same facing slopes and aspects under a similar environmental condition to minimize spatial variation in soil properties. 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 (NPK, PK, Control) × 2 blocks × 3 replicated plots] in a mature red pine stand. Treatment plots (plot size=10 x 10m) were randomly assigned with a 5 m buffer zone between each plot. The combination of fertilizer types was based on the guideline (N:P:K = 113:150:37 kg ha^{-1}) of forest fertilization in Korea forests (Joo et al., 1982) and without nitrogen fertilizer (P:K=150:37 kg ha^{-1}). Urea, fused superphosphate, and potassium chloride fertilizers were used as sources of nitrogen (N), phosphorus (P), and potassium (K), respectively.

Fresh foliage samples following fertilizer application were collected at three times (1st of July, 8th of September, and 7th of November, 2011) with pole pruners from the mid-crown of two or three dominant trees per each treatment plot. The foliage samples with plastic zipper bags were transported to the laboratory and sorted by several foliage age classes (current-year-old, one-year-old, and two-year-old needles) from twigs or small branches. For each treatment, three repetitions of 10 fascicles were counted and weighted. Needle leaf area was measured by fresh needle samples by using scanned leaf meter (CI-202 area meter CID, Inc. USA). Specific leaf area of needle was determined by leaf area (cm^2) and dry mass (mg) of ratio (Bauer et al., 1997).

The needle leaf samples were oven-dried at 65℃ for 48 h and the dried samples were ground in a Wiley mill to pass a 40-mesh stainless steel sieve. Carbon and nitrogen concentrations from the ground materials were determined on an elemental analyzer (Thermo Scientific, Flash 2000, Italy).

All data were analyzed by three-way analysis of variance (ANOVA) to determine the significance of main effects [fertilizer type (F), sampling month (M), needle age (A)] and their interactions (F×M, M×A, F×A, F×M×A). The model describing the data analysis is as follows:

$$Y_{ijk} = u + F_i + M_j + A_k + (FM)_{ij} + (FA)_{ik} + (MA)_{jk} + (FMA)_{ijk} + e_{ijk}$$

where u is the overall mean effect, F is fertilizer type (k=1, 2, 3), M is sampling month (i=1, 2, 3), and A is needle age (j=1, 2, 3). All ANOVA were executed

Table 1. Stand characteristics in the study site (n=6).

Treatments	Stand density	DBH†	Basal area
	trees ha^{-1}	cm	m^2ha^{-1}
Control	1200	15.51	22.35
	(141)‡	(0.81)	(1.96)
PK	1283	16.32	22.61
	(149)	(1.48)	(2.01)
NPK	1150	15.80	20.62
	(193)	(1.11)	(2.38)

†DBH: diameter at breast height (1.2 m). ‡Standard errors in parenthesis.

Table 2. Soil properties of the study site before fertilizer application (n=6).

Treatment	Sand	Silt	Clay	C	N	K^+	Ca^{2+}	Mg^{2+}
	%					$cmol_ckg^{-1}$		
Control	45	43	12	2.40	0.07	0.09	1.35	0.43
	(3.5)†	(3.0)	(1.0)	(0.28)	(0.01)	(0.01)	(0.19)	(0.05)
PK	42	45	13	2.66	0.08	0.09	2.10	0.65
	(2.0)	(1.9)	(1.8)	(0.27)	(0.01)	(0.01)	(0.26)	(0.05)
NPK	42	44	14	2.82	0.09	0.09	1.77	0.54
	(2.9)	(1.8)	(1.0)	(0.21)	(0.01)	(0.01)	(0.17)	(0.04)

†Standard errors in parenthesis.

using the General Linear Models procedure in SAS (SAS Institute Inc., 2003). The main and interaction effects on the growth and carbon and nitrogen status in foliage were tested at *P*=0.05 and a Tukey's test used for the mean separation.

Results

Morphological growth response According to ANOVA results, morphological growth responses (dry mass, leaf area, specific leaf area) of foliage were not significantly (*P* > 0.05) affected by fertilizer application (Table 3, Fig. 1). Needle dry mass and leaf area showed a significant two-factor interaction between sampling month and needle age (Table 3). Needle dry mass and leaf area of July were significantly lower in current-year-old than in one-year-old or two-year-old needles of September or November (Fig. 2). However, there was a significant main effect of specific leaf area on sampling month and needle age with no significant two- or three-factor interaction (Table 3).

