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Retranslocation of foliar nutrients of deciduous tree seedlings in different soil condition under free-air O₃ enrichment

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Retranslocation is the amount of an element that is depleted from old plant components and is provided for new growth. Leaf senescence is usually accelerated at elevated O₃ (eO₃), and leaf shedding is influenced by soil nutrient availability (and acidification). In this study, we focused on the net retranslocation and allocation dynamics of foliar nutrients (N, P, Mg, K, Ca, Mn, Fe and Al) to investigate the effect of eO₃ on birch (*Betula platyphylla* var. *japonica*), oak (*Quercus mongolica* var. *crispula*), and beech (*Fagus crenata*) seedlings grown in different soil conditions. Seedlings of the 3 species were planted in a free-air O₃ enrichment system under 3 soil types (brown forest soil, serpentine soil, volcanic ash soil) for one growing season. All tree species were grown with 3 replications per each plot at elevated O₃ (about 80 ppb) and ambient condition (O₃ ranging 25-35 ppb). Leaf samples were taken from the top part of seedlings during the growing season in mid-September, and senescing leaves were sampled in mid-November. Both were collected for chemical composition analysis. Retranslocation rate of P was markedly increased by eO₃ in birch and significantly differed among soil types in oak seedlings, while was constant across treatments in beech seedlings. Retranslocation of N in oak seedlings was significantly affected by soil type. Retranslocation of other elements was most sensitive to both eO₃ and soil type in beech seedlings. The influence of differential growth patterns among species in modulating the physiological response of seedlings to high levels of ozone and different soil conditions are discussed.

Keywords: Retranslocation, Foliar Nutrients, Ozone, Volcanic Ash Soil, Serpentine Soil

Introduction

Retranslocation of foliar nutrients can be considered as the amount of certain elements depleted from aged plant organs and used for production in other parts of the plant (Salifu & Timmer 2001, Killingbeck 2004). It is one of the most essential processes in nutrient dynamics and ecosystem function via decomposition processes, especially for broadleaf species (Koike 2004).

Retranslocation can be quantified by the

parameter of nutrient resorption efficiency (NURE), which is defined as the percentage of the foliar nutrient pool absorbed (Huang et al. 2007, Farahat & Linderholm 2015). Positive NURE values indicate that plants are able to reuse rather than lose their internal nutrients with senescence, therefore plant growth, reproduction and competitive ability will be facilitated and vice versa for negative NURE values (Farahat & Linderholm 2015).

Retranslocation has been characterized as an important strategy for trees to conserve nutrients, which eventually affects nutrient competition, absorption and productivity (Salehi et al. 2013). It is an essential mechanism for improving plant growth as well as nutrient competitiveness and species conservation (Salifu & Timmer 2001, Uddling et al. 2006).

These processes of nutrient retranslocation in plants are likely to be affected by plant genetic make-up or plant species, soil fertility, as well as other environmental conditions (Hagen-Thorn et al. 2004). Despite the fact that research has been conducted on the retranslocation response of foliar nutrients, most studies has mainly targeted one or various species (Helmisaari 1992, Salehi et al. 2013) under different soil statuses, for instance, water irrigation and nitrogen fertilization (Salifu & Timmer 2001, Zhao et al. 2013). Therefore, little information is available for understanding the effects of elevated ozone (hereafter: eO₃) and its relations and interactions with soil fertility (or acidification) on the retranslocation process of foliar nutrients (Uddling et al. 2006).

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Recently, ground level O₃ concentration has been continuously increasing in north-east Asia and is negatively affecting the forest health (Serengil et al. 2011, Koike et al. 2013). Furthermore, elevation of O₃ is expected to continuously increase for at least several decades in the foreseeable future (Ohara et al. 2007). In addition, eO₃ can accelerate foliar senescence (Hoshika et al. 2012) and reduce the retranslocation rate of nitrogen (N – Kam et al. 2015), resulting in nutrient deficiencies in leaves caused by the decline of N acquisition (Karnosky et al. 2007).

Brown forest soil (mostly Cambisols, pH 5.4) which is a common soil type for deciduous broad-leaved forests, contains the necessary elements for plant growth (Kayama et al. 2011, Kim et al. 2011). Serpentine soil (pH 7.4) is derived from weathered serpentine rock (Brady et al. 2005, Kayama & Koike 2015) and is characterized by excessive Mg and heavy metals but a low contents of Ca and several essential nutrients (Brady et al. 2005). It has a high pH value and is also known to be widely distributed throughout Japan (Kayama & Koike 2015). Volcanic ash soil (pH 3.6) is an acidic soil that contains high levels of aluminum (Al) and iron (Fe), but low contents in magnesium (Mg), calcium (Ca), manganese (Mn), nitrogen (N) and potassium (K – Kayama et al. 2011, Watanabe et al. 2010). As the response of retranslocation seems to be influenced by soil fertility (Xu & Timmer 1999) as well as soil acidification (Gjengedal 1996, Adams et al. 2006), we have planted seedlings on brown forest soil, volcanic ash soil and serpentine soil. These soil conditions represent rich fertility with neutral soil, medium fertility with acidic soil and poor fertility with alkaline soil, respectively.

