Foliar nitrogen dynamics of representative woody plants seedlings grown under elevated ozone with a free-air system

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Abstract

To clarify effects of ozone (O_3) on foliar nitrogen (N) contents dynamics in three representative woody plant seedlings: birch (*Betula platyphylla* var. *japonica*), oak (*Quercus mongolica* var. *crispula*), and beech (*Fagus crenata*) grown in elevated O_3 (eO_3), we investigated N contents in live and senescing leaves, the relation of N content differences and leaf mass per area (LMA) of each species. The 3 species seedlings were planted in a free-air O_3 enrichment system for one growing season with 3 replicated plots exposed to the air or O_3 at about 2.5 times the ambient. In our study, about 60 % of foliar N was retranslocated under eO_3 conditions in birch; nearly 70 % of N in live leaves of oak was decreased by O_3; and negative correlations were found between LMA and N at ambient. Based on the results, we discussed plausible understanding physiologically and biochemically to conclude that foliar N contents of birch is more sensitive than beech in response to O_3, especially for the senescing leaves; on the other hand, LMA may be considered as an index parameter in speculation of the changes in foliar N contents at ambient O_3 concentrations.

Key Words: foliar nitrogen, elevated O_3, free-air, woody plants

▪ Introduction

Ground level ozone (O_3) is strongly oxidizing, can be regarded as a significant atmospheric pollutant that may reduce the vigor and health of forests (Koike et al., 2013). In Japan, ground level of O_3 is increasing due to the increasing emissions of precursor gases and will continuously become higher for several decades in the foreseeable future (Paoletti and Manning, 2007). Despite significant increases in atmospheric O_3, we don't understand the effect it has on plant growth. Previous research has found contrasting results that some suggested that eO_3 increased the compounds for the biosynthesis of protein and enhanced the N metabolism through the increased activities of enzymes in leaves (e.g. Yamaguchi 2007). While others reported that eO_3 may accelerate foliar senescence (Hoshika et al., 2012) and can adversely affect foliar Nitrogen (N) dynamics, resulting in decline of N acquisition, thereby influence foliar physiological and biochemical processes (Karnosky et al. 2007, Yamaguchi et al., 2010). The handful of studies about the effects of eO_3 on the foliar N changes have not clarified the debate, and only a few were carried out with free-air system on filed-grown trees; much remains unclear on interaction between eO_3 and foliar N alternation among different species (e.g. Yamaguchi et al., 2010).

Japanese white birch (birch: *Betula platyphylla* var. *japonica*), Mizunara oak (oak: *Quercus mongolica* var. *crispula*), and Siebold's beech (beech: *Fagus crenata*) were selected in this study since they represent the deciduous species with different O_3 sensitivities in the Japanese northern forests (Koike, 1995). Yamaguchi et al. (2011) reported that birch is the most sensitive species to O_3 while oak is the most tolerant among the 3 species.

Our objective was to test the hypothesis that O_3 sensitivity is related to changes in foliar N contents, and therefore we will discuss it with the specific traits of the three species. In addition, we investigated the effects of eO_3 on N contents in live and senescing leaves, as well as the relation of N contents and leaf mass per area (LMA).

▪ Material and methods

▪ *Experimental site and sample measurements*

The plant materials and experimental site are same as the experiment we reported in our previous paper (Shi et al., 2016). Therefore, we described briefly as follow: seedlings of Japanese white birch, oak, and beech with 3 duplicates for each species were planted in mid-July 2014 at the experimental nursery of Hokkaido University, Japan. The seedlings were planted in a free-air O_3 enrichment system for one growing season with 3 replication plots exposed to either air (26.5 ppb) or eO_3 (80 ± 7 ppb). When taken together, the experiment was set up with 3 replicate seedlings in 3 plots for 9 individuals for one species at one treatment.

The collection was conducted in two-stages, mid-September for the higher nutrient activities of plants and mid-November for the beginning of plant senescence. Especially for live leaf samples, we collected the top crown or the second leaf counted from the shoot-top (Shi et al., 2016). All the collected samples were dried at 70 ℃ for about one week and weighed. We then measured the leaf area and calculated the leaf mass per unit area (LMA). The foliar N concentration were determined by the combustion method using a NC analyzer (NC-900, Sumica, Osaka, Japan).