Carbon and nitrogen responses There were significant main effects of carbon responses (concentration and content) on sampling month and needle age with no significant

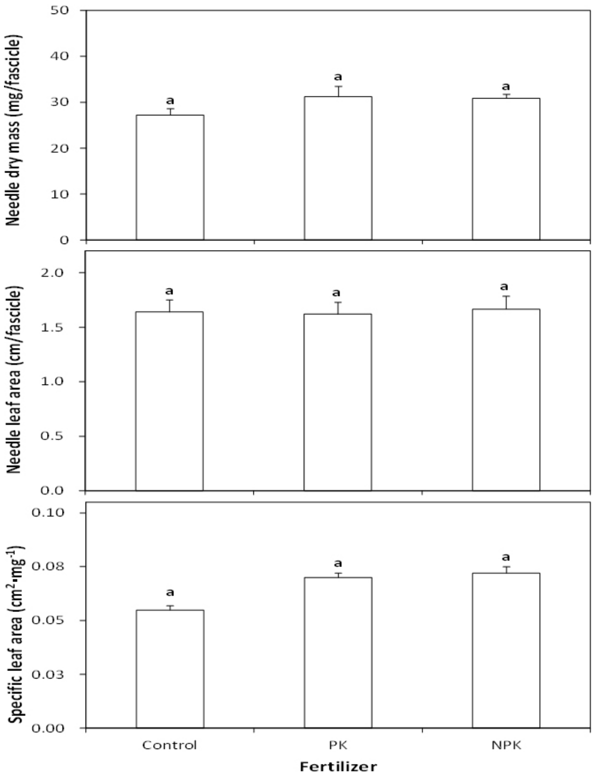

Fig. 1. Dry mass, leaf area and specific leaf area of foliage by fertilizer application in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

Table 3. *P*-value from results of ANOVA on sampling month, needle age and fertilizer type on growth and carbon and nitrogen responses of foliage in red pine stands.

Component	Needle dry mass	Leaf area	Specific leaf area	Carbon concentration
Sampling month (M)	**<0.001**[†]	**<0.001**	**<0.001**	**<0.001**
Needle age (A)	**<0.001**	**0.001**	**<0.001**	**<0.001**
Fertilizer (F)	**0.086**	0.389	0.056	0.617
M×A	**0.035**	**<0.001**	0.699	0.059
M×F	0.095	0.718	0.183	0.568
A×F	0.604	0.734	0.267	0.558
M×A×F	0.991	0.547	0.905	0.366

Component	Carbon content	Nitrogen concentration	Nitrogen content	Carbon/ Nitrogen ratio
Sampling month (M)	**<0.001**	0.673	**<0.001**	0.750
Needle age (A)	**<0.001**	**0.002**	**<0.001**	**0.003**
Fertilizer type (F)	0.109	**0.001**	**0.004**	**0.011**
M×A	**0.046**	0.654	**<0.001**	0.258
M×F	0.081	0.359	0.182	0.388
A×F	0.740	0.288	0.364	0.575
M×A×F	0.998	0.956	0.968	0.967

[†]Bold values denote a significance at *P*=0.05.

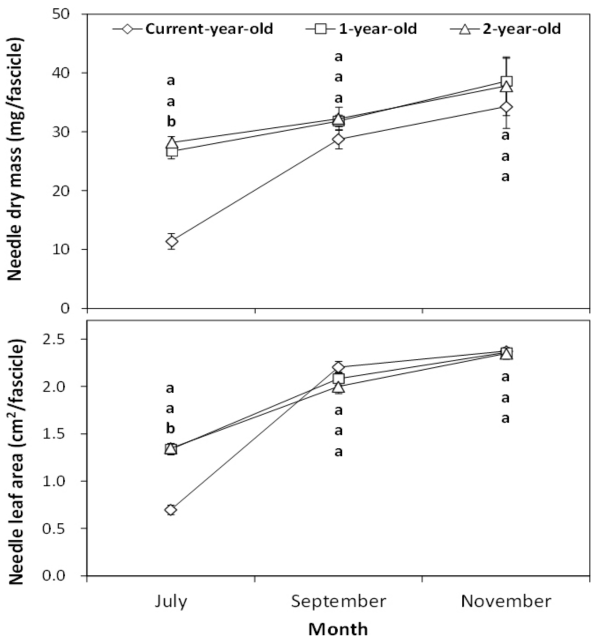

Fig. 2. Dry mass and leaf area of foliage by sampling month and needle age in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