As the representative of deciduous species in the northern forests of Japan, Japanese white birch (henceforth birch: *Betula platyphylla* var. *japonica*), Mizunara oak (oak: *Quercus mongolica* var. *crispula*), and Siebold's beech (beech: *Fagus crenata*) were chosen, since they all provide social, economic and environmental benefits that are important to the cool temperate forests of Japan (Koike 1995, Koike et al. 1998). According to Yamaguchi et al. (2011), O₃ tolerance capacity of these 3 species can be ranked from low to high as follows: birch < beech < oak. In general, most early successional tree species retranslocate less nutrients from their leaves before shedding, compared to late successional trees, especially in plants grown in infertile soil condition (Killingbeck 2004, Koike 2004).

In this study, we focus on the retranslocation and allocation dynamics of foliar nutrients (N, P, Mg, K, Ca, Mn, Fe and Al) to achieve the following major objectives: (i) to determine how O₃ concentration affects foliar nutrients retranslocation in birch, beech and oak; (ii) to quantify foliar nutrient retranslocation of element N, P, Mg, K, Ca, Mn, Fe and Al, as well as to examine

foliar nutrients retranslocation in different soil sites; (iii) to evaluate the combined effects of both O₃ and soil on foliar nutrients retranslocation in birch, beech and oak. Based on the achieved results, we also discuss the plausible understanding of the ecophysiological meaning of retranslocation of each element in leaves in relation to specific traits of O₃ effect.

Materials and methods

Plant materials

Two-year old seedlings of Japanese white birch (birch: *Betula platyphylla* var. *japonica*), Siebold's beech (beech: *Fagus crenata*) and Mizunara oak (oak: *Quercus mongolica* var. *crispula*) were obtained near Sapporo (Hokkaido, Japan), and were planted in mid-July 2014 at the nursery of Hokkaido University, Sapporo. These 3 deciduous tree species are the major components of cool temperate forests in Japan (Koike 1995, Koike et al. 1998). All the seedlings were grown in the same soil-environment and growth media.

Experimental site description

The experimental site was located in Experimental Nursery of Hokkaido University Forests in the central part of Sapporo, northern Japan (43° 04' N, 141° 20' E, 15 m a.s.l.) with about a population of 2 million people. Annual mean temperature and the total precipitation in 2014 were 8.2 °C and 1129 mm respectively. A free-air O₃ enrichment system was utilized at the university experimental nursery with each system having 3 soil types (brown forest soil, serpentine soil, volcanic ash soil) and with each soil type being about 30 cm in depth. The enrichment lasted for one growing season. The experimental site consists of 3 control and 3 treatment circular plots 6.5 m in diameter, and with each system being surrounded by 5 m high dispersal pipes (Kim et al. 2011). Details of the O₃ exposure in field station as well as the plot design can be found in Watanabe et al. (2013). O₃ concentration of 80 ± 7 nmol mol⁻¹ was applied during daylight hours (about 7 h per day) from early-August to mid-November in treatment plots, while at the same time, the control plots were subjected to ambient daytime O₃ concentration of 26.5 nmol mol⁻¹ (Kam et al. 2015).

Each of the free-air enrichment plot was unevenly divided into 3 parts, replaced with 3 different soil types: about 84% of the plot area was divided into 2 parts, each equally consisting of brown forest soil (B) and volcanic ash soil (V); about 16% of the area was serpentine soil (S). All of these soil types are widely distributed across northern Japan (Kim et al. 2011). Brown forest soil is native to the Sapporo Experimental nursery, immature volcanic ash soil was brought from the Tomakomai Experimental Forest of Hokkaido University (Kim et al. 2011) and serpentine soil was brought from the eastern part of Teshio Experimental

Forest (Watanabe et al. 2012), where serpentine soil is dominant (Kayama & Koike 2015).

When taken together, the system was set up with 6 factorial combination treatments of O₃ exposure and soil types in the experimental nursery as follow: ambient O₃ × B (AB), eO₃ × B (EB), ambient O₃ × S (AS), eO₃ × S (ES), ambient O₃ × V (AV), eO₃ × V (EV).

Collection of samples and measurements

Samples of live and senescing leaves were taken from 3 seedlings per species in each treatment (4 trees per replication) in two-stages: for live leaves, samples were taken in mid-September during the peak nutritional activities of seedlings, while for senescing leaves samples were taken in mid-November (Shi et al. 2016). The top crown or second leaf counted from the shoot-top was collected as live leaves. After rinsing their surface with distilled water, both collected live and senescing leaves were dried at 70 °C for at least 5 days. Afterward, all the leaves were mill ground into powder to be used for further analysis.