▪ *Statistical analysis*

All the statistical analyses were performed by SPSS software (21.0, SPSS, Chicago, USA). Two way analysis of variance (ANOVA) was used to evaluate the difference of O_3, species and their interactions on the foliar N content changes and Tukey's HSD test was applied when interaction term among the 3 species with the 2 gas treatments was significant. Significance of differences of foliar N content and the retranslocation rate (RR) between ambient and O_3 for each species was tested by the Student's *t*-test. Correlation analysis between N contents in live or senescing leaves and LMA were quantified and tested with Pearson's correlation coefficients.

▪ Results and discussion

Generally, as a water-soluble element, N is easily transported via phloem from older to younger leaves due to its mobility and availability in plants (Marschner, 2012). Therefore, foliar N is usually regarded to be an unstable nutrient element to commonly transport for the new growth and is decreased in senescing leaves (Helmisaari, 1992). This is also in agreement with our results (Table 1): foliar N in live leaves typically stayed higher than N in senescing leaves for each species under the same O_3 condition.

As a key species in boreal forests, birch has a high photosynthetic and responds rapidly to the

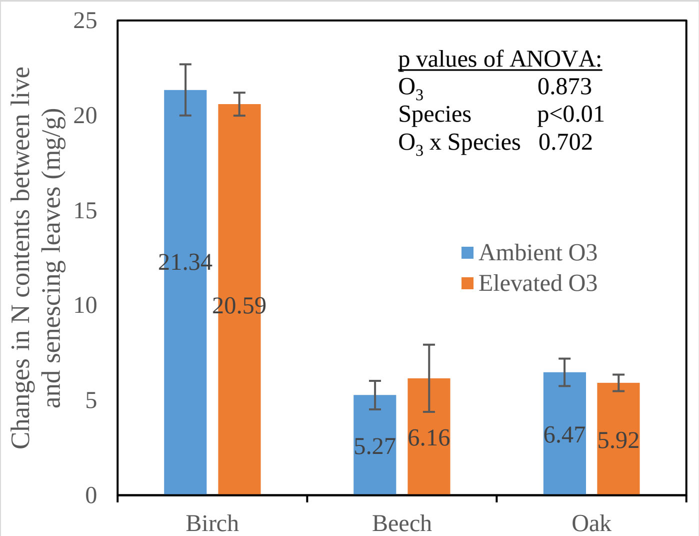

Figure 1. Changes in N contents (mg/g) between live and senescing leaves of Birch, Beech and Oak seedlings grown under ambient O3 and elevated O3 as measured within the growing season. Data are represented as mean ± SD (n=7-9). P values for the main effects of O3 treatments, species and their interactions on the foliar N content changes by two-way ANOVA. Different letters above the bar indicate the significant difference among the 3 species with the 2 O3 treatments (Tukey's HSD test, P<0.05)

environment (Koike, 1995), especially when eO_3 accelerate the process of senescence, therefore it make birch has to recycle the N more quickly before senescence as a self-protection strategy (Hoshika et al., 2013). In this study, although no significant differences were observed between ambient and eO_3 treatment for foliar N content differences in each species in Figure 1, the significantly higher values in birch than either beech or oak were observed in changes in N contents between live and senescing leaves at both O_3 treatments. In addition to the results of the birch at eO_3 shown in Table 1, nearly 62% foliar N was retranslocated (N RR = (Nlive – Nsenescing)/Nlive), suggesting that birch is relatively sensitive to eO_3 compared to the other 2 species.

For beech, foliar N contents were affected by eO_3 in senescing leaves (Table 1). As compared to the other species, beech is distributed further south in Japan (Hoshika et al. 2012) where temperature is relatively high and soil microorganisms are relatively active, resulting in a longer growing season.

For oak species, eO_3 significantly led to about 70 % lower foliar N contents in live leaves (Table 1), suggesting that eO_3 promoted oak to adopt the strategy to recycle N beforehand in leaf vigorous stage during the growing season. Although oak was labeled as the least sensitive to O_3 among the 3 species in Yamaguchi et al. (2011), the present result in our study shows that live leaves of oak were very sensitive to eO_3.