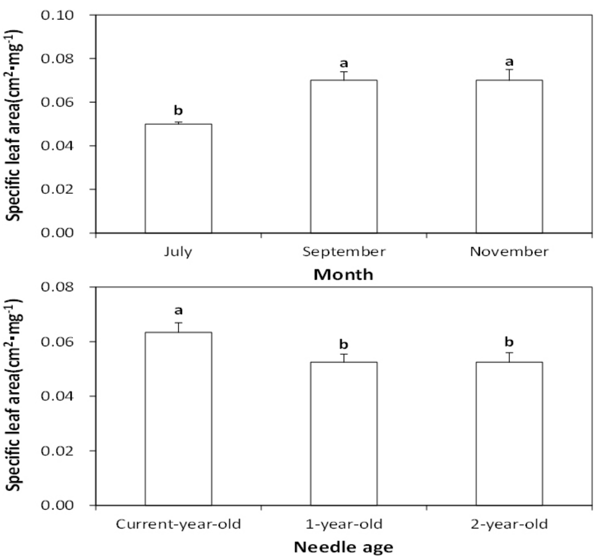

Fig. 3. Specific leaf area of foliage by sampling month and needle age in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

three-factor interaction (Table 3). Nitrogen and C/N ratio of foliage were significantly affected ($P < 0.05$) by fertilizer application. There was a significant interaction effect of sampling month and needle age on carbon and nitrogen content (Table 3). Carbon concentration of foliage was significantly higher in July than in September and November, while significantly lower in current-year-old than in one-year-old or two-year-old needles (Fig. 4). However, carbon concentration of foliage was not significantly affected by fertilizer application (Fig. 4). In contrast to carbon concentration, nitrogen concentration and content of foliage were significantly higher ($P < 0.05$) in the NPK than in the PK or control treatments (Fig. 5). Carbon and nitrogen content of foliage was closely related to dry mass of foliage. Carbon and nitrogen ratio was significantly lower in the NPK than in the PK or control treatments (Fig. 6).

Discussion

Because nutrient availability is generally considered the major environmental factor limiting growth in many forest tree species (Binkley, 1986), foliage growing with fertilization would have greater dry mass than those growing without fertilization. However, dry mass of fascicle was not significantly greater in the fertilizer (NPK: 30.99 mg/fascicle; PK: 31.20 mg/fascicle) than in the control (27.22 mg/fascicle) treatments. Needle or specific leaf areas were also not significantly different between the fertilizer and control treatments because both factors are closely related to dry mass of foliage (Fig. 1). Growth and development in foliage following fertilizer application could be regulated by physiological responses such as uptake and allocation of carbon and nutrient in tree components (Gough et al., 2004). Therefore, fertilization response potential can be estimated from increase of foliage biomass in tree levels rather than from dry mass of each fascicle (Gough et al., 2004). Similarly, nitrogen fertilization increased the number of fascicles of loblolly pine trees (Zhang et al., 1997). In contrast to this result, PK fertilization increased the dry mass of foliage in Scots pine stands suffered from the PK deficiency sites (Silfverberg and Moilanen, 2008). The lack of significant fertilization effects on morphological growth response of foliage in this study could be due to primordial characteristics of foliage initiated in the previous year prior to fertilization (Zhang et al., 1997) or other nutrient imbalances caused by nitrogen fertilization (Amponsah et al., 2005).

Dry mass by foliage was significantly lower in the current-year-old (24.80 mg/fascicle) than in one-year-old or two-year-old (32.37 - 32.31 mg/fascicle) needles with the two-factor interaction of needle age and sampling time. Dry mass in current-year-old needles was significantly lower in July than in September or November samplings