In order to analyze the projected elements (N, P, Mg, K, Ca, Mn, Fe and Al), the powdered leaf samples were digested by nitric acid, hydrogen peroxide method and the concentration was measured with an Inductively Coupled Plasma Mass Spectrometry (ICP-MS, IRIS/IRIS Advantage ICAP, Thermo Fisher Scientific Inc., MA, USA) analysis. N concentration was determined by the combustion method using a NC analyzer (NC-900, Sumica, Osaka, Japan).

For evaluating the retranslocation rate and determining the proportion of the foliar nutrients resorbed before leaf abscission, the nutrient resorption efficiency (*NURE*) from senescing leaves was calculated as follows (eqn. 1):

$$NURE = (C_{live} - C_{senescing}) / C_{live}$$

where *NURE* is the nutrient resorption efficiency, *C_{live}* is the nutrient concentration of live leaves and *C_{senescing}* is the nutrient concentration of senescing leaves (Helmissaari 1992, Huang et al. 2007, Salehi et al. 2013). For each specific nutrient *X*, (*X*)RE represents the resorption efficiency of nutrient *X*, where *X* = N, P, Mg, K, Ca, Mn, Fe and Al.

Statistical analysis

Parameter values for each species and each treatment were averaged over at least 3-4 replications, in order to provide the sample estimate for the actual replicate. Each *NURE* was subjected to linear mix model as follows (eqn. 2);

$$NURE = GAS + Soil + GAS \times Soil + Plot$$

where *GAS* is the treatment of ozone (ambient and eO₃), *Soil* is the treatment of soil (B, S and V), *GAS* × *Soil* is the interac-

Tab. 1 - Mean (\pm standard deviation) nutrient resorption efficiency (NURE) of Mg, K, Ca, Mn, Fe and Al in birch seedlings grown on 3 soil types (B: brown forest soil; S: serpentine soil; V: volcanic ash soil) under 2 gas treatments (A: ambient O₃; E: elevated O₃). Two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range tests were used to compare the NURE differences among the 6 treatments (3 replicates each). Different letters within each row (nutrient) indicate significant differences among treatments ($\alpha = 0.05$). (ns): treatment effect not significant; (*): $p < 0.05$; (**): $p < 0.01$; (***) : $p < 0.001$ (ANOVA).

Birch	AB	EB	AS	ES	AV	EV	Gas	Soil	Gas \times Soil
Mg	-0.472 \pm 0.086 ^a	-0.010 \pm 0.314 ^a	-0.177 \pm 0.249 ^a	-0.510 \pm 0.173 ^a	-0.227 \pm 0.098 ^a	-0.472 \pm 0.066 ^a	ns	ns	ns
K	0.261 \pm 0.009 ^a	0.257 \pm 0.031 ^a	0.239 \pm 0.046 ^a	0.242 \pm 0.015 ^a	0.334 \pm 0.026 ^a	0.157 \pm 0.084 ^a	ns	ns	ns
Ca	-0.624 \pm 0.160 ^{cd}	-0.744 \pm 0.078 ^d	-0.242 \pm 0.075 ^a	-0.364 \pm 0.021 ^{abc}	-0.258 \pm 0.077 ^{ab}	-0.541 \pm 0.064 ^{bc}	*	**	ns
Mn	-0.650 \pm 0.269 ^a	-0.605 \pm 0.113 ^a	-0.611 \pm 0.043 ^a	-0.508 \pm 0.057 ^a	-0.328 \pm 0.049 ^a	-1.050 \pm 0.065 ^b	ns	ns	*
Fe	-3.180 \pm 0.504 ^b	-3.224 \pm 0.517 ^b	-1.262 \pm 0.039 ^a	-1.579 \pm 0.073 ^a	-1.513 \pm 0.275 ^a	-1.854 \pm 0.224 ^a	ns	***	ns
Al	-4.575 \pm 0.371 ^b	-2.503 \pm 0.268 ^a	-2.250 \pm 0.312 ^a	-2.649 \pm 0.139 ^a	-2.597 \pm 0.363 ^a	-2.186 \pm 0.058 ^a	*	**	**

tion of ozone and soil, and *Plot* is the location of each circular plot. We used *GAS*, *Soil* and *GAS* \times *Soil* as fixed factors, and *Plot* as a random factor. The effects of each fixed factors were analyzed by likelihood ratio test, and we applied Tukey's post-hoc test when the likelihood ratio test was significant ($p < 0.05$). These analyses were performed separately on each species. These statistical analyses were performed using R (version 3.1.0 – <http://www.r-project.org/>) with the "lmer" function of lme4 package (Version 1.1-7 – <http://CRAN.R-project.org/package=lme4>).