Table 1: N contents (mg/g) in live and senescing leaves and their retranslocation rate (RR) in birch, beech and oak seedlings grown under ambient and elevated O3 as measured within the growing season. Data are represented as mean ± SD (n=7-9). t-test was applied for evaluate the difference of foliar N content and the retransloaction rate between ambient and elevated O3; n.s. no significant, *P<0.05, **P<0.01

	Birch			Beech			Oak		
	Live	Senescing	RR%	Live	Senescing	RR%	Live	Senescing	RR%
Ambient O_3	37.62 (±1.55)	16.28 (±0.78)	58.00 (±0.62)	19.79 (±0.43)	14.52 (±0.34)	25.47 (±1.20)	67.17 (±0.89)	47.75 (±1.33)	28.02 (±4.07)
Elevated O_3	34.99 (±1.10)	14.39 (±0.73)	61.82 (±1.62)	18.85 (±0.95)	12.69 (±0.86)	34.20 (±2.99)	20.2 (±0.21)	14.28 (±0.64)	30.08 (±1.38)
t-test	n.s	**	*	n.s	*	**	**	n.s	ns

LMA is considered as the essential indicator for leaf trait analysis in the leaf morphology of those species (Hayashi et al., 2005). Generally, LMA is decreased with the natural process of

Table 2: Pearson's correlation coefficients (r) between N concentrations in leaves (dependent variable) and the corresponding LMAs (independent variable) of three species (n=35; Pooled = the main effects of the gas treatments).

Foliar N	Ambient O_3				Elevated O_3			
	Birch	Oak	Beech	Pooled	Birch	Oak	Beech	Pooled
N_{live}	-0.29*	-0.14**	0.02	-0.46**	-0.07	0.009	0.23*	0.27
$N_{senescing}$	-10.51*	5.47	2.03	-0.56**	-0.34	-3.63	2.34	0.41

*P<0.05, **P<0.01

leaf senescence due to the reduction of dry mass and the decline of the nutrient in senescing leaves, but O_3-induced modification in leaf traits may reduce the leaf size and thus enhance the LMA values (Paoletti, 2007).

With regard of foliar N contents at ambient O_3 levels, there were significant negative correlations between foliar N and LMA of birch and oak in live leaves, as well as for birch in senescing leaves. Accordingly, N_{live} at ambient in birch and oak was higher compared to that at eO_3 which was regulated by their relatively lower LMA values at ambient air. And those results in Table 2 are also consistent with the results in Table 1. Similarly, LMA of senescing leaves in birch at ambient was a lower value than that in either live leaves or at eO_3. Based on the negative correlations in birch in Table 2, we expected a higher $N_{senescing}$ at ambient compared to eO_3 which also in agreement with the result in Table 1.

The only significant positive correlation observed between foliar N and LMA was for the live leaves of beech under eO_3 (Table 2). However, LMA in live leaves was expected a higher value compared to senescing leaves as well as a lower value compared to eO_3 at the same time. Higher N_{live} at ambient in Table 1 indicated that eO_3 did not affect much on the correlations between LMA and foliar N in this study. Alternatively, eO_3 did not simply increase the LMA, the changes on LMA may also be controlled by other factors that may need to be further tested.

With regard of the main effects on gas treatments, the highly significant negative correlations were observed at ambient between LMA and foliar N, for both in live and senescing leaves (Table 2). As a consequence, LMA can be utilized as an index parameter in speculation of the foliar N dynamics at ambient.

▪ Conclusions

In conclusion, different species may affect the foliar N dynamic in response to exposure O_3 differently. Foliar N dynamics of birch species is also accelerated by eO_3 due to the sensitivity to O_3 compared to beech and oak. Both birch and beech adopt the recycling of N at late stage of the growing season. Moreover, although the correlations between LMA and the effect of O_3 on foliar N dynamics are unclear, LMA is capable to be utilized as one of the indicators in speculation of the foliar N dynamics at ambient condition. (This study is supported in part by

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