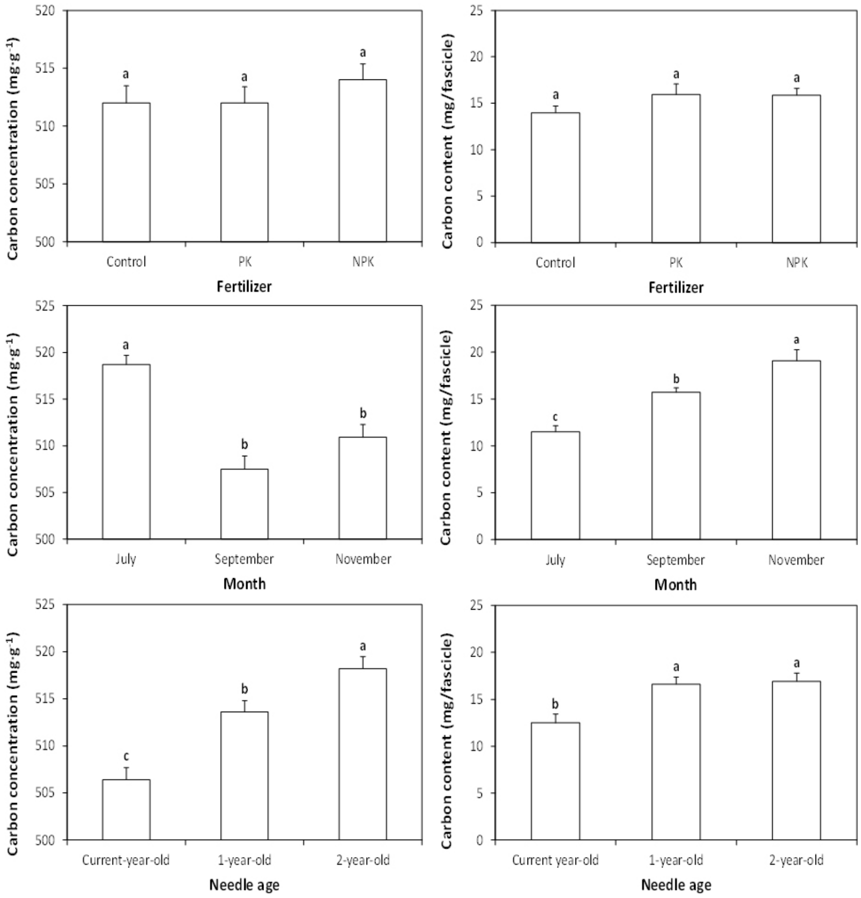

Fig. 4. Carbon concentration and content area of foliage by fertilizer application, sampling month, and needle age in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

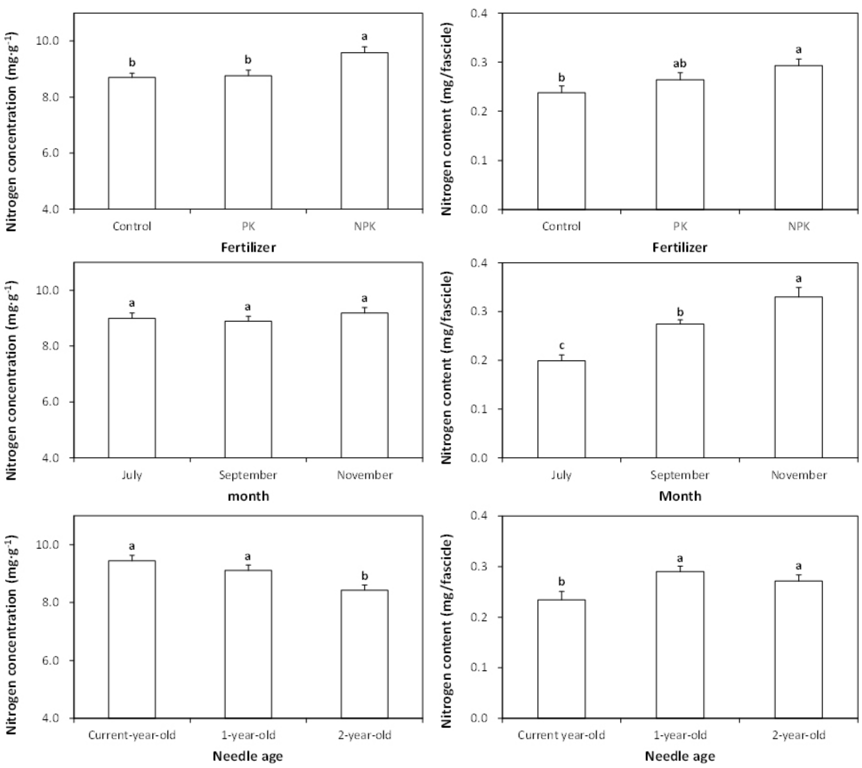

Fig. 5. Nitrogen concentration and content area of foliage by by fertilizer application, sampling month, and needle age in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