For the mineral elements Mg, K, Ca, Mn, Fe and Al, two-way analysis of variance (ANOVA) was used to test the effects of O₃ exposure and soil types as well as their interaction. Results were considered significant when $p < 0.05$. Duncan's Multiple Range tests were utilized to compare the NURE differences among the 6 treatments. These statistical analyses were performed using the software package SPSS® ver. 21.0 (IBM, NY, USA).

Results and discussion

In general, foliar elements are transported through xylem and phloem, and their content results from the balance between influx via xylem and phloem, and efflux via phloem (Larcher 2003). Water soluble elements such as N, P and K are known to be mobile in the phloem and readily retranslocated from older to younger leaves (Marschner 2012). Consequently, N, P and K become unstable during leaf senescence, and are usually transported to other parts of the plant, resulting in a decrease of their concentration in senescing leaves (Helmisaari 1992).

On the other hand, the non-mobile mineral elements Ca, Mn, Fe and Al are transported from roots to leaves through xylem but are poorly mobile in phloem (Schulze et al. 2005, Marschner 2012, Shi et al. 2016). Therefore, content deficiencies of these elements commonly occur in younger leaves.

Mg is the central atom of chlorophyll pigment, and is essential for ribosome aggregation. In addition, it is often regarded as one of the indicators for leaf greenness and many physiological functions (Killingbeck 2004). Because of its mobility and

availability in plants (Gjengedal 1996), Mg is usually considered to decrease with leaf senescence (Helmisaari 1992). Therefore, positive values for N, P, K and Mg, along with negative values of NURE for Ca, Mn, Fe and Al may be the expected results.

Birch seedlings

Notwithstanding the above considerations, (Mg)RE in birch was unexpectedly negative under every treatment (Tab. 1). This result is in agreement with the study of Carr et al. (2003), who reported an increased Mg concentration in aged leaves. It is possible that the dynamics of Mg in live leaves was suppressed by the increased foliar K, as we detected a negative correlation between Mg and K (Kayama & Koike 2015).

Previous research suggested that nutrient retranslocation in late successional

species can increase at nutrient-poor sites (Koike 2004). In this study, similar results were obtained at ambient O₃ for 5 elements (N, P, Ca, Fe, Al – Fig. 1, Tab. 1) and at eO₃ condition for (Ca)RE and (Fe)RE (Tab. 1). However, the contrasting result observed for (N)RE at eO₃ (Fig. 1a) suggests that NURE may be independent of the gradient of the nutrients in the soil (Nelson et al. 1995). Alternatively, NURE is likely decreased at nutrient-poor sites by the interaction effects of eO₃ and soil fertility, as (N)RE was found to be significant in interaction of eO₃ and soils (Fig. 1a). In any case, NURE was likely to be limited by the availability of nutrient gradients, as NURE of N, Ca and Fe at eO₃ and NURE of P, Ca, Fe and Al at the ambient site did not show significant differences in both V and S soils (Fig. 1 and Tab. 1).

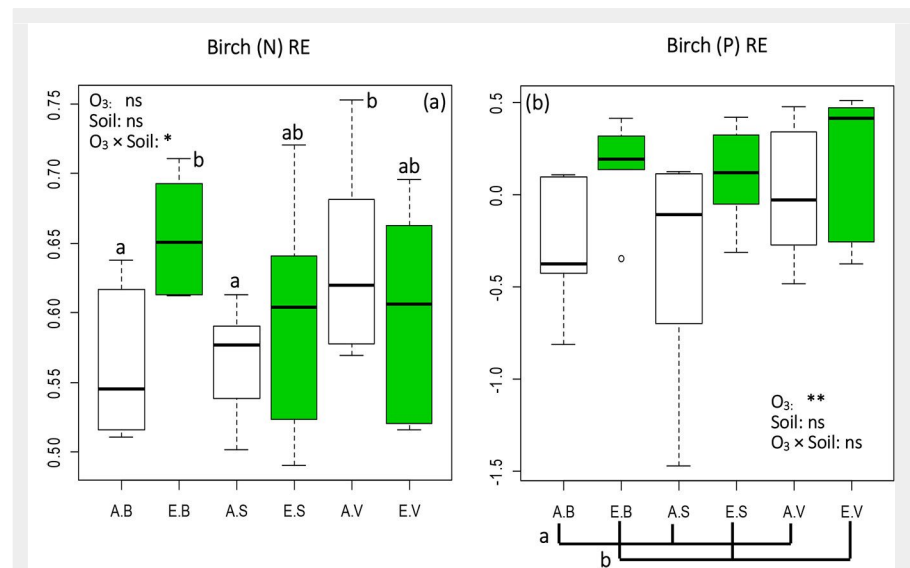


Fig. 1 - Box-plot of nutrient resorption efficiency (NURE) values of (a) Nitrogen [(N)RE] and (b) Phosphorus [(P)RE] in birch seedlings subjected to 6 treatments (3 replicates each). Green boxes indicate elevated O₃ treatments (EB, ES, EV), white boxes ambient O₃ treatments (AB, AS, AV). B, S, and V indicate different soil types (see Tab. 1). Effects of each fixed factors were tested by the likelihood ratio (LR) test, and Tukey's post-hoc test was used when LR test was significant. Different letters indicate significant pairwise differences between treatments ($\alpha=0.05$). Box: inter-quartile range; whiskers: lower and upper quartiles; thick horizontal line within boxes: median; circles: extreme values. (ns): treatment effect not significant; (*): $p < 0.05$; (**): $p < 0.01$.

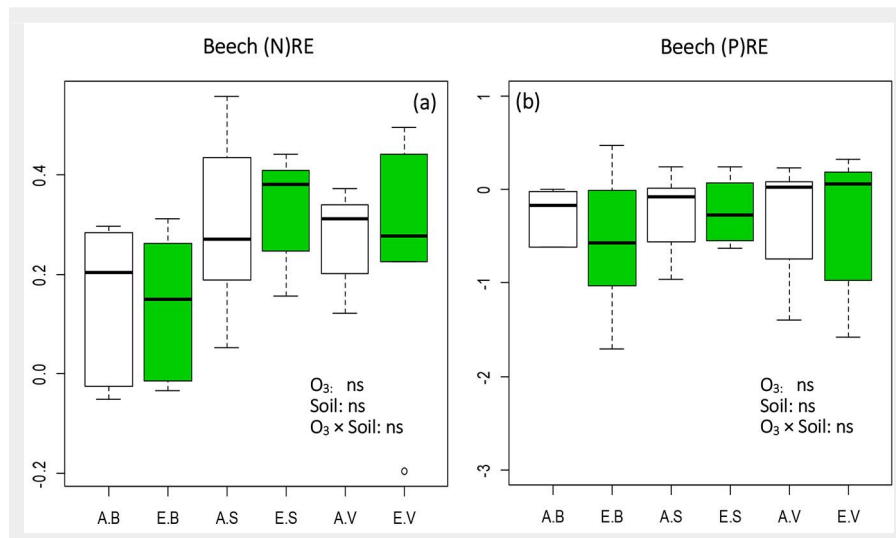


Fig. 2 - Box-plot of nutrient resorption efficiency (NURE) values of (a) Nitrogen [(N)RE] and (b) Phosphorus [(P)RE] in beech seedlings subjected to 6 treatments (3 replications each). Green boxes indicate elevated O₃ treatments (EB, ES, EV), white boxes ambient O₃ treatments (AB, AS, AV). B, S, and V indicate different soil types (see Tab. 2). Effects of each fixed factors were tested by the likelihood ratio (LR) test, and Tukey's post-hoc test was used when LR test was significant. Different letters indicate significant pairwise differences between treatments ($\alpha=0.05$). Box: interquartile range; whiskers: lower and upper quartiles; thick horizontal line within boxes: median; circles: extreme values. (ns): treatment effect not significant.

We expected that O₃ would be detrimental to plant photosynthetic performances (Uddling et al. 2006, Koike et al. 2013) and nutrient dynamics, resulting in a lower NURE at eO₃ due to the O₃-induced reduction of translocation from older to younger leaves. As an example, the (Mn)RE value of plants grown in the V soil (Tab. 1) was significantly decreased by eO₃. Such decrease may be attributed to the reduction of Mn in live leaves caused by the decline of photosynthetic functions and by the low Gs induced by eO₃ (Yamaguchi et al. 2011, Shi et al. 2016). Contrastingly, NURE of N, P (Fig. 1a, Fig. 1b) and Al (Tab. 1) were markedly increased by eO₃. According to Hoshika et al. (2013b), live leaves show a higher capacity of avoidance for O₃-induced low Gs, thus being less susceptible to O₃ stress than senescing leaves in birch (Hoshika et al. 2012, 2013b). Therefore, the decreased concentration of the above elements in senescing leaves at eO₃ may be the result of their increased NURE.

Furthermore, Mn showed a significantly higher accumulation in senescing leaves of plants grown in V soil at eO₃, as compared with other soils at eO₃ (Tab. 1). According to the literature (Kitao et al. 1998), Mn tends to have a lower mobility when it is limiting to the plant, thus the deficiency of Mn in the V soil may explain its higher accumulation in the leaves of plants grown under eO₃.