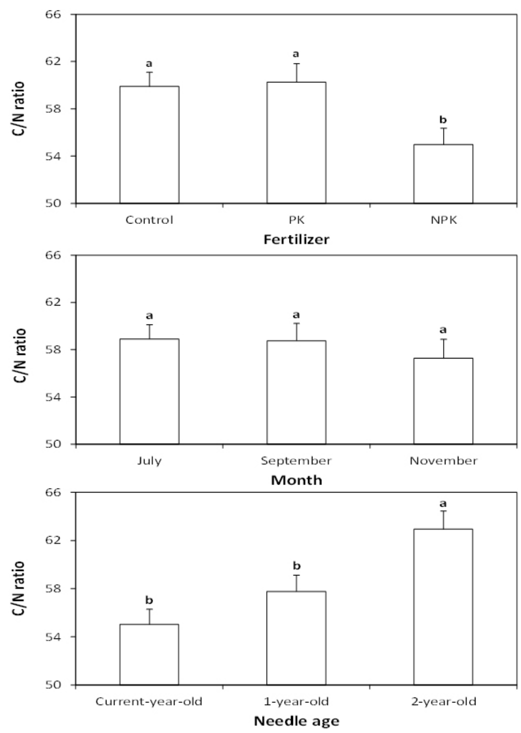

Fig. 6. Carbon (C) and nitrogen (N) ratio of foliage by fertilizer application, sampling month, and needle age in a red pine stand. Different letters on the bars indicate a significant difference among treatments at *P*=0.05.

(Fig. 2). Seasonal patterns of foliage dry mass could be attributed to needle maturation or allocation of resources between woody (root, stemwood, and branches) and photosynthetic compounds (Mugasha et al., 1999, Gough et al., 2004). Needle and specific leaf area by sampling month were significantly lower in July than in September or November (Fig. 2, Fig. 3) because of relationship between needle maturation and dry mass of foliage in this season. In addition, low needle leaf area in July indicated that current-year-old needle was not fully to be elongated in this season. The variations of specific leaf area under identical conditions of the same species could be attributed to multi-factorial influences that occur during the leaf life-span, such as light environment, nutrients, temperature and water supply (Wilson et al., 1999).

Carbon concentration and content in foliage were not affected by (P > 0.05) the fertilizer application (Fig. 4). Similarly, carbon concentration in black pine seedlings was a poor indicator of fertilizer response (Jeong et al., 2010) because the inter- and intra- specific variations of carbon concentration in tree species were determined by genetic and environmental factors (Bert and Danjon, 2006; Zhang et al., 2009). In contrast to this result, carbon concentration of tree components was generally greater in nutrient deficient conditions compared with trees grown in better nutrient conditions because of low mineral concentration of tree components or the difference of carbon allocation (Poorter and De Jong, 1999).

Carbon concentration in foliage was significantly higher in July (519 mg g^{-1}) than in September (508 mg g^{-1}) or November (511 mg g^{-1}), while carbon content was lower in July than in September or November (Fig. 4). Seasonal patterns of carbon concentration and content in foliage were due to needle maturation related to translocation of carbohydrates and other cellular materials to active growing tissues (Mugasha et al., 1999).

Nitrogen concentration and content in foliage increased significantly following the NPK fertilization (9.6 mg g^{-1}), while insignificant increases in foliage nitrogen occurred following the PK fertilization (8.8 mg g^{-1}) compared with control (8.7 mg g^{-1}) treatments (Fig. 5). A high concentration of nitrogen in foliage with NPK application is likely due to increased nitrogen uptake after fertilizer application (Mugasha et al., 1999; Gough et al., 2004), as tree species with high nitrogen availability tend to produce foliage with high nitrogen concentration (Sariyildiz and Anderson, 2005). In contrast to the NPK treatment, low nitrogen concentration of foliage found in the PK and control treatment may be deficient and limiting to tree growth in this study site. Nitrogen concentration and content in foliage were not related to sampling season or needle age, although the decrease in foliar nitrogen concentration during needle elongation was a result of dilution due to greater relative accumulation of carbohydrates (Silfverberg and Moilanen, 2008).

The fertilizer application was associated with the change in C/N ratio of foliage because of high nitrogen concentration and content of foliage with the NPK fertilizer compared with the PK or control treatments (Fig. 6). The C/N ratio was not significantly different among sampling month, while C/N ratio among needle age was significantly higher two-year-old than current-year-old or one-year-old needles. High C/N ratio in two-year-old needles could be due to nitrogen retranslocation before heavy litterfall season (Hagon-Thorn et al., 2006).

Conclusion

Growth characteristics such as dry mass, leaf area, and specific leaf area in foliage were not affected by NPK or PK fertilizer application, while NPK fertilizer produced high nitrogen concentration and content of foliage in a red pine stand. The C/N ratio in foliage was associated with increased nitrogen concentration following NPK fertilizer application. However, carbon concentration and content in foliage was little affected by fertilizer application compared with sampling month or needle age. The result suggests that nitrogen concentration and content in foliage may be an essential tool for needle analysis following fertilizer application in a red pine stand.

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