Beech seedlings

(N)RE and (P)RE were decreased under eO₃ in beech, with the exception of (N)RE in the AS and ES treatments. Since no significant effects of O₃ and soil (or their interaction) on N and P were detected (Fig. 2), the observed NURE decrease was likely due to the leaching of N and P in senescing leaves under eO₃ (Helmisaari & Mälikönen 1989). In contrast, (N)RE in the ES treatment reached the highest value compared to other treatments (Fig. 2a), suggesting that more N was allocated to live leaves of

beech for plant growth. This is especially important for plants grown in poor fertility soils like S soil in this study.

Yamaguchi et al. (2007) reported that the sensitivity of growth and photosynthetic parameters to O₃ in beech became high at higher concentrations of N in the soil. Moreover, Hoshika et al. (2013b) concluded that late leaves in birch were more susceptible to O₃ stress. In this context, the higher NURE values observed at eO₃ in high fertility soils may be a consequence of the lower retention of elements in senescing leaves. Indeed, we found NURE values at eO₃ markedly higher in the B soil than in other soils for K, Ca, Mn and Al (Tab. 2).

In addition, Fe and Al NURE were significantly enhanced by eO₃ treatments in both V and S soils and in V soil alone, respectively (Tab. 2). Fe and Al were indicative of the reduced accumulation of elements in senescing leaves at specific treatments. It has recently been shown that N deficiency-induced leaf senescence (as in the case of V and S soil types) enhances Fe mobilization in old leaves, thereby leading to the decline of foliar Fe accumulation (Barunawati et al. 2013, Shi et al. 2012). Regarding the higher Al(RE) observed under eO₃ in beech, the possible explanation is similar to that discussed above for birch. However, the O₃-induced reduction of Al concentration in older leaves of beech (Hoshika et al. 2013b, Yamaguchi et al. 2007) may not be adequately counterbalanced by the increase in foliar Al during leaf senescence in plants growing on acidic soils (Gjengedal 1996), and this may explain the increase of (Al)RE in the treatment EV observed in this study (Tab. 2).

On the other hand, a decrease in (Mg)RE in B and S soils, NURE of K and Ca in S and V soils, (Mn)RE in B and V soils were observed in plants grown under eO₃ (Tab. 2). Beside the aforementioned O₃-induced reduction of translocation, the concentration of those nutrients in live leaves could be affected by leaching (Helmisaari 1992, Gjengedal 1996, Vergutz et al. 2012). Previous research demonstrated that foliar leaching of Ca, K and Mg can be observed on acidified soil and is believed to occur via cation exchange (Gjengedal 1996). These results may suggest that foliar leaching is independent from soil acidification, at least in

Tab. 2 - Mean (\pm standard deviation) nutrient resorption efficiency (NURE) of Mg, K, Ca, Mn, Fe and Al in beech seedlings grown on 3 soil types (B: brown forest soil; S: serpentine soil; V: volcanic ash soil) under 2 gas treatments (A: ambient O₃; E: elevated O₃). Two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range tests were used to compare the NURE differences among the 6 treatments (3 replicates each). Different letters within each row (nutrient) indicate significant differences among treatments ($\alpha = 0.05$). (ns): treatment effect not significant ; (*): $p < 0.05$; (**): $p < 0.01$; (***) : $p < 0.001$ (ANOVA).

Beech	AB	EB	AS	ES	AV	EV	Gas	Soil	Gas × Soil
Mg	-0.020 ± 0.174 ^{ab}	-0.849 ± 0.863 ^c	0.436 ± 0.006 ^a	-0.449 ± 0.207 ^{bc}	0.202 ± 0.055 ^{ab}	-0.135 ± 0.049 ^{abc}	**	ns	ns
K	0.216 ± 0.053 ^a	0.238 ± 0.083 ^a	0.310 ± 0.069 ^a	-0.063 ± 0.070 ^b	0.144 ± 0.017 ^a	-0.020 ± 0.012 ^b	**	*	**
Ca	0.043 ± 0.028 ^a	-0.093 ± 0.041 ^{ab}	-0.099 ± 0.073 ^{ab}	-0.345 ± 0.085 ^c	0.068 ± 0.021 ^a	-0.150 ± 0.049 ^b	**	**	ns
Mn	0.487 ± 0.035 ^a	-0.039 ± 0.043 ^b	-0.630 ± 0.066 ^c	-0.352 ± 0.097 ^c	0.135 ± 0.072 ^b	-0.455 ± 0.171 ^c	**	***	**
Fe	-0.148 ± 0.017 ^a	0.095 ± 0.125 ^a	-1.082 ± 0.343 ^b	0.005 ± 0.058 ^a	-1.420 ± 0.246 ^b	-0.416 ± 0.105 ^a	***	**	ns
Al	-0.276 ± 0.058 ^{ab}	0.013 ± 0.030 ^a	-0.561 ± 0.179 ^b	-0.353 ± 0.053 ^b	-1.265 ± 0.239 ^c	-0.467 ± 0.088 ^b	***	***	*

beech. Moreover, the Al toxicity may also cause the inhibition of some mineral nutrients resorption during senescence (Schulze et al. 2005).

Oak seedlings

No significant effects of O₃ on the NURE of N and P were detected in oak seedlings, though an increase of (N)RE and (P)RE in plants grown in both S and V soils under elevated O₃ was observed (Fig. 3). Also, (N)RE and (P)RE were clearly higher in leaves from plants grown in the V soil than in the S soil. Helmisaari (1992) emphasized that foliar NURE does not vary in response to soil fertilization. Therefore, we hypothesized that higher (N)RE and (P)RE in V soil may reflect soil depletion and acidification. Indeed, depletion-driven losses of N and P in the soil are associated with a decreased retention of N and P in senescent leaves and strongly increased NUREs (Hayes et al. 2014). Accordingly, the higher NURE of N and P in V soil (Fig. 3) may result from the high depletion-driven loss in V (acidic) soil, as output N had a high negative correlation with soil pH (Dise & Wright 1995).

Similarly, the markedly higher (Ca)RE observed in the V soil compared to the S soil in the ambient O₃ treatments (Tab. 3) suggests that higher depletion-driven loss in V soil may also affect Ca. In contrast, significantly lower (Ca)RE in V than in S soil was observed in treatments under eO₃, likely due to soil type-O₃ interactions. In particular, the enhanced NURE caused by soil was not adequately compensated by O₃-induced detrimental effects on NURE. Such combined influences may largely affect other elements in oak plants.

(Al)RE in high fertility soil (B soil) was the highest in controls (ambient O₃) but clearly the lowest under eO₃ (Tab. 3). Retranslocated nutrients are generally essential for the production of new tissues and organs throughout all stages of plant development. This suggests that soil and environmental factors that facilitate plant growth also promote nutrient retranslocation and vice versa (Nambiar & Fife 1991). As a result, NURE is expected to increase in high-fertility soils but to decrease under eO₃. Therefore, the markedly highest (Mn)RE and (Al)RE in AB (Tab. 3) may be the result of the relatively high-fertility soil conditions

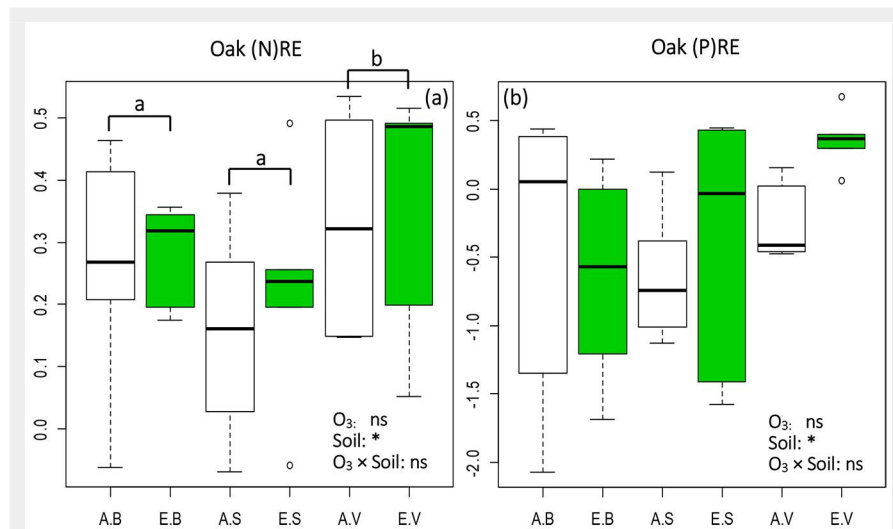


Fig. 3 - Box-plot of nutrient resorption efficiency (NURE) values of (a) Nitrogen [(N)RE] and (b) Phosphorus [(P)RE] in oak seedlings subjected to 6 treatments (3 replications each). Green boxes indicate elevated O₃ treatments (EB, ES, EV), white boxes ambient O₃ treatments (AB, AS, AV). B, S, and V indicate different soil types (see Tab. 3). Effects of each fixed factors were tested by the likelihood ratio (LR) test, and Tukey's post-hoc test was used when LR test was significant. Different letters indicate significant pairwise differences between treatments ($\alpha=0.05$). Box: interquartile range; whiskers: lower and upper quartiles; thick horizontal line within boxes: median; circles: extreme values. (ns): treatment effect not significant; (*): $p < 0.05$.

(B soil) where plants were grown, whereas the significant low (Fe)RE and (Al)RE in EB (Tab. 3) may be due to the effect of interactions between O₃ and soil. Nevertheless, the declined NURE caused by eO₃ may be aggravated by the relative high N in soil and finally results in the low NURE values (Yamaguchi et al. 2007).

Comparisons among birch, beech and oak seedlings

Considering the overall impacts of the eO₃ exposure on the foliar mineral nutrients investigated (Mg, K, Ca, Mn, Fe and Al), beech was the most affected (Tab. 2), followed by oak (Tab. 3) and birch (Tab. 1). This was inconsistent with previous studies reporting birch as the most and beech as the least sensitive species to eO₃ (Yamaguchi et al. 2011). These contrasting results may be attributable to differences in patterns of leaf development and growth among species (Hoshika et al. 2013a, Kiku-

zawa & Lechowicz 2011). Indeed, birch shows an indeterminate growth pattern (Koike 1995, Hoshika et al. 2013b), while beech and oak typically have determinate (Kitao et al. 2009) and semi-determinate patterns (Holbrook & Zwieniecki 2011), respectively. However, it is worth noting that such classification of shoot growth patterns is not clear-cut and considerable overlaps do exist in between classes. In any case, indeterminate growth patterns may promote the production of new leaves to compensate the damage caused by eO₃ (Hoshika et al. 2013a, 2013b), and this may explain why birch is the least affected species in terms of effects of eO₃ on mineral nutrients, as compared with beech and oak (Tab. 1).

In addition, beech seedlings were the most affected by soil type concerning the mineral nutrients investigated (Tab. 2), whereas oak was the most tolerant species in terms of impact of soil conditions on

Tab. 3 - Mean (\pm standard deviation) nutrient resorption efficiency (NURE) of Mg, K, Ca, Mn, Fe and Al in oak seedlings grown on 3 soil types (B: brown forest soil; S: serpentine soil; V: volcanic ash soil) under 2 gas treatments (A: ambient O₃; E: elevated O₃). Two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range tests were used to compare the NURE differences among the 6 treatments (3 replicates each). Different letters within each row (nutrient) indicate significant differences among treatments ($\alpha = 0.05$). (ns): treatment effect not significant; (*): $p < 0.05$; (**): $p < 0.01$; (***) : $p < 0.001$ (ANOVA).

Oak	AB	EB	AS	ES	AV	EV	Gas	Soil	Gas × Soil
Mg	-0.166 ± 0.046 ^a	0.269 ± 0.222 ^a	-0.317 ± 0.252 ^a	0.075 ± 0.058 ^a	0.172 ± 0.122 ^a	-0.167 ± 0.179 ^a	ns	ns	ns
K	-0.189 ± 0.022 ^a	-0.007 ± 0.062 ^a	0.068 ± 0.051 ^a	0.053 ± 0.183 ^a	0.073 ± 0.062 ^a	-0.308 ± 0.230 ^a	ns	ns	ns
Ca	-0.055 ± 0.064 ^{bc}	-0.109 ± 0.026 ^{bc}	-0.280 ± 0.020 ^d	0.148 ± 0.160 ^a	0.049 ± 0.033 ^{ab}	-0.157 ± 0.021 ^{cd}	ns	ns	***
Mn	-0.753 ± 0.330 ^b	-0.444 ± 0.203 ^{ab}	-1.759 ± 0.444 ^c	0.100 ± 0.010 ^a	-2.121 ± 0.033 ^c	0.255 ± 0.034 ^a	***	ns	**
Fe	-0.208 ± 0.059 ^a	-1.730 ± 0.490 ^b	-0.722 ± 0.181 ^a	-0.312 ± 0.343 ^a	-0.327 ± 0.040 ^a	-0.085 ± 0.048 ^a	**	*	**
Al	-0.241 ± 0.092 ^a	-1.481 ± 0.085 ^d	-0.593 ± 0.037 ^b	-0.164 ± 0.090 ^a	-0.984 ± 0.069 ^c	-0.513 ± 0.115 ^b	ns	***	***

nutrient retranslocation (Tab. 3). Although beech is a typical late-successional tree species (Koike 2004, Kitao et al. 2009), it often retranslocates more foliar nutrients before leaf shedding when growing on infertile soils, compared to early or mid-successional species such as birch and oak (Killingbeck 2004, Koike 2004, Eguchi et al. 2008). The results obtained in this study further support such evidence.

Conclusion

In summary, NURE is deeply affected by eO₃ and soil types in saplings of 3 Japanese representative tree species and considerable differences in nutrient acquisition and loss in leaves were found among species (Koike 2004, Hayes et al. 2014). The effect of ozone and soil varied across species with different growth patterns. The retranslocation of mineral nutrients was more efficient in beech, which has a determinate shoot growth pattern and showed a relatively high sensitivity of foliar NURE to either eO₃ or nutrient content in the soil. Based on our results, foliar NURE may be considered a good indicator of the physiological conditions of plants to be monitored in field surveys. Studies on the retranslocation of foliar nutrients may help develop appropriate strategies of nutrient management in plantations (Salehi et al. 2013).